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Understanding the Effect of Single- and Twin-Screw Extrusion Processing Parameters on Physical and Physico-Chemical Properties of Sprouted Quinoa and Sprouted Proso Millets

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UNDERSTANDING THE EFFECT OF SINGLE - AND TWIN-SCREW EXTRUSION PROCESSING PARAMETERS ON PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF SPROUTED QUINOA AND SPROUTED PROSO MILLETS

BY

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DISSERTATION ACCEPTANCE PAGE

Gabriela John Swamy

This dissertation is approved as a creditable and independent investigation by a candidate for the Doctor of Philosophy degree and is acceptable for meeting the dissertation requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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I dedicate this dissertation to

- My mom, MATILDA
- My husband, ARONE
- My princess, SASHA

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CONTENTS

ABBREVIATIONS

°C - Degree Celsius

a* - Redness/greenness

ANN - Artificial Neural Network

ANOVA - Analysis of Variance

b* - Yellowness/Blueness

BD - Bulk Density

CV - Coefficient of Variance

Db - Dry basis

Df - Degrees of freedom

ER - Expansion Ratio

L* - Brightness/Darkness

L_s - Screw length

R^2 - Regression Coefficient

Rpm - revolutions per minute

RSM - Response Surface Methodology

SS - Sum of Squares

WAI - Water Absorption Index

w.b. - Wet basis

WSI - Water Solubility Index

ΔE - Color change

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ABSTRACT

UNDERSTANDING THE EFFECT OF SINGLE - AND TWIN-SCREW EXTRUSION PROCESSING PARAMETERS ON PHYSICAL AND NUTRITIONAL PROPERTIES OF SPROUTED QUINOA AND SPROUTED PROSO MILLETS

GABRIELA JOHN SWAMY

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According to Mordor Intelligence Research, the new compositional research and moisture content extrusion process are helping the growth of the plant protein market. The demand for plant proteins is growing at a fast rate, owing to change in lifestyle, lack of balanced dietary intake, and improved research and development in order to develop new kinds of plant-protein enriched products. It is necessary to identify the right plant protein sources and choose the right processing methodology to create highly digestible foods which can be consumed by infants and elderly as well. In addition, it is important to support local farmers and help them make value added products.

The primary objective of this study was to investigate the feasibility of developing extruded foods from protein rich sprouted quinoa and proso millet flours with high protein and starch digestibility. The secondary objective was to understand the effect of extrusion processing conditions on the systems parameters and on the physical and physico-chemical properties of the extruded product.

Quinoa and Proso millet were chosen as the grains of interest due to their high protein content as compared to wheat, rice, corn, amaranth and buckwheat. The study was broadly then divided in to three parts. In the first part, pre-treatment methods such as soaking and sprouting were analysed for effective reduction of saponins and phytic acid and increased starch and protein digestibility. The sprouting of quinoa increased the starch digestibility from 55.6% on Day 0 to 78.2% on Day 4. A similar increase in the protein digestibility was observed from Day 0 (42.2%) to Day 4 (75.5%). Sprouting also produced similar effects in proso millet where the starch digestibility increased from 51.7% (Day 0) to 77.1%. The breakdown of the phytates during sprouting of proso millet increased the protein digestibility from 46.4% on Day 0 to 76.8% on Day 4. Simultaneously the reduction of saponin content in quinoa and phytic acid content in both grains were observed. The saponin content in raw quinoa of 0.8g/100g was reduced to 0.1g/100g samples (Day 4) by germination. The phytic acid content in quinoa reduced from 1.1g/100g to 0.1g/100g (Day 4) and in proso millet reduced from 1.5g/100g to 0.2g/100g (Day 4). The color of the flour produced from the sprouted grains were significantly different from the unprocessed flour respectively. The L* value of sprouted quinoa flour was darker (L*=61.2) as compared to the control sample (L* = 82.6). Similarly, the sprouted proso millet flour was darker (L*=70.1) than the unprocessed proso millet flour (L*=84.2)

In the second part, the extrusion process of sprouted quinoa was divided into three experiments. The first experiment is the single screw extrusion of sprouted quinoa. Using a response surface design to understand the influence of feed moisture content (15-25% w.b), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of sprouted quinoa extrudates was studied. The following responses were obtained: bulk density (116-154 kg/m3), hardness (1.05-1.8 N), water solubility index (11.5-16.5%), water absorption index (2.36-3.51), total color difference ΔE (14.8-21.7), expansion ratio (2.52-3.75), protein digestibility

(80.5-86.5%) and starch digestibility (80.1-85.8%). The die temperature and germination time played a significant role in product responses.

The second experiment was designed to create a puffed product with an inclusion of corn meal. Corn meal is the most common ingredient of expanded snacks in the food market. Because of its composition, ratio of vitreous to floury endosperm, and particle size, under optimal extruding conditions corn meal makes for a light, highly expanded, crunchy and soft product. Single-screw extrusion processing of sprouted quinoa-corn meal blend was studied using a response surface design to understand the influence of feed moisture content (15-25% w.b), die temperature (80-140°C), screw speed (90-220 rpm), corn meal ratio (0-30%) and quinoa time (Days 0-4) on the physical and physico-chemical properties of sprouted quinoa- corn meal blend extrudates. The following responses of the extrudates were measured and the values ranged from: bulk density (102-145 kg/m3), hardness (1.03-1.62 N), water solubility index (12.3-19.9%), water absorption index (2.44-3.79), total color difference ΔE (14.1-21.4), expansion ratio (2.75-3.97), protein digestibility (78.4-85.8%) and starch digestibility (77.5-85.6%). The addition of corn meal improved the color difference and expansion ratio of the extrudates.

The third experiment employed a twin-screw extruder to understand the influence of feed moisture content (15-25% w.b), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of sprouted quinoa flour extrudates. The bulk density (132-175 kg/m3), hardness (1.56-2.14 N), water solubility index (14.4-18.5%), water absorption index (2.93-3.41), ΔE (16.7-20.8), expansion ratio (2.28-2.83), protein digestibility (78.4-84.1%) and starch digestibility (77.1-83.4%) were measured. All independent parameters had statistically significant effects on all the extrudate characteristics. Twin screw extrusion did not improve the extrudate characteristics significantly. Poor expansion ratio was observed as compared to single-screw extrusion.

Similar experiments were carried out for sprouted proso millet flour. Single-screw extrusion processing of sprouted proso millet flour was studied using a Box Behnken response surface design to understand the influence of feed moisture content (15-25% w.b), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of proso millet extrudates. The following responses of the extrudates were measured and the values ranged from: bulk density (101-137 kg/m3), hardness (1.01-1.3N), water solubility index (14.8-18.7%), water absorption index (4.0-4.41), ΔE (13.0-17.1), expansion ratio (3.28-3.75), protein digestibility (78.2-86.6%) and starch digestibility (80.9-87.7%). Both feed moisture content and extruder die temperature had statistically significant effects on all the extrudate characteristics. Extruder screw speed had minimal effect on the extrudate properties.

It was followed by a single-screw extrusion processing of sprouted proso millet-corn meal blend with feed moisture content (15-25% w.b.), die temperature (80-140°C), screw speed (90-220 rpm), corn meal ratio (0-30%) and proso millet germination time (Days 0-4) on the physical and physico-chemical properties of sprouted proso millet-corn meal blend extrudates. The following responses of the extrudates were measured and the values ranged from: bulk density (100-143 kg/m3), hardness (0.84-1.59 N), water solubility index (12.5-20.1%), water absorption index (2.55-3.90), total color difference ΔE (13.07-20.37), expansion ratio (2.74-3.96), protein digestibility (78.5-85.9%) and starch digestibility (77.7-85.8%). The addition of 30% corn meal significantly increased the expansion ratio from 3.29-3.96.

Last study was to investigate the effects of twin-screw extrusion processing of sprouted proso millet with feed moisture content (15-25% w.b), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of sprouted proso millet extrudates. The responses from the experiment include: bulk density (132-175 kg/m3), hardness (1.56-2.14 N), water solubility index (14.4-18.5%), water absorption index (2.93-3.41), ΔE (16.7-20.8), expansion ratio (2.28-2.83), protein digestibility (78.4-84.1%) and starch digestibility (77.1-83.4%). The expansion ratio was significantly poor when compared to the single screw extrusion of sprouted proso millet.

In the third and last part, a comparative study on the efficacy of Response Surface Methodology (RSM) and Artificial Neural Network (ANN) in modelling of single and twin-screw extrusions were conducted. We optimized the neural network topology to predict the error and regression coefficient and root mean square error using artificial neural network and compared the results with response surface methodology for single screw (sprouted quinoa, sprouted quinoa-corn meal blend, sprouted proso millet, sprouted proso millet-corn meal blend) and twin screw (sprouted quinoa and sprouted proso millet) extrusion processes. Irrespective of the ingredient composition and blend for all extrusion processes, ANN predictions have regression coefficients greater than 0.9 and RSM predictions are greater than 0.8. Similarly, the root mean square error values were low in all ANN predictions are compared to RSM. Based on error analysis results, the prediction capability of ANN model is found to be the best of all the prediction models investigated, irrespective of food composition and extrusion processes.

CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1 Introduction

A plant-based diet is a diet consisting largely or entirely of foods derived from plants, together with vegetables, grains, nuts, seeds, legumes and fruits, and with few or no animal products. According to the Good Food Institute, from 2009 to 2018, approximately $17.1 billion has been invested into the plant-based food industry from 229 unique investors (Cameron & O’Neill, 2019). Recent innovations have opened the door to delivering complete proteins in a myriad of snack applications. As snacking becomes an integral part of the American diet, it will be increasingly important for snacks to deliver nutrition benefits, including high-quality protein. This development has food manufacturers looking beyond just bars and shakes to deliver high-protein snacks. According to a report by Research and Markets (2019), the global extruded snack food market reached a value of around USD 80.6 billion in 2018, growing at a CAGR of around 3% during 2011-2018.

Proso millet (*Panicum milliaceum*) is a warm season grass with a growing season of 60–100 days and is harvested depending on the seed maturity required for end-use. (Why this much variation?). It is an extremely nutritive cereal grain used for human consumption, bird seed, and/or ethanol production. Unique characteristics, such as drought and heat tolerance, make proso millet a promising alternative cash crop for the United States (Colosi & Schaal, 1997). Development of proso millet varieties tailored to dryland farming regions might offer growers a much-needed choice for diversifying their preponderantly wheat-based cropping systems (Habiyaremye et al., 2017). On the other hand, the nutritional value of proso millet is equivalent to that of wheat. It is rich in

Vitamin B6, folic acid and above all is gluten free (Amadou, Gounga, & Le, 2013). The US food industry is interested in proso millet for its health benefits, mild flavor, high protein and gluten-free characteristics.

Quinoa is an annual grain crop. It is a pseudocereal unlike wheat and rice (Jacobsen, 2003). Quinoa is gluten-free, high in protein and one of the few plant foods that contain sufficient amounts of all nine essential amino acids (Repo-Carrasco, Espinoza, & Jacobsen, 2003). It is also high in fiber, magnesium, B vitamins, iron, potassium, calcium, phosphorus, vitamin E and various beneficial antioxidants (James, 2009). While quinoa is being considered important for its high protein content, several studies have helped to clarify some key strengths of this food from a protein standpoint. Equally important, within the protein are plentiful amounts of many amino acids (the building blocks of protein). For all amino acid requirements declared by the World Health Organization (WHO), quinoa delivers between 70-360% of each required amino acid (using the milligrams/gram standard by WHO) (Ruales & Nair, 1992). When combined together, the high total protein content of quinoa and its outstanding amino acid composition make it an excellent source of plant protein in any meal plan (Wright, Pike, Fairbanks, & Huber, 2002).

Phytic acid is considered both an antioxidant and an anti-nutrient. It is technically called a hexa-phosphoinositol and is a powerful chelator, which means it binds with other minerals and makes it unavailable (Khattak, Zeb, Bibi, Khalil, & Khattak, 2007). This could be positive, toxin-reducing, and cancer-fighting, or negative, prohibiting the system from absorbing minerals (Liang, Han, Nout, & Hamer, 2008). Phytates are the bond holding phytic acid. They are the “salt of the phytic acid,” which can be broken in several ways. in the gut with stomach acid via germination under the enzyme phytase (Tabekhia & Luh, 1980). Similarly, saponins are naturally occurring phytochemicals that coat the outside of unwashed quinoa, giving it a bitter taste (Woldemichael & Wink, 2001). Saponins serve as natural insecticides when quinoa is growing. However, when consumed by humans, they can pose a risk and cause irritation is in the stomach (Kuljanabhagavad, Thongphasuk, Chamulitrat, & Wink, 2008).

To reduce the phytate and saponin content of proso millet and quinoa, germination appears to be a feasible and economic method (Kozioł, 1992). Sprouting is the natural germination process by which seeds, or spores put out shoots, plants produce new leaves or buds, or other newly developing parts experience further growth. The germination process takes a few days and can be done at home manually, as a semi-automated process, or industrially on a large scale for commercial use. Typically, the seeds are first rinsed to remove soil, dirt and the mucilaginous substances produced by some seeds when they encounter water. Then they are soaked for 20 minutes to 12 hours, depending on the type and size of the seed (Lorenz & D'Appolonia, 1980). The soaking increases the water content in the seeds and brings them out of quiescence (Yang, 2001). After draining and then rinsing seeds at regular intervals, the seeds then germinate, or sprout. The metabolic activity of resting seeds increases as soon as they are hydrated during soaking. Complex biochemical changes occur during hydration and subsequent sprouting (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012). The reserve chemical constituents, such as protein, starch and lipids, are broken down by enzymes into simple compounds that are used to make new compounds. Sprouting grains causes increased activities of hydrolytic enzymes, improvements in the contents of total proteins, fat, certain essential amino acids, total sugars, B-group vitamins, and a decrease in dry matter, starch and anti-nutrients (Chavan, Kadam, & Beuchat, 1989). The increased contents of protein, fat, fibre and total ash are only apparent and attributable to the disappearance of starch (Bartnik & Szafrańska, 1987). However, improvements in amino acid composition, B-group vitamins, sugars, protein and starch digestibility and decrease in phytates and protease inhibitors are the metabolic effects of the sprouting process (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013). Very complex qualitative changes are reported to occur during soaking and sprouting of seeds. The conversion of storage proteins of cereal grains into albumins and globulins during sprouting may improve the quality of cereal proteins (Chavan et al., 1989). An increase in proteolytic activity during sprouting is desirable for nutritional improvement of cereals because it leads to hydrolysis of prolamins and the liberated amino acids such as glutamic and proline are converted to limiting amino acids such as lysine (Hamad & Fields, 1979). Sprouting can improve levels of gamma-aminobutyric acid (GABA), a compound involved in the regulation of blood pressure, and promoted the liberation of bioactive peptides (Maisont & Narkrugsa, 2010).

Extrusion processing has become an important food process in the manufacture of pasta, ready-to-eat cereals, snacks, pet foods, and textured vegetable protein (Muthukumarappan & Swamy, 2018). An extruder consists of tightly fitting screw rotating within a stationary barrel. Ground and conditioned ingredients enter the screw where they are conveyed, mixed, and heated by a variety of processes. The product exits the extruder through a die where it usually puffs and changes texture from the release of steam and normal forces.

Extrusion has been a very useful tool to convert highly nutritious ingredients in nutritious food. The screw exerts shearing action on starch and protein- based materials during the extrusion process. In addition, high temperature transforms the material to a viscoelastic mass. At the exit of the die, the pressure drops suddenly, and the emerging material expands. The physical properties of the product (density, expansion, texture, etc.) relies on the material composition (such as presence of protein, starch, fiber and moisture), and the processing conditions during extrusion. However, there is a challenge when a high protein ingredient is being extruded. The study has been directed towards extruding a high protein proso millet and quinoa in single and twin-screw extruders to create a healthy snack product. In addition, sprouting causes a breakdown of complex ingredients and hence the digestibility of the extrudates has also been investigated.

1.2 Objectives

The main objective of this study was to optimize the germination time of proso millets and quinoa to increase the protein content and digestibility and to understand the significant impacts of extrusion processing on physical and nutritional properties of the millet and quinoa extrudates.

The specific objectives of this study were to investigate the:

1. Impact of the germination process on the phytic acid content, starch and protein digestibility of the sprouted proso millet and quinoa flours (CHAPTER 2)
2. Effect of moisture content, temperature and screw speed and germination time on sprouted quinoa extrudates and evaluate the physico-chemical properties such as color, bulk density, water absorption index, water solubility index, expansion ratio, hardness, starch digestibility and protein digestibility in a single screw extruder (CHAPTER 3)

3. Effect of moisture content, temperature and screw speed, corn meal ratio and germination time on sprouted quinoa-corn meal blend extrudates and evaluate the physico-chemical properties such as color, bulk density, water absorption index, water solubility index, expansion ratio, hardness, starch digestibility and protein digestibility in a single screw extruder (CHAPTER 4)

4. Effect of moisture content, temperature and screw speed and germination time on (i) sprouted quinoa extrudates and evaluate the physico-chemical properties such as color, bulk density, water absorption index, water solubility index, expansion ratio, hardness, starch digestibility and protein digestibility in a twin screw extruder (CHAPTER 5)

5. Impact of single screw extrusion process variables such as moisture content, temperature and screw speed and germination time on sprouted proso millet extrudates and evaluate the physical (color, bulk density, expansion ratio, hardness), functional (water absorption index, water solubility index), and nutritional (starch digestibility and protein digestibility) properties of extrudates (CHAPTER 6)

6. Impact of single screw extrusion process variables such as moisture content, temperature and screw speed, corn meal ratio and germination time on sprouted proso millet-corn meal extrudates and evaluate the physical (color, bulk density, expansion ratio, hardness), functional (water absorption index, water solubility index), and nutritional (starch digestibility and protein digestibility) properties of extrudates (CHAPTER 7)

7. Impact of twin screw extrusion process variables such as moisture content, temperature and screw speed and germination time on sprouted proso millet extrudates and evaluate the physical (color, bulk density, expansion ratio, hardness), functional (water absorption

index, water solubility index), and nutritional (starch digestibility and protein digestibility) properties of extrudates (CHAPTER 8)

8. Develop an Artificial Neural Network model of single and twin-screw extrusion of sprouted quinoa and proso millet (CHAPTER 9)

1.3 Literature Review

1.3.1 Raw materials for extrusion processing

1.3.1.1 Proso millet

Proso millet (*Panicum miliaceum*) is a grain crop, also called as broomcorn millet, common millet, hog millet, Kashfi millet, red millet and white millet. Proso millet is extensively cultivated in China, India, Nepal, Russia, Ukraine, Belarus, the Middle East, Turkey, Romania, and the United States. In 2018, US farmers produced 15 million bushels of proso millet on 4.1 million acres (USDA, 2019). The Central Great Plains states consisting of Colorado, Nebraska and South Dakota are the major producers of proso millet as shown in Fig 1.1. Kansas, Wyoming, Minnesota and North Dakota are the other states producing proso millet (Habiyaremye et al., 2017). Proso production in the U.S. has dramatically increased in the past decade and has been primarily used as a rotational crop. The crop has an extremely short lifespan, with some varieties producing grain just 60 days after planting. In addition, it has low water requirements and produces grains more efficiently per unit of moisture than any other grain species tested (Nielsen & Vigil, 2017). In the United States, it is primarily grown for livestock feed. As a fodder, it is very deficient in lysine and needs complementation. It also has a low leaf-to-stem ratio and has a possible irritant effect due to its hairy stem. However, the demand for more diverse and healthier cereal-based foods is create new markets for proso millet products in human nutrition.

Protein content in proso millet grains is comparable with that of wheat as shown in Table 1.1, but the share of essential amino acids (leucine, isoleucine and methionine) is substantially higher in proso millet (Richard W Jones, AC Beckwith, U Khoo, & GE Inglett, 1970b). In addition, health-promoting phenolic compounds contained in the grains are readily bio-accessible and their high calcium content favor bone strengthening and dental health. Among the most commonly consumed products are ready-to-eat breakfast cereals made purely from millet flour as well as a variety of noodles and bakery products, which are, however, often produced from mixtures with wheat flour to improve their sensory quality (Romero, Santra, Rose, & Zhang, 2017).

1.3.1.2 Quinoa

Quinoa is native American crop cultivated for centuries in the high Andes of Peru, Bolivia, and Chile. It was staple food of the Incas since 3000 B.C. (FAO, 2011). Primary production occurs in Chile and Peru, its highest production, quinoa's yield equals that of wheat. The Faro variety performs well in Oregon; the Milahue variety is well-suited to California valleys. The Temuco variety grows well in Washington, California, and New Mexico. The variety Isleuga, a native of Chile, grows successfully in the United States. Several varieties have been grown in the Rocky Mountains, the interior Northwest, and the northern Pacific coast. In Colorado, researchers have obtained yields of 1,200 pounds per acre (Alandia, Rodriguez, Jacobsen, Bazile, & Condori, 2016).

Quinoa (*Chenopodium quinoa*), which is considered a pseudo cereal has been recognized as a complete food due to its protein quality. It has remarkable nutritional properties; not only from its protein content (15%) but also from its great amino acid balance (Ranhotra, Gelroth, Glaser, Lorenz, & Johnson, 1993). It is an important source of minerals and vitamins and has also been found to contain compounds like polyphenols, phytosterols, and flavonoids with possible nutraceutical benefits. Quinoa's composition is 10-15% protein, 4.5% fat, 63% carbohydrates, 4.1% fiber, 12.6% water, and 3.4% ash (Navruz-Varli & Sanlier, 2016). It is rich in unsaturated oils and is source of calcium, iron, and essential amino acids. It has some functional (technological) properties like solubility, water-holding capacity (WHC), gelation, emulsifying, and foaming that allow diversified uses. Besides, it has been considered an oil crop, with an interesting proportion of omega-6 and a notable vitamin E content (Mahoney, Lopez, & Hendricks, 1975). Quinoa starch has physicochemical properties (such as viscosity, freeze stability) which give it functional properties with novel uses. Quinoa has a high nutritional value and has recently been used as a novel functional food because of all these properties; it is a promising alternative cultivar (Scanlin & Stone, 2009).

Saponins are a wide group of glycosides found in plants. Their name comes from the plant genus Saponaria, whose root was used as soap; so they are water soluble and form foaming solutions (Estrada, Redmond, & Laarveld, 1997). These compounds have a bitter taste and are considered toxic in large amounts. They are present in the whole quinoa plant; where their natural function is to defend the plant from the external medium. In general, quinoas contain saponins in the seed coat except sweet varieties, without saponin or containing less than 0.11% (Mizui, Kasai, Ohtani, & Tanaka, 1990). Saponins are the main antinutritional factor present in the seed cover. Studies in rats revealed that animals fed with unwashed quinoa diets showed growth damage and reduced food conversion efficiency (Gee et al., 1993). According to their chemical structure, saponins can be partially removed by washing with water, but even after washing some saponin remains in the seed. Alkaline water rather than neutral water can be used to debitter. Bitter taste imparted by saponins could potentially be reduced by extrusion and roasting processes (Brady, Ho, Rosen, Sang, & Karwe, 2007).

1.3.1.3 Corn and Corn meal

Corn is a cereal grain first domesticated by indigenous peoples in southern Mexico. Corn has become a staple food in many parts of the world, with the total production of corn surpassing that of wheat or rice. However, little of this corn is consumed directly by humans: most is used for corn ethanol, animal feed and other corn products, such as corn starch and corn syrup. Corn is the most widely grown grain crop throughout the Americas, with 361 million metric tons grown in the United States in 2018 (Capehart & Proper, 2019). Approximately 40% of the crop—130 million tons—is used for corn ethanol. Corn is popular in Midwestern states such as Indiana and Illinois; in the latter, it was named the state's official grain in 2017.

The corn plant is a tall annual grass with a stout, erect, solid stem. The large narrow leaves have wavy margins and are spaced alternately on opposite sides of the stem. Varieties of yellow and white corn are the most popular as food, though there are varieties with red, blue, pink, and black kernels, often banded, spotted, or striped. Many industrial varieties of corn are genetically modified for resistance to the herbicide glyphosate or to produce proteins from *Bacillus thuringiensis* (Bt) to kill specific insect pests. In addition, some strains have been genetically engineered for greater drought tolerance. Genetically modified corn made up 93% of the corn planted in the United States in 2018.

Corn is an inexpensive and versatile ingredient that is used to prepare a wide range of foods. Cornmeal is a meal (coarse flour) ground from dried corn. It is a common staple food, and is ground to fine, medium, and coarse consistencies. In the United States, very finely ground cornmeal is referred to as corn flour. Corn starch is the starch derived from the endosperm of the kernel. Corn starch is a common food ingredient, used in thickening sauces or soups, and in making corn syrup and other sugars. High fructose corn syrup is used in soda, condiments, packaged desserts, candy, granola bars, fruit snacks and cereal. Most kinds of whiskey are produced using corn ingredients.

1.3.2 Pre-treatment Methods

1.3.2.1 Soaking

Soaking is an essential step in the production of most cereal based foods as it activates the enzyme phytase. This enzyme then works to break down phytic acid which binds minerals like iron, calcium and zinc. Water is rapidly absorbed through porous structure of grains; therefore, cooking time is shortened. Soaking temperature, time and solvent are the factors that have a greater impact on the quality of grains. In general, soaking offers the following benefits:

- Promotes production of beneficial enzymes like phytase that reduce phytic acids
- Reduces anti-nutrients like phytic acids, saponins, oxalates and tannins
- Improves the body's ability to absorb minerals such as iron, zinc and calcium
- Removes flatulence causing compounds
- Decreases cooking time and improves food texture

- Breaks down gluten

The impact of soaking on commonly use cereals grains such as wheat, millet, rice, barley and corn have been studied extensively. Several researchers attempted to find out the optimum soaking condition, incubation condition and drying temperature to obtain GBR with high content of nutrients and bioactive compounds. However, the information about characteristics of quinoa and proso millet affected by soaking conditions is still limited in literature.

1.3.2.2 Germination

Germination is a common household technique carried out at low cost without the use of any sophisticated and expensive equipment. It reduces antinutrients thereby improving nutritional and functional properties of millet and the mousy odor of damp millet is eliminated. The protein quality is also improved by germination by increasing GABA amino acid (Nelson et al., 2013). Gamma-aminobutyric acid (GABA), which has a significant role in neurotransmission, is one of the important bio-functional substances produced during germination.

Germination or malting of cereal grains may result in some biochemical modifications and produce malt with improved nutritional quality that can be used in various traditional recipes. Germination of millet grains increases the free amino acids and total sugars and decreases the dry weight and starch content. Increases in lysine, tryptophan, and non-protein nitrogen also occur. Germination also appreciably improved the in vitro protein (14% to 26%) and starch (86% to 112%) digestibility in millet, and the improvement by germination was significantly higher (Lorenz & D'Appolonia, 1980). The improvement in protein digestibility after germination, soaking, de-branning, and dry heating can be attributed to the reduction of antinutrients such as phytic acid, tannins, and polyphenols, which are known to interact with proteins to form complexes (Donkor et al., 2012). Also, the changes in nutrient contents of grains after germination can be attributed to the utilization by growing sprouts. It has also been found that the in vitro extractability and bio-accessibility of minerals such as calcium, iron, and zinc were increased in millet by germination; however, the antinutritional factors such as phytic acid were decreased (Hung, Maeda, Yamamoto, & Morita, 2012). Furthermore, the relative in vitro solubility of iron was doubled by the germination of millet grains (Hemalatha, Platel, & Srinivasan, 2007). The effect of germination on the physico-chemical properties of grains is described in Table 1.2.

1.3.3 Extrusion Processing

Extrusion is a process which combines several unit operations including mixing, cooking, kneading, shearing, shaping and forming. Food ingredients are fed through a hopper and the feed moves along various sections with the help of a screw and exits through a die (Sun & Muthukumarappan, 2002). During the journey inside the extruder, the ingredients are formed into a dough with partial cooking. Extrusion is operated in both batch and continuous methods. This technique is extensively operated as a continuous process in cooking of food materials, texturization and shaping of food, impregnation of fibrous materials, and fractionation of solid–liquid media (Muthukumarappan & Swamy, 2018). The industrial application has generated numerous foods of the modern world such as 3D snacks, snack and feed pellets, three dimensional breakfast cereals, pellet-to-flakes cereals, partially filled cereals, crispy flat bread, puffed foods, textured vegetable proteins

(TVP) and encapsulated flavors (Maskan & Altan, 2016). Table 1.3 shows the effect of extrusion on cereal grains.

Extruders are classified based on the design and function as illustrated in Fig 1.2. The food industry employs both single screw and intermeshing co-rotating twin screw extruders. In particular, the cereal processing industry extensively use the extruders to generate products of various shapes, sizes and textures. Extrusion technology transforms cereal flours to prepare ready-to-eat (RTE) food products and functional ingredients by a series of steps such as kneading, cooking, forming, and texturizing functions (Altan & Maskan, 2011). This thermo-mechanical process is very useful in producing low-fat snacks and has the advantage of increasing protein and starch digestibility, solubilizing fiber, inactivating toxins, anti-nutritional factors, and undesirable enzymes, such as lipo-oxygenases and peroxidases (Gopirajah & Muthukumarappan, 2018). The effect of extrusion temperature, moisture content, and screw speed need to be evaluated for further applications of extrusion processing. Table 1.4 shows the effect of extrusion on quinoa and proso millet.

1.3.3.1 Single-screw Extruder

Single-screw extruders are readily available in a number of shapes and sizes, and the barrel, screw configuration, and screw can be varied to suit a particular variety of product characteristics (Riaz, 2000). Fig 1.3 shows the parts of a single screw extruder. The main advantages of single-screw over twin screw extruders are that they are mechanically very simple and the cost is half the price of similar-sized twin-screw extruders (Guy, 2001). Because of this, single-screw extruders are used wherever possible in the industry and in academic research. The material is conveyed along the length of the screw by a drag flow mechanism, where drag is directly proportional to screw speed. In general, single-screw extruders possess poor mixing ability, which necessitates premixing of ingredient prior to extrusion (Bruin, Van Zuilichem, & Stolp, 1978).

1.3.3.2 Twin-screw Extruder

This comprises two screws rotating either in the same direction (co-rotating) or in the opposite direction (counter-rotating). Fig 1.4. shows the design of a twin-screw extruder. Twin screw extruders are more flexible in operation than single-screw extruders, but they are more expensive. Some of the advantages of twin-screw extruders include ability to handle a variety of materials (viscous, oily, sticky, and wet) and a wide range of particle sizes, non-pulsating feed, positive pumping action, self-cleaning, and scaling up (Guy, 2001).

1.3.3.3 Comparison of single-screw extrusion and twin-screw extrusion

Compared to single-screw extruders, twin- screw extruders are more flexible in controlling both product and process parameters. They have a flexible design permitting easy cleaning and rapid product changeover. Single-screw extruders are limited to a maximum fat level in the formula of 12–17%. Greater fat levels reduce friction because of their lubrication effects, thus not allowing the hardware to transform mechanical energy into heat for cooking purposes. Comparatively, fat levels in recipes for twin-screw extruders can be as high as 18–22% while still maintaining the required mechanical energy. Moisture is another key factor. A twin-screw extruder can process a much wider range of moisture content in the feedstock than a single-screw extruder.

Processors should consider twin-screw extruders in the following situations:

- Frequent product changeovers

- Products with high internal fat content (above 17%)
- Addition of a high level of fresh meat in the product (up to 35%)
- Uniform size and shapes
- Ultra-small product sizes (less than 1.5 mm)
- Products made with low density powder
- Special formulations

1.3.3.4 Variables in food extrusion

The variables involved in extrusion processing are shown in Figure 1.5. Screw speed, barrel temperature, screw and barrel configuration, die opening, and feed rate are some of the parameters that affect extruder performance (Muthukumarappan & Swamy, 2018). Extruder operation depends on pressure build-up in the barrel (prior to exiting the die), slip at the barrel wall (transportation), and the degree of filling (Karunanithy & Muthukumarappan, 2010).

Screw speed

In general, screw speed is responsible for the rate of shear development and the mean residence time of the feed. The heat dissipation from the mechanical energy input to dough depends on screw speed, which in turn influences dough viscosity (Muthukumarappan & Karunanithy, 2012). In some cases completion of texture formation and chemical reactions within the barrel require a long residence time, which corresponds to slow screw speed (Doğan & Karwe, 2003).

Temperature

In order to avoid plugging and back-flow of material, the feed zone temperature is low and barrel temperature ramps up as the material travels down the screw (Harper & Clark, 1979). Barrel temperature usually has a positive effect on the degree of starch gelatinization and extrudate expansion, whereas it has a negative effect on product color especially at elevated temperatures. Several studies have indicated that elevated temperature leads to more moisture evaporation when exiting the die, and thus results in more expanded products (Guy, 2001) (Mason & Hoseney, 1986) (Sun & Muthukumarappan, 2002).

Extruder feed rate depends on the type of screw element, screw speed, type of feeding element, and feed moisture (Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006). Feed rate has an influence on residence time, torque requirement, barrel pressure, and dough temperature (Akdogan, 1999).

Feed composition

Feed composition is one of the factors that have the greatest effects on extrusion and extrudates (Őzer, İbanoğlu, Ainsworth, & Yağmur, 2004). The typical composition of any blend consists of water, starch, protein, lipid/fat, and fiber, which all contribute to product quality (Thakur & Saxena, 2000). Water is an important medium in extrusion. It is needed for starch gelatinization and ingredient dispersion. In the formation of a viscous fluid, it is conveyed and cooked. Air cell creation and expansion by evaporation at the die exit also depends on the optimum moisture content of raw materials. Principal differences between twin- and single-screw extruders are that the former may be more suitable for handling wet material. Moisture is always listed as a separated variable in addition to feed ingredients because it is often controlled separately in the extruder. Moisture can be added directly to the feed, injected into the barrel, or added in the form of steam to the pre-conditioner or barrel; it will also affect the temperature of the feed material.

Starch is the main component of the final product; it provides the underlying structure. Starch is contained in a large variety of plant crops, such as cereals (50-80% starch), legumes (25-50% starch) and tubers (60-90% starch). Starch granules are gelatinized and dispersed during extrusion, resulting in the formation of a continuous phase of the melt inside the extruder. Average molecular weight is decreased, which allows for optimum formation and stability of air cells at the die exit. Both amylose and amylopectin are needed to give the best expansion characteristics. Starch degradation usually reduces product expansion. It is essential that infant and weaning foods have high starch digestibility, which is largely dependent on full gelatinization.

Single- and twin-screw extrusion of protein has been the subject of several studies (Zhao et al., 2019) (Matthey, Hanna, & Technology, 1997). Textured Plant Protein (TPP) has been popular in recent times with the increasing number of novel plant protein ingredients such as pea, navy beans, millet, quinoa and so on. Textured products manufactured by this process are increasingly being sold as meat analogues in globally. Given the nutritional factor content of plant proteins, a range of different products high in plant protein are preferred by consumers.

Lipid levels over 5–6% act as a lubricant, reducing slip within the barrel and resulting in poor product expansion (Riaz, 2000). If the production of porous and expanded product is not the target, then a fat level of 15–18% can be used in single-screw extruders and a fat level of 20–22% in twin-screw extruders (Muthukumarappan & Swamy, 2018). The lipid content of the extruded product is low. Rancidity is an issue for extruded products during storage because of lipid oxidation, which causes rapid deterioration of sensory and nutritional qualities (Camire, Camire, & Krumhar, 1990).

Sugar and salt (functional ingredients) have more effects on wear than other ingredients. In cereal processing, sugar concentration has a negative effect on viscosity and high sugar concentration inhibits gelatinization, requiring higher temperatures to achieve the same degree of product expansion (Hsieh, Peng, & Huff, 1990). Salt will assist in obtaining uniform moisture migration after drying of third-generation pellets during moisture equilibration (Jin, Hsieh, & Huff, 1995). In general, salt reduces water activity, which leads to poor product expansion (Pitts, Favaro, Austin, & Day, 2014).

Generally, fiber is a non-interacting component that contributes to low expansion, cohesiveness, durability, and water stability (Robin, Schuchmann, & Palzer, 2012). High fiber content usually results in high screw wear. Fiber has the nutritive value in food products and it has related to a healthy modern diet. Fibrous materials such as bran can be part of the dispersed phase of extruded products, included in the starchy continuous phase. Fiber is chemically unchanged by the extrusion process and influences the expansion of the product. Fibrous fragments disrupt the starchy film of air cell walls, reducing their formation and swelling, and altering air cell size.

Moisture content

Moisture is a critical variable that has multiple functions in starch gelatinization, protein denaturization, barrel lubrication, and final product quality. Processing is uneconomical at in-barrel moistures below 20% and results in undesirable nutritional quality. However, a dry extruder can process materials with 8–22% moisture with no additional drying of extrudates (Miller, 1985). In general, a medium shear stress extruder can handle food with 16–30% in barrel moisture, whereas a low shear stress extruder can handle food with more than 30% in-barrel moisture (pasta dough has 31%) (Singh, Sekhon, & Singh, 2007). An increase in moisture content will have a pronounced effect on the rheological properties of the melt in the barrel. High-moisture feeds decrease the mechanical energy requirement and reduce the wear and thereby operating cost. However, most extruded snacks have a moisture content between 8 and 12% and require additional drying to impart the desired texture and mouth-feel (Lin, Huff, & Hsieh, 2002). High moisture reduces vitamin loss during extrusion due to limited thermal degradation (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014).

Particle size

As a general rule of thumb that the extruder feed should not have particles larger than one-third the diameter of die holes (Garber, Hsieh, & Huff, 1997). Particle size also plays an important role not only in moisture distribution, heat transfer, and viscosity but also in final product quality (Onwulata & Konstance, 2006). Coarse ingredient particles have more effect on wear than fine particles. A product composed of fine particles will have good water stability, water absorption index, expansion, and floatability (Carvalho, Takeiti, Onwulata, & Pordesimo, 2010).

Table 1.1: Nutritional composition of wheat, proso millet and quinoa

Nutrient/100g	**Wheat**	**Proso Millet**	**Quinoa**
Protein (g)	13.6	11.0	13.1
Fat (g)	1.5	4.2	5.8
Carbohydrates (g)	71.2	73.0	63.2
Fiber (g)	12.2	8.5	7.5
Sugars (g)	0.4	0	0.6
Vitamin B_6 (mg)	0.3	0.4	0.3
Folic acid (μg)	38.0	85.0	77.0
Riboflavin (μg)	284	420	320

Table 1.2: Effect of germination on the physico-chemical properties of grains

Product	**Germination time**	**Results**	**Reference**
Proso millet	1-7 days	Overnight soaking and germination up to 7 days significantly increased the free amino acids and total sugars while the content of dry weight and starch decreased The protein content increased gradually and was 14.3% at 7th day of germination The percent protein in germinated grains was higher than in the initial grain as a result of dry matter loss during germination Increases in albumin and globulin and large decreases in prolamin accompanied sprouting There was an increase in lysine, tryptophan and non-protein nitrogen contents during germination	(Parameswaran & Sadasivam, 1994)

		No change was noticed in methionine content.	
Finger millet	96 h	There were significant decreases in antinutritional factors, with tannins and phytates decreasing to undetectable levels Trypsin inhibitor activity decreased threefold Changes in proximate composition were also significant. By 48 h of germination, there were high decreases in viscosity and starch content accompanied by large increases in sugar content Significant changes also occurred in in vitro protein digestibility and 13.3% of the seeds' dry matter was lost over the 96 h sprouting period Based on these changes in nutrients, it was concluded that it was not necessary to prolong sprouting of finger millet meant for weaning foods beyond 48 h Longer sprouting times would only result in high losses in dry matter through respiration without corresponding significant overall nutritional benefits	(Mbithi-Mwikya, Van Camp, Yiru, & Huyghebaert, 2000)

Finger millet, pearl millet and foxtail millet	Soaking time - 24 h Finger millet - upto 96 h Pearl and foxtail millet - 48 h	Germination resulted in a slight decrease in total protein and minerals, a fall in phytate-phosphorus and a significant increase in the ascorbic acid content of the millets An increase in lysine and tryptophan but no appreciable changes in threonine and sulfur amino acid content of the millets were observed as a result of germination The protein efficiency ratio values of ungerminated control seeds, 48 h germinated green malt and kilned malt were not significantly different.	(Malleshi & Desikachar, 1986)
Foxtail millet	48 h	The results indicated that with increase in soaking time (ST), germination time (Gt) and temperature (GT), AoxA, TPC (free/bound) and TFC (free/bound) of foxtail millet increased significantly The best combination of germination bioprocess variables for producing optimized germinated foxtail millet flour with the highest AoxA (90.5%), TPC (45.67 mg gallic acid equivalent (GAE)/100 g sample) and TFC (30.52–43.96	(Sharma, Saxena, & Riar, 2015)

		mg RU/g sample) were found with soaking time of 15.84 min having germination temperature of 25°C The optimized germinated foxtail millet flour was nutritionally rich as it produced higher protein (14.32 g/100 g), dietary fibre (27.42 g/100 g), calcium (25.62 mg/kg), iron (54.23 mg/kg), magnesium (107.16 mg/kg) and sodium (69.45 mg/kg) per kg as compared to un-germinated foxtail millet flour	
Pearl millet	25^{o}C for 48, 54, 60 h 30^{o}C for 36, 42, 48 h 35^{o}C for 36, 42,48 h	Germination reduce the phytic acid content of pearl millets Germination at 30^{o}C for 48 h yielded the highest protein content	(Kumar & Chauhan, 1993)
Rice Maize	144 h	Phytase activity was high (0.21–0.67 U g-1) in all samples Phytate content ranged between 5.6 and 6.2 mg g^{-1} During germination, the level of phytase activity increased and reached its maximal value after seven (16-fold), six (5-fold), five (7-fold), seven (3-fold)	(Azeke, Egielewa, Eigbogbo, & Ihimire, 2011)

Millet Sorghum Wheat		and eight (6-fold) days of germination for rice, maize, millet, sorghum and wheat respectively. After this initial increase, phytase activity declined slightly ($P < 0.05$) The increase in phytase activity during germination was accompanied by a significant reduction in phytate ($P < 0.05$) and a small but significant increase in total phosphorus	
Quinoa	72 h	Germination resulted in a 2-fold increase in antioxidant activity measured as DPPH radical scavenging activity, after 3 days of germination The amounts of HPLC identified phenolic acids and flavonoids increased 8.57-fold, and 4.4 fold respectively	(Carciochi, Manrique, & Dimitrov, 2014)
Amaranth, quinoa, buckwheat and	Germination time was 96, 110, 98 and 82 h for buckwheat,	The total phenol content amongst the seed extracts were significantly higher in buckwheat (323.4 mg GAE/100 g) and decreased in the following order: buckwheat > quinoa > wheat > amaranth.	(Alvarez-Jubete, Wijngaard, Arendt, &

wheat	wheat, amaranth and quinoa, respectively	Analysis by liquid chromatography coupled with diode array detector revealed the presence of phenolic acids, catechins, flavanol, flavone and flavonol glycosides Quinoa and buckwheat seeds and sprouts represent potential rich sources of polyphenol compounds for enhancing the nutritive properties of foods such as gluten-free breads	Gallagher, 2010)
Quinoa	42 h	The use of desirability methodology showed that the optimum conditions to maximise the content of total phenolic content (TPC) and antioxidant activity in sprouted quinoa were 20 °C for 42 h Sprouts produced under these conditions exhibited increases of 80% and 30% in TPC and antioxidant activity, respectively, compared to un-germinated seeds, and contained high γ-aminobutyric acid (GABA) concentration	(Paucar-Menacho, Martínez-Villaluenga, Dueñas, Frias, & Peñas, 2018)

Table 1.3: Effect of extrusion on cereal grains

Cereal	Extruder type	Process parameters	Results	Reference
Chenopodium quinoa Wild.	Twin screw	Feed rate - 3.0 kg/hr Feed moisture - 15, 20 and 25% w.b., Temperature - 120, 140, 160 °C Screw speed - 300, 400, 500 rpm.	• The following characteristics of the extrudates were observed: expansion ratio (1.17–1.55 g/cm3), unit density (0.45–1.02 g/cm3), water absorption index (WAI) (2.33–3.05 g/g), and water solubility index (WSI) (14.5–15.87%) • The quinoa flour had relatively low direct expansion compared to cereal grains such as corn or wheat, suggesting that it is not well suited for the making of direct expanded products	(Kowalski, Medina-Meza, Thapa, Murphy, & Ganjyal, 2016)
Quinoa	Twin screw	Feed rate - 300 g/min	• Responses were most affected by changes in	(Doğan & Karwe,

		Feed moisture 16-24% Temperature 130-170°C Screw speed 250-500 rpm	feed moisture content and temperature, and to a lesser extent by screw speed • The best product, characterised by maximum expansion, minimum density, high degree of gelatinization and low water solubility index, was obtained at 16% feed moisture content, 130°C die temperature, and 375 rpm screw speed, which corresponds to high SME input	2003)
Quinoa-corn grits	Twin screw	Corn grits - 10, 20, 30%	• Quinoa addition produced unprocessed and extruded products which were higher in protein, fiber, ash and some amino acids than 100% corn grit products. the products containing quinoa had a greater nitrogen solubility and a somewhat lower in-vitro	(Coulter & Lorenz, 1991)

			digestibility than the products containing only corn grits - Sensory evaluation of the extruded blends indicated that the products were acceptable.	
Corn-base containing amaranth/quinoa/kaniwa (20% of solids)	Twin screw	Moisture 15–19% Screw speed 200–500 rpm Temperature 150–170 °C	- Expansion ratio increases at higher screw speed for corn-based extrudates containing quinoa - Extrudates containing kañiwa presented the lowest hardness (28 N mm−1), while extrudates containing quinoa and amaranth presented hardness values of 39 and 53 N mm^{-1}, respectively - Extrudates containing amaranth and quinoa maintained a light colour while kañiwa conferred a dark colour to extrudates	(Diaz et al., 2013)

			• The hexanal formation was considerably higher in milled samples than in the whole extrudates (exposed to either 11 or 76% RH prior to storage), except for extrudates containing quinoa	
Quinoa (Cherry vanilla and Bolivian Royal)	Twin screw	Feed moisture - 15%, 20%, and 25% (wet basis) Barrel temperature - 140 °C Screw speeds -100, 150, and 200 rpm	• The degermed Bolivian Royal showed the highest expansion in comparison to all other varieties, attributed to its significantly low levels of fat, fiber, and protein • The Cherry Vanilla resulted in the lowest expansion ratio. This was attributed to the increase in the protein content from the removal of the outer layer • The results indicate that all the varieties	(Aluwi et al., 2016)

			performed differently in the extrusion process due to their modification processes as well as the individual variety characteristics	
Proso millet	Twin screw	Moisture 17–25% Screw speed 170–250 rpm Temperature 90–150 °C	• Moisture and screw speed were the most influential variables affecting millet extrusion: their linear, quadratic and interaction terms accounted for more than 50% of the variability in all responses except for b* • Expansion was greatest at severe conditions of low moisture and high screw speed	(Paridhi Gulati, Steven Weier, Dipak Santra, Jeyamkondan Subbiah, & Devin Rose, 2016)

Table 1.4: Effect of extrusion on germinated cereal grains

Material	**Extrusion type**	**Results**	**References**
Brown rice	Twin screw	• The puffed pre-germinated brown rice contained more oryzanol, inositol, total ferulic acid and total dietary fibers compared with the un-puffed polished rice • The product prepared by the co-extrusion of pre-germinated brown rice (90%) and beer yeast (10%) contained more free amino acids, such as GABA, glycine, alanine, aspartic acid and glutamic acid, compared with polished rice, brown rice and puffed pre-germinated brown rice. • Extrusion cooking was shown to sterilize the germinated brown rice by the incubation test, which would lead to the development of consumer-oriented rice products in terms of food safety • The wheat bread prepared with 30% of the puffed pre-germinated brown rice contained more GABA, free sugars, such as maltose, compared with the ordinary wheat bread. The	(Ohtsubo, Suzuki, Yasui, & Kasumi, 2005)

		extrudate bread was shown to be sweeter ($P < 0.05$) and equivalently palatable ($P < 0.05$) as a result of organoleptic test.	
Wheat	Twin screw	• Germination also significantly increased the protein content, reducing sugar and total soluble sugar content in GW, while extrusion had much increasing impact on reducing sugar content in extruded samples. • Extrusion significantly increased the starch digestibility but decreased the protein digestibility in extrudates. • A 15% extruded germinated wheat (350 rpm) addition in 85% WW showed significant increase of γ-aminobutyric acid content in tortilla compared to the control (100% WW).	(Zhu, Adedeji, & Alavi, 2017)
Wheat	Twin screw	• Extrusion increased protein, soluble arabinoxylans, β-glucan content while reduced phytic acid content. • The chemical properties (crude protein, fat, ash, reducing sugar, γ-aminobutyric acid, soluble arabinoxylans, β-glucan and phytic acid) were affected by the germination step and extrusion process	(Singkhornart, Edou-ondo, & Ryu, 2014)

		• The difference of die temperatures, screw speed and CO_2 injection had slight effect on the chemical properties	
Rice	Twin screw	• Results indicated that 175 °C and 14 g 100 g^{-1} of moisture were the most appropriate conditions to obtain expanded products and precooked flours based on germinated rice • Selected extruded product presented less PA content (821.6 9 ± 10.3 versus 695.2 0 ± 1.6 mg 100 g^{-1}) and higher Fe bio-accessibility, FPC (45.2 9 ± 1.61 versus 66.3 5 ± 3.35 mg GAE g^{-1}) and antioxidant capacity compared with WR (34.9 5 ± 0.8 versus 54.6 3 ± 1.6 µmol trolox g^{-1}). • Combining germination–extrusion processes could be a strategy to obtain expanded products or precooked flours based on WR with enhanced health benefits	(Albarracín, De Greef, González, & Drago, 2015)
Brown rice	Twin screw	• Increasing the levels of pre-germinated glutinous brown rice flour increased the expansion ratio ($P <0.01$), brittleness, WSI (P ? 0.01) and the GABA content ($P <0.01$) • It decreased the density, hardness ($P <0.01$) and WAI ($P <0.01$), while the feed moisture content and screw speed had no significant effect on the GABA content ($P > 0.05$)	(Chanlat, Songsermpong, Charunuch, & Naivikul, 2011)

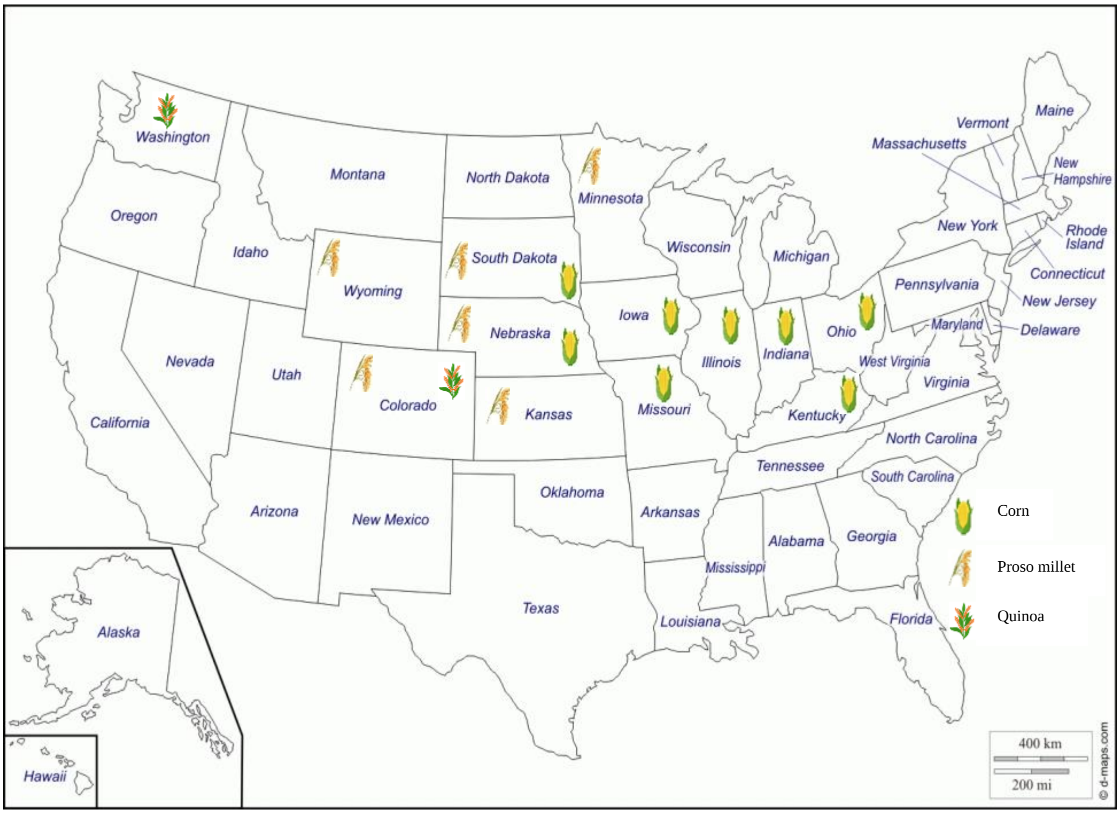


Figure 1.1: Cultivation of corn, proso millet and quinoa in USA

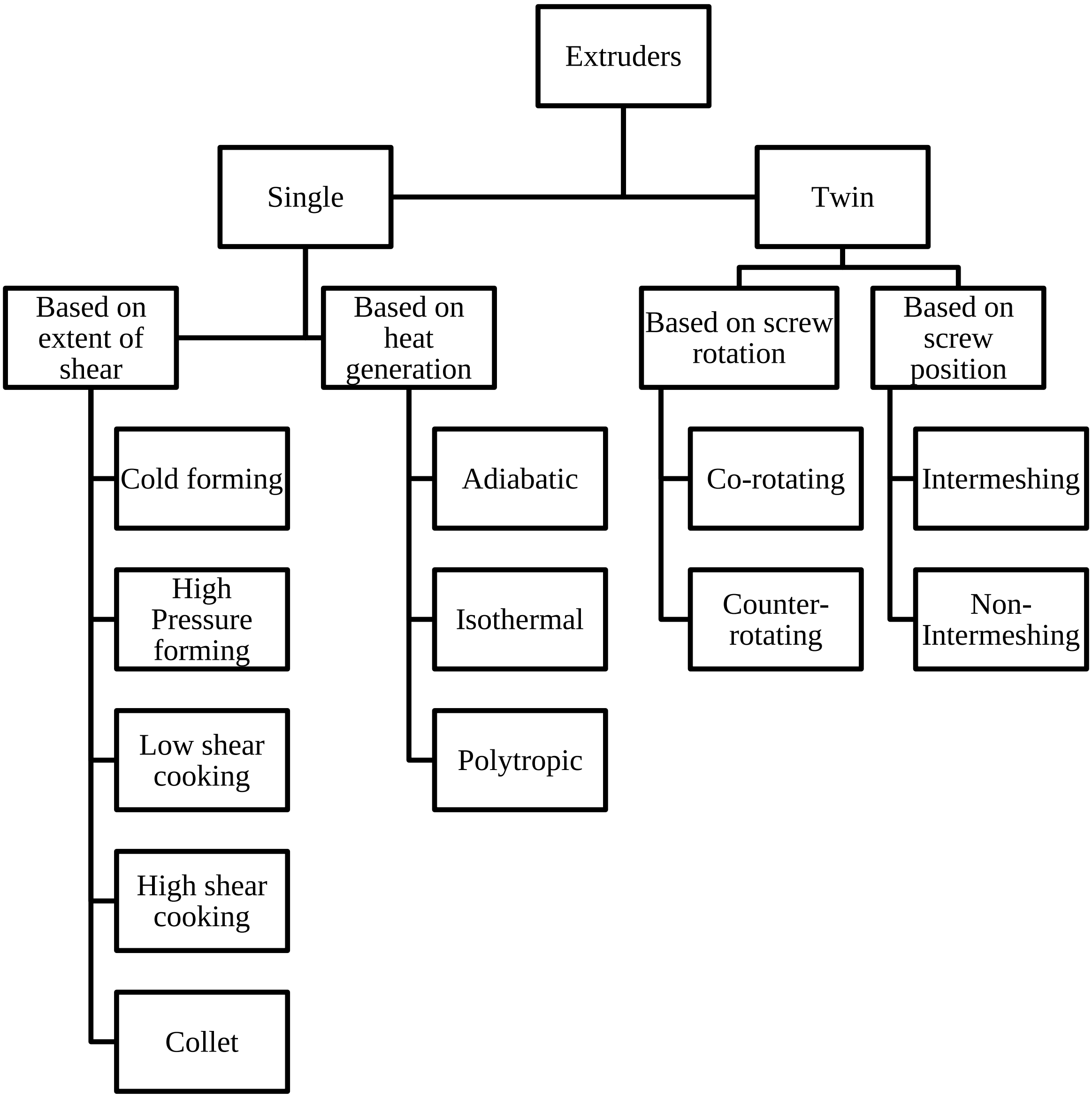
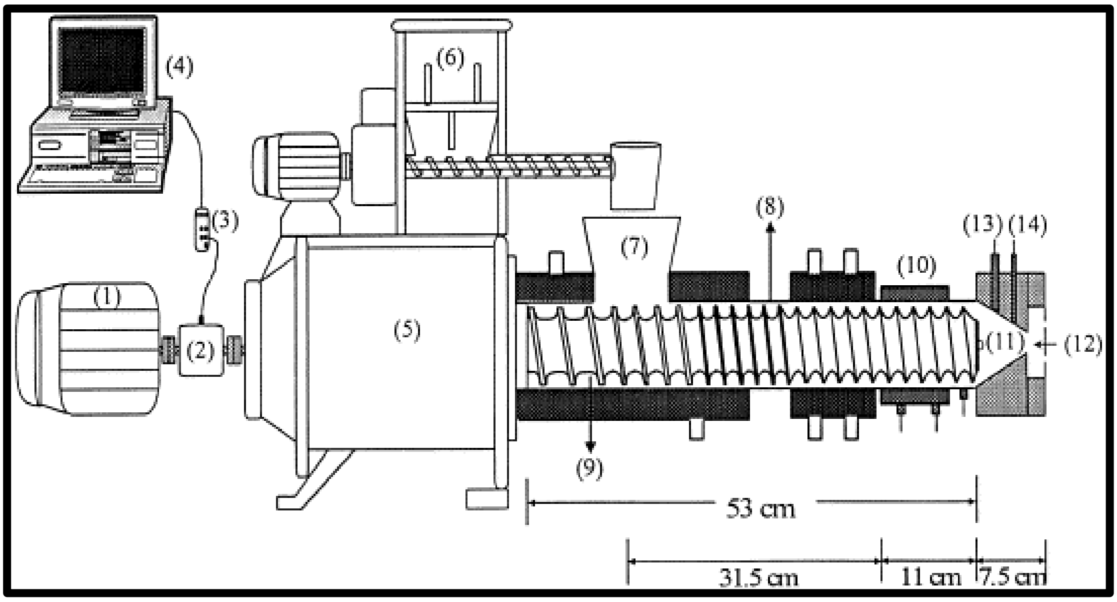


Figure 1.2: Classification of extruders based on design and function



(1) Motor; (2) Torque Transducer; (3) Amplifier; (4) Personal Computer; (5) Gear Box; (6) Feeder; (7) Hopper; (8) Barrel; (9) Screw; (10) Heater; (11) Die Plate; (12) Die Space; (13) Thermocouple; (14) Pressure Gauge.

Figure 1.3: Single screw extruder design

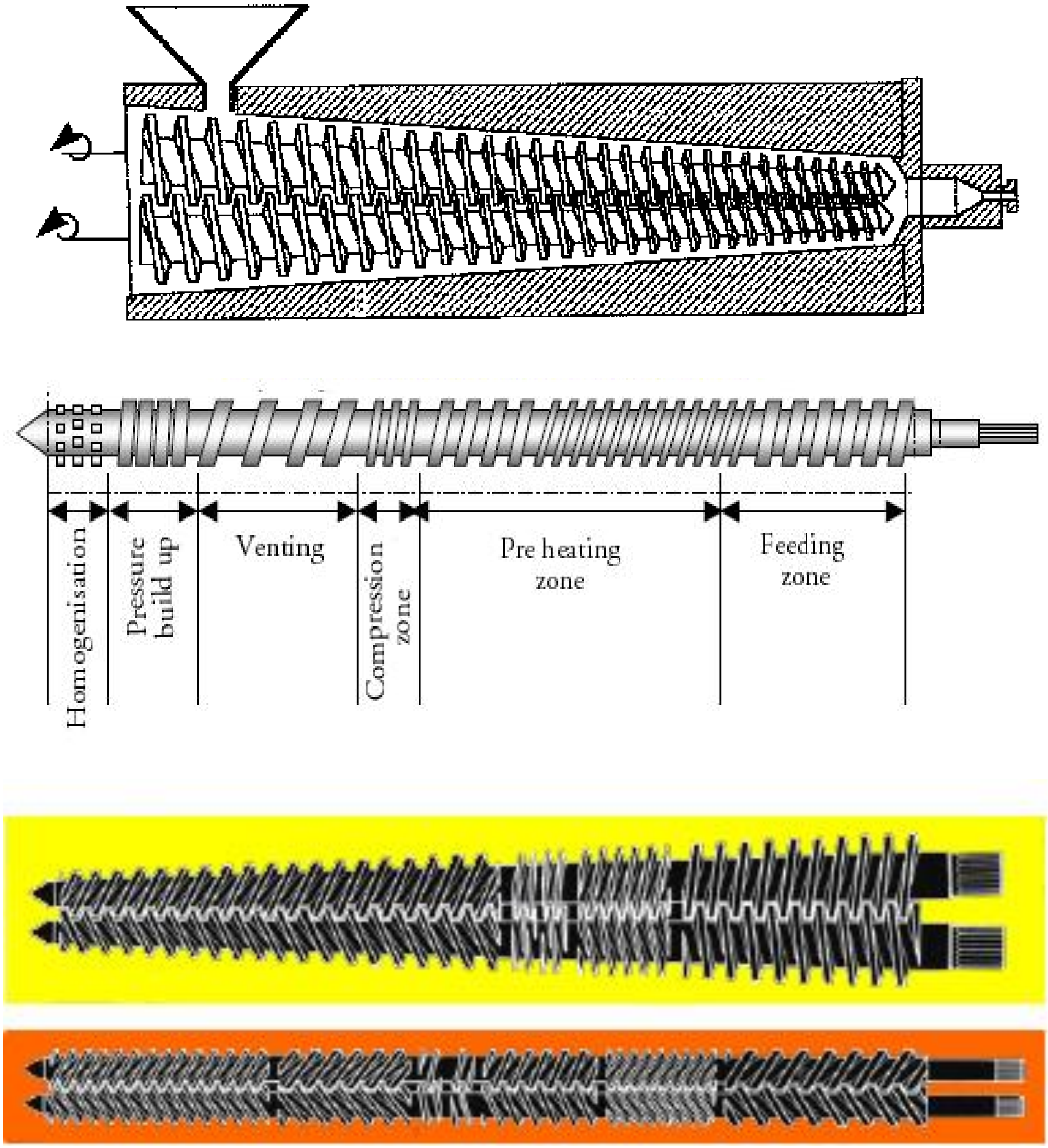


Figure 1.4: Twin Screw Extruder design (A) Twin screw extruder basic design (B) Twin screw sections (C) Conical and parallel twin screw design

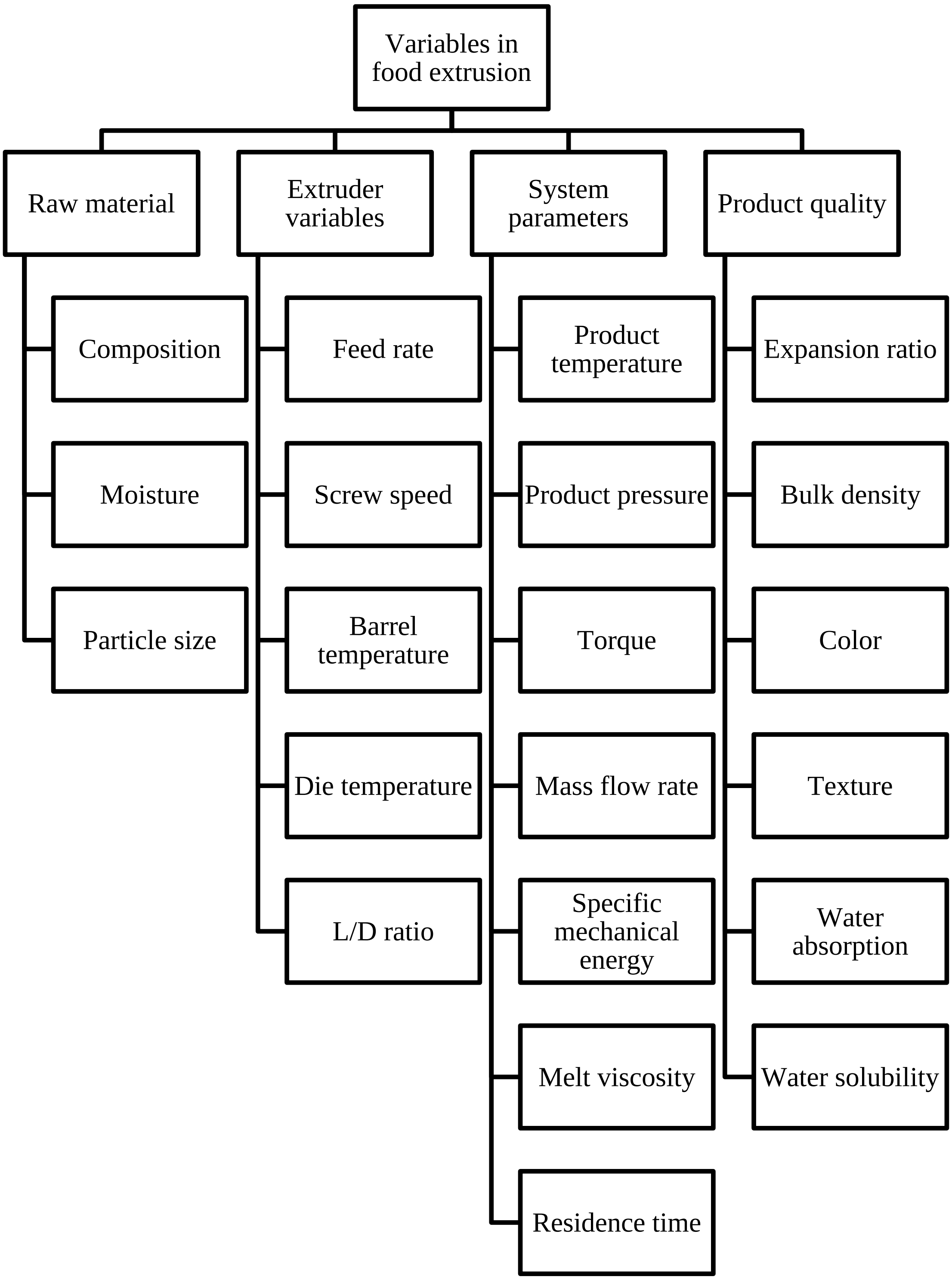


Figure 1.5: Schematic representation of variables involved in extrusion processing of foods

CHAPTER 2: EFFECT OF SOAKING AND GERMINATION ON THE PHYSICO-CHEMICAL PROPERTIES OF QUINOA AND PROSO MILLET

Abstract

In the first part, pre-treatment methods such as soaking and sprouting were analysed for effective reduction of saponins and phytic acid and increased starch and protein digestibility. The sprouting of quinoa increased the starch digestibility from 55.6% on Day 0 to 78.2% on Day 4. A similar increase in the protein digestibility was observed from Day 0 (42.2%) to Day 4 (75.5%). Sprouting also produced similar effects in proso millet where the starch digestibility increased from 51.7% (Day 0) to 77.1%. The breakdown of the phytates during sprouting of proso millet increased the protein digestibility from 46.4% on Day 0 to 76.8% on Day 4. Simultaneously the reduction of saponin content in quinoa and phytic acid content in both grains were observed. The saponin content in raw quinoa of 0.8g/100g was reduced to 0.1g/100g samples (Day 4) by germination. The phytic acid content in quinoa reduced from 1.1g/100g to 0.1g/100g (Day 4) and in proso millet reduced from 1.5g/100g to 0.2g/100g (Day 4). The color of the flour produced from the sprouted grains were significantly different from the unprocessed flour respectively

Keywords: Soaking, Sprouting, Quinoa, Proso Millet, Saponin, Phytic Acid

2.1 Introduction

Consumer lifestyle has shifted towards healthy living and healthier food. Consequently, food demand is more oriented towards diets rich in protein, characterized by higher amount of bioactive molecules (Chavan et al., 1989). The term “sprouted seeds” involves different types of products obtained from seeds, depending on the part of the plant collected and consumed—whether the seed is comprised or removed—and on the growing substrate and environmental conditions during sprouting (Kyriacou et al., 2016). For each of these products several ambiguous commercial definitions occur (i.e., microgreens, shoots, baby greens, cress, wheatgrass), widespread even in the scientific literature, and the same term could refer to different types of product (Di Gioia, Renna, & Santamaria, 2017). This frequently leads to misunderstanding, depriving specialists of the basic terminology on which it is necessary to point out, since the only legal definition in Western countries is given for “sprouts” and “sprouted grains”. “Sprouted grains” are defined by the American Association of Cereal Chemists (AACC) with the endorsement of the United States Department of Agriculture (USDA) as follows: “malted or sprouted grains containing all of the original bran, germ, and endosperm shall be considered whole grains as long as sprout growth does not exceed kernel length and nutrient values have not diminished. These grains should be labelled as malted or sprouted whole grain (Benincasa, Falcinelli, Lutts, Stagnari, & Galieni, 2019).

Soaking is an important pre-treatment which is often underestimated. Soaking grains before cooking can help neutralize the phytic acid, removing barriers to proper absorption of these minerals (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002). The principal reason for soaking is to gelatinize the starch in the grain (Kashiri, Kashaninejad, & Aghajani, 2010). It can be achieved either through conditioning below the gelatinization temperature and then cooking above the gelatinization temperature, or through direct cooking above the gelatinization temperature (Yamakura et al., 2005). Understanding water absorption in grains during soaking is of practical importance since it governs the subsequent operations and quality of the final product.

Millets are one of the cereals asides from the major wheat, rice, and maize. Although the protein content of proso is like wheat, the grain of proso is significantly richer in essential amino acids (leucine, isoleucine, methionine) (Richard W Jones, AC Beckwith, U Khoo, & GE Inglett, 1970a). The phytic acid content of the unmalted pearl millet grain ranged from 2.91% to 3.30% (Badau, Nkama, & Jideani, 2005). The composition of free and bound lipids in proso millet (Panicum miliaceum) flours and brans was analyzed by (Bagdi et al., 2011) and found that in the free lipids, hydrocarbons, sterol esters, triacylglycerols, diacylglycerols, and free fatty acids were present. The predominant fatty acids in the free lipids were linoleic, oleic, and palmitic acids, though, in the bound lipids, monogalactosyl diacylglycerols, digalactosyl diacylglycerols, phosphatidylethanolamine, phosphatidyl serine, and phosphatidyl choline.

Quinoa is a flowering plant in the amaranth family. It is an herbaceous annual plant grown as a crop primarily for its edible seeds; the seeds are rich in protein, dietary fiber, B vitamins, and dietary minerals in amounts greater than in many grains (Jacobsen, 2003). The amino acids of quinoa are well balanced and comparable to those of soy protein, casein, and wheat (Alandia et al., 2016). Most quinoa starch granules exist in perisperm cells (Ahamed, Singhal, Kulkarni, Pal, & Bulletin, 1998). Lipids in quinoa seed is about 6.9%, which is higher compared to other cereals (FAO, 2011). Most minerals in quinoa have higher content compared to that in wheat, which is deficient in iron, copper, manganese, and zinc (Kozioł, 1992). There are several anti-nutritional factors in quinoa, including saponins (0.1-5%), phytic acid (1.05-1.35%), and protease inhibitors (Gee et al., 1993). These factors could cause negative effects to the nutritional, sensory, and quality aspects of quinoa products. Bioactive compounds such as phenolic compounds, flavonoids, and carotenoids in quinoa are the critical antioxidants in diet and provide vital health benefits.

Phytic acid is present in grains and seeds of cereals, legumes, oilseeds, and nuts as the main phosphorus storage form (Oatway, Vasanthan, & Helm, 2001). Phytic acid is shaped like a snowflake and acts like a locked storage container in grains and seeds. It is found in the bran and germ portion and holds mainly phosphorus, but often times calcium, magnesium, iron and zinc too. At pH values normally occurring in foods, as well as under physiological conditions, phytic acid is negatively charged and has the potential to bind cations or other positively charged functional groups of molecules (Cheryan & Rackis, 1980). Phytic acid forms complexes with minerals and trace elements in vitro and can influence bioavailability in vivo as shown in Fig 2.1 (Bosscher et al., 2001). High bioavailability of minerals and trace elements from the diet is of special importance for the rapidly growing child; however, complementary foods based on cereals are usually high in phytic acid, resulting in low iron bioavailability (Egli et al., 2002). Removal or degradation of phytic acid has been reported to increase absorption of both iron and zinc (Hurrell, 2004).

A phytase (myo-inositol hexakisphosphate phosphohydrolase) is any type of phosphatase enzyme that catalyses the hydrolysis of phytic acid (myo-inositol hexakisphosphate) as shown in Fig 2.2 – an indigestible, organic form of phosphorus that is found in grains and oil seeds – and releases a usable form of inorganic phosphorus (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2003). Lower inositol phosphates have less binding capacity and have little influence on iron and zinc bioavailability in humans (Simpson & Wise, 1990). Phytic acid in complementary foods and soy formula can be completely degraded by addition of microbial phytase or by the native phytase present in the cereals (Hurrell, Reddy, Juillerat, & Cook, 2003). Cereal grains and legume seeds contain widely different phytase activities, and the phytases can be active during the production of low phytic acid complementary foods by traditional food processing methods such as soaking and germination (Khattak et al., 2007; Liang et al., 2008; Sattar, Durrani, Mahmood, Ahmad, & Khan, 1989)

A bitter taste compound called saponin is in the outer layers of quinoa seeds. This protects them from birds and insects. Saponins are glycoside compounds which occur in two groups. According to the nature of the sapogenin moiety they are conjugated with hexoses, pentoses, or uronic acids. The sapogenins are steroids (C27) or triterpenoids (C30). Quinoa saponins are soluble in methanol or water. They have strong detergent properties which form very stable foam in water solutions and reduce the superficial tension of aqueous solutions. They also show hemolytic activity and are in general toxic to cold-blooded animals which obtain oxygen directly from water. Saponins are also present in common foodstuffs such as peanuts, asparagus, garlic, onion, and spinach. The amount of saponins present depends on the variety of quinoa. It is higher in bitter-flavor varieties than in sweet, or low-saponin, varieties. Quinoa comprises saponins from 0.1 to 5%. The saponins of quinoa seeds are reduced to low levels after dry polishing or washing with water. These levels are apparently non-toxic to humans.

The aim of this research is to pre-treat proso millets and quinoa to increase the digestibility of the grains while reducing the phytic acid content and saponin content respectively. Soaking and germination have been experimented with various grains (Hemalatha et al., 2007), however, limited data is available on the effects of soaking and germination on the physico- chemical properties of proso millet and quinoa.

2.2 Materials and Methods

2.2.1 Raw materials

Quinoa seeds (white) were obtained from a national grain processor (Bob's Red Mill Natural Foods, Milwaukie, OR). Proso millet seeds (white) were obtained from Foundation Seed Stock in Agronomy, Horticulture and Plant Science department at South Dakota State University, SD. For the proximate composition analysis of proso millet and quinoa seeds and flour, the following AACC methods were used: Moisture -Oven drying at 135°C (method no. 44–19), Lipids - defatting in a Soxhlet apparatus with petroleum ether (method no. 30–25), Protein - micro Kjeldahl (N 6.25) (method no. 46–13), Carbohydrate – sugars and starch (method no. 80–04, 76-13) and total fiber - (method no. 32–07). Saponin content and phytic acid content (hexaphosphate equivalents) were quantified using the method described by (Rathod & Annapure, 2017). All chemicals were purchased from Sigma–Aldrich, USA. All chemicals and reagents used were of analytical grade. All experiments were performed in triplicates.

2.2.2 Soaking and Germination

The quinoa (11 pounds) and proso millets (11 pounds) were soaked overnight (8 pm-8 am) for 12 h in tap water (1:5 w/v) at room temperature. The soaked samples were then drained and washed. Excess water was drained, and the samples were wrapped in a moist cloth for 5 days (0 h – Day 0, 24 h - Day 1, 48 h - Day 2, 72 h – Day 3, 96 h – Day 4) at room temperature. All experiments were performed in triplicates. To inhibit further sprouting, the sprouted quinoa and sprouted proso millets were subjected to a heat treatment of 80°C for 5 hours. The moisture was completely removed from the sprouts during this period. The dried sprouts were then milled using a lab mill. The sprouted quinoa flour and sprouted proso millet flour were then packed in air-tight bags and stored in the refrigerator at 4°C until extrusion process was carried out.

2.2.3 Color

The color of the grains and germinated flours was measured using colorimeter (LabScan XE, HunterLab, Reston, VA) in triplicate in terms of Hunter Lab values (L^*, a^*, b^*). The Hunter Lab value 'L^*' signifies lightness with 0 for dark and 100 for bright. The value 'a^*' measures the degree of green color in the range from -100 to 0 and red in the range 0 to 100. The 'b^*' value represents blue color in the range from -100 to 0 and yellow in the range from 0 to 100.

where $\Delta L = L^* - L_0$, $\Delta a = a^* - a_0$, and $\Delta b = b^* - b^*_0$; the subscript ''0'' represents initial color values before processing. All readings were taken in triplicate.

2.2.4 Starch and Protein Digestibility of sprouted quinoa and proso millet flours

The starch digestibility and protein digestibility of the flour was measured. The measurements were done in triplicate. The in-vitro starch digestibility was determined in the flour (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20 °C for 2 h according to the method of (Rathod & Annapure, 2017). At the end of the incubation period, 2 ml of 3,5-dini-trosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard.

A system consisting, 100 mg (0.1 g) dry and defatted samples were incubated at 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 °C for 3 h. Further it was neutralized with 7.5 ml 0.2N NaOH. Then 4 mg pancreatin in 7.5 ml phosphate buffer (pH 8) was added and incubated for 37 °C for 24 h, further 5 mg of TCA was added, centrifugated at 5000 rpm for 10 min. After that the supernatant dry residue at 40–50 °C was discarded and the micro Kjeldahl process (Rathod & Annapure, 2017) for protein estimation was carried out. The percent in vitro protein digestibility was calculated using the following equation (Rathod & Annapure, 2017):

$$Protein\ digestibility\ (\%) = \frac{Total\ protein - Residual\ protein}{Total\ protein} * 100 \qquad (2.1)$$

2.3. Results and Discussion

2.3.1 Proximate analysis of quinoa and proso millet

The proximate analysis tests were carried out to understand the nutritional properties of proso millet and quinoa. The results are presented in Table 2.1. Quinoa is considered as a whole-grain food which means quinoa will digest slower compared to its refined grain counterparts (Ahamed et al., 1998). This makes the blood sugar level more stable that results in lesser fluctuations in the level of energy (Kozioł, 1992). Complex carbohydrates are those that consists of a longer molecule chain compared to simple carbohydrate. This is the reason attributed to the slow breakdown and absorption by the body (James, 2009). Quinoa is a complete protein, which means it contains all the nine essential amino acids that your body requires (Doğan & Karwe, 2003). The proteins in quinoa offer a wide range of amino acids. Amino acids are vital for supporting muscle development and immune activity, among other essential functions. Quinoa, unlike many other grains, is also an excellent source of lysine (Brady et al., 2007). This is an essential amino acid as it is vital for the synthesis of proteins. Although deficiency is rare, it can cause a range of medical issues, as lysine plays a role in processes such as growth and development. Quinoa contains two times more fiber than other grains like buckwheat and amaranth (Alvarez-Jubete et al., 2010). Fiber is well known or relieving constipation. It reduces high blood pressure and keeps heart disease at bay. Fiber also aids in lowering glucose and cholesterol levels. It can even aid in losing weight because it takes more time to chew compared to other foods and tends to make you feel full for longer (Jacobsen, 2003). It is also less energy dense. This means it contains lesser calories for the same amount food.

Millet products have found use in diets of patients with celiac diseases because the protein complex does not content gluten-forming proteins (Jones et al., 1970b). While the protein is deficient in lysine like common cereals, proso millet has higher component of essential amino acids than barley, oat, rye and wheat (Habiyaremye et al., 2017). Therefore, millet protein together with other proteins could be a basis for the development of new foods. Grains of proso millet are a rich source of starch, trace elements, dietary fibre and vitamins (P. Gulati, S. A. Weier, D. Santra, J. Subbiah, & D. J. Rose, 2016). Seeds also contain components with healing benefits, which decrease the level of low-density lipoprotein cholesterol in blood and injury to the liver (L. Zhang, Liu, & Niu, 2014). Phenolic compounds like antioxidants and beta glucans are present too (D. D. J. T. i. n. c. Baltensperger & uses, 2002). However, compounds decreasing the nutritional value of the foodstuff like tannins, phytates or oxalates are included (Bagdi et al., 2011).

2.3.2 Impact of soaking and germination on quinoa and proso millet

Soaking or hydration is the process of simultaneous absorption of water and swelling. This is basically a diffusion process and the result of different phenomena, such as, molecular absorption, capillary absorption and hydration. Germination incorporates those events that begin with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryo axis, usually the radicle, which extends to penetrate the structures that surround it. The subsequent mobilization of the major storage reserves is associated with the growth of seedling. The white color hypocotyls appeared in the quinoa seeds on Day 1. This was confirmed by a visual check. The germination ratio was more than 95%. The nutritional properties of the sprouted quinoa are shown in Table 2.2 and the nutritional properties of proso millet are shown in Table 2.3 respectively. The physical and biochemical process underlie this process, i.e., weakening of seed covers, turning on of metabolic activity, activation of gene transcription, relaxation of the embryonic cell walls, and reassembly and biogenesis of organelles. Briefly, during a first phase there is a rapid imbibition of water by the dry seeds until all the matrices and cell contents are fully hydrated. Then, a second phase like a plateau phase involves a limited water uptake but a strong metabolic reactivation. The increase in water uptake associated with the third phase is associated with cell elongation leading to completion of germination. Upon imbibition, the quiescent dry seeds rapidly restore their metabolic activity, including remobilization, degradation and accumulation, which imply important biochemical, nutritional and sensorial changes in the edible products. The outcoming primary and secondary metabolites exert differential biological health effects when consumed compared with non-germinated seeds. The reduction of saponin content in quinoa and phytic acid content in both grains were observed. The saponin content in raw quinoa of 0.8g/100g was reduced to 0.1g/100g samples (Day 4) by germination. The phytic acid content in quinoa reduced from 1.1g/100g to 0.1g/100g (Day 4) and in proso millet reduced from 1.5g/100g to 0.2g/100g (Day 4).

2.3.4 Influence of germination on color

The color development and change within food products is a complex phenomenon. Color is a very important factor that relates to consumer acceptance of food and, therefore, it is important to understand how it may change through the germination process. An increase in the activities of amylases and maltase during sprouting causes a gradual decrease in starch with a concomitant increase in reducing and non-reducing sugars during sprouting of cereal grains. The results of color change in the sprouted quinoa flour and sprouted proso millet flour is shown in Table 2.4 The change in color of the sprouted flour also indicates the breakdown of phytates. The color of the flour produced from the sprouted grains were significantly different from the unprocessed flour respectively. The L* value of sprouted quinoa flour was darker (L*=61.2) as compared to the control sample (L* = 82.6). Similarly, the sprouted proso millet flour was darker (L*=70.1) than the unprocessed proso millet flour (L*=84.2).

2.3.4 Influence of germination on starch and protein digestibility

The starch and protein digestibility results are presented in Table 2.4. The sprouting of quinoa increased the starch digestibility from 55.6% on Day 0 to 78.2% on Day 4. A similar increase in the protein digestibility was observed from Day 0 (42.2%) to Day 4 (75.5%). Sprouting also produced similar effects in proso millet where the starch digestibility increased from 51.7% (Day 0) to 77.1%. The breakdown of the phytates during sprouting of proso millet increased the protein digestibility from 46.4% on Day 0 to 76.8% on Day 4. Enhancement of starch digestibility during soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility. The degree of starch gelatinisation of germinated samples Day 4 is higher than in Day 0 and it is thus more readily hydrolysed. The rupture of starch granules of sprouted proso millet, making the substrate more accessible, facilitated the amylolysis. On the other hand, reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility. The results show that both protein digestibility and starch digestibility of the both flours increased significantly by soaking and germination. Germination produced a more significant improvement of protein digestibility and starch digestibility in both quinoa and proso millet.

2.4. Conclusions

Pre-treatments are the key for developing a highly nutritional product. Soaking and germination serve as excellent pre-treatment methods for increasing the starch and protein digestibility of quinoa and proso millet. The reduction of saponin content in quinoa and phytic acid content in both grains were observed. The saponin content in raw quinoa of 0.8g/100g was reduced to 0.1g/100g samples (Day 4) by germination. The phytic acid content in quinoa reduced from 1.1g/100g to 0.1g/100g (Day 4) and in proso millet reduced from 1.5g/100g to 0.2g/100g (Day 4). The sprouting of quinoa increased the starch digestibility from 55.6% on Day 0 to 78.2% on Day 4. A similar increase in the protein digestibility was observed from Day 0 (42.2%) to Day 4 (75.5%). Sprouting also produced similar effects in proso millet where the starch digestibility increased from 51.7% (Day 0) to 77.1%. The breakdown of the phytates during sprouting of proso millet increased the protein digestibility from 46.4% on Day 0 to 76.8% on Day 4.The improvement in nutritional quality, desirable changes in evident and functional properties of fresh sprouts, as well as meals obtained from dried sprouts of cereals can be made use of in the preparation and consumption of various traditional and processed foods. New and unconventional foods are accepted by the consumers if enough information on how to use them and their nutritional advantages are carefully impressed upon them. Extrusion processes can further increase the nutritional properties and digestibility of the sprouted quinoa and proso millet flours.

Table 2.1: Composition of quinoa seed (white) and proso millet

Composition	Quinoa	Proso Millet
Carbohydrates	63.2	70.1
- Sugar	0.6	0.8
- Starch	62.6	69.3
Protein[a]	13.1	12.2
Fat[a]	5.8	3.2
Moisture[a]	11.1	11.4
Fiber[a]	7.5	9.2

[a] g/100 edible material

Table 2.2: Impact of soaking and germination on the nutritional properties of quinoa

	Raw quinoa	Day 0	Day 1	Day 2	Day 3	Day 4
Total Protein[a]	13.1±0.5	13.1±0.15	13.2±0.03	13.5±0.05	13.7±0.01	13.8±0.07
Carbohydrates[a]	63.2±0.16	63.2±0.16	63.3±0.03	63.4±0.07	63.4±0.09	63.6±0.02
Moisture[a]	11.1±0.2	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid[b]	1.1±0.07	0.69±0.05	0.55±0.05	0.46±0.02	0.33±0.04	0.21±0.02
Saponin[b]	0.8±0.04	0.4±0.11	0.4±0.06	0.3±0.04	0.2±0.02	0.1±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 2.3: Impact of soaking and germination on the nutritional properties of proso millet

	Raw proso millet	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Total Protein[a]	12.5±0.5	12.6±0.09	12.7±0.04	12.9±0.05	13.2±0.01	13.3±0.07
Carbohydrates[a]	65.2±0.6	65.2±0.7	66.3±0.07	66.9±0.15	67.4±0.03	67.9±0.02
Moisture[a]	11.3±0.6	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid[b]	1.5±0.07	1.5±0.05	1.16±0.07	0.91±0.07	0.53±0.04	0.23±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 2.4: Impact of soaking and germination on the color, protein digestibility and starch digestibility of quinoa and proso millets

	Color			**Starch Digestibility**	**Protein Digestibility**
	L*	**a***	**b***	**(%)**	**(%)**
Quinoa					
Day 0	82.6	2.9	29.6	55.6	42.2
Day 1	66.8	3.6	21.1	60.1	50.7
Day 2	64.6	3.8	20.4	68.7	59.8
Day 3	62.4	3.6	20.0	72.6	69.9
Day 4	61.2	3.9	19.5	78.2	75.5
Proso Millet					
Day 0	84.2	0.5	21.8	51.7	46.4
Day 1	73.4	0.7	19.8	61.2	56.4
Day 2	72.6	0.6	22.1	69.4	67.3
Day 3	71.1	0.6	21.3	73.2	73.1
Day 4	70.1	0.8	20.5	77.1	76.8

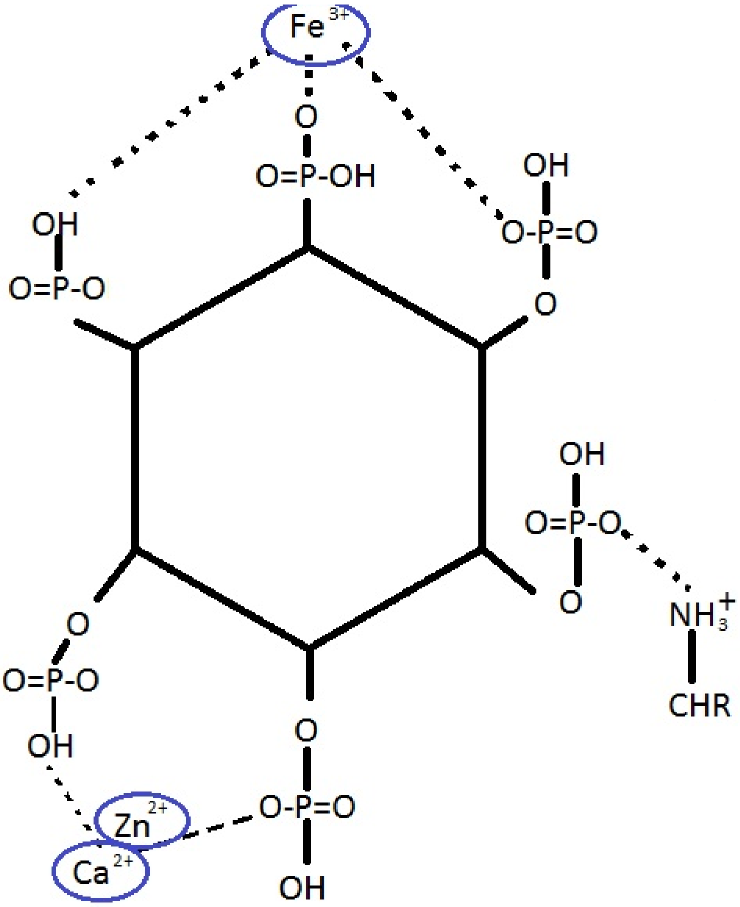


Figure 2.1: Mineral absorption by phytic acid

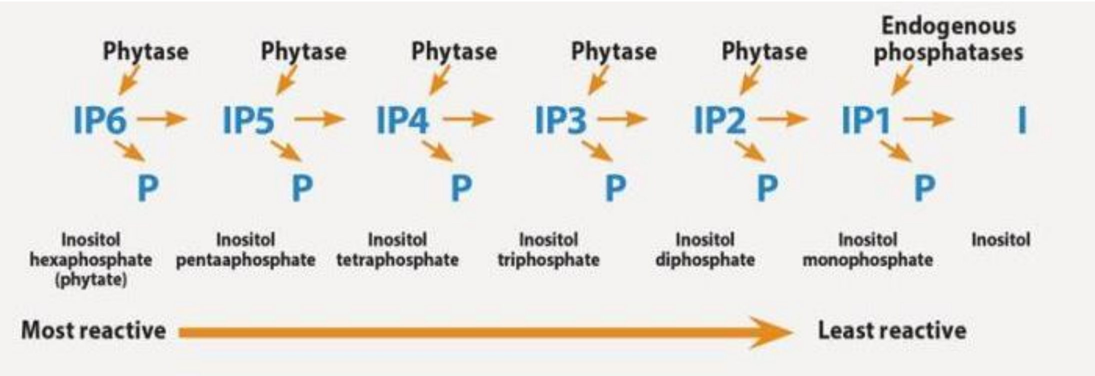


Figure 2.2: Breakdown of phytate to inositol by phytase action

CHAPTER 3: EFFECT OF SINGLE-SCREW EXTRUSION PROCESS PARAMETERS ON THE PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF SPROUTED QUINOA EXTRUDATES

Abstract

Soaking and germination pre-treatments of quinoa were carried out to reduce the saponin and phytic acid content and therefore increase protein and starch digestibility. Single-screw extrusion processing of sprouted quinoa flour was studied using a response surface design to understand the influence of feed moisture content (15-25% w.b.), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of sprouted quinoa extrudates. The following responses of the extrudates were measured and the values ranged from: bulk density (116-154 kg/m^3), hardness (1.05-1.8 N), water solubility index (11.5-16.5%), water absorption index (2.36-3.51), total color difference ΔE (14.8-21.7), expansion ratio (2.52-3.75), protein digestibility (80.5-86.5%) and starch digestibility (80.1-85.8%). Both feed moisture content and extruder die temperature had statistically significant effects on all the extrudate characteristics. Extruder screw speed had minimal effect on the extrudate properties. As the germination time increased, the starch and protein digestibility of the extrudates increased. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility.

Keywords: Quinoa, Saponin, Gluten-Free, Soaking, Germination, Digestibility

3.1. Introduction

According to the US Food Allergy Research and Education, a survey of 40,000 adults assessed that more than 26 million people have a food allergy (Gupta et al., 2019). An additional 8% believed to have a food allergy but had symptoms from food intolerance. More than 70 foods are reported to cause food allergies (Wróblewska &

Jędrychowski, 2009). The chief reasons for gluten free diets are celiac disease, gluten intolerance, gluten ataxia, and gluten avoidance for non-medical reasons (Lorenz & D'Appolonia, 1980). Coeliac disease, also known as gluten-sensitive enteropathy, is one of the common food intolerances on a global scale. Tiny hair-like projections in the small intestine called villi are injured through an immune-mediated response which leads to mal-absorption of vital nutrients like iron, folic acid, calcium and fat-soluble vitamins, when exposed to gluten (Thompson, 2009). Due to lack of any treatment, food allergic and food intolerant people need to avoid the foods to which they are allergic or intolerant. However, individuals who follow an allergy-free diet may be at danger of several nutrient deficiencies.

Instead of wheat, rye, barley, or other gluten-containing cereal grains, there are numerous other edible seeds that can be consumed, including amaranth, buckwheat, quinoa, teff and wild rice, as well as cereal grains that do not contain gluten, including corn, millet, rice, and sorghum (Thompson, 2009). For example, quinoa is a pseudo cereal and has been cultivated in the Andean region. It is an annual plant, 1–2 m tall with deep penetrating roots. It can be grown from sea level up to an altitude of 3800 m (Alandia et al., 2016). The plant has tolerance to frost, salinity and drought, and be cultivated on marginal soils (Jacobsen, 2003). Quinoa has outstanding protein quality and a wide range of minerals and vitamins, which makes it popular for consumption. In addition, it is rich in amino acids like lysine and methionine that are deficient in cereals. Quinoa is an excellent source of fiber, iron, calcium, magnesium, and B vitamins (Navruz-Varli & Sanlier, 2016). Because of their nutritive properties, edible seeds other than the gluten-containing cereal grains and pseudocereals are viable candidates to formulate gluten-free diets.

One of the factors which limit the widespread utilization of quinoa is its bitter taste caused by the presence of saponins. Saponins are triterpene glucosides that consist of a linear arrangement of one to six hexose or pentose glycoside units joined to the sapogenin aglycone, which can be a steroidal or a triterpenoid aglycone (Kuljanabhagavad et al., 2008). Quinoa saponins are soluble in methanol or water. They produce stable foams in aqueous solutions. They may form chemical complexes with iron and reduce its absorption. Saponins are located on the outer layers of the seeds and can be removed by polishing and washing with water (Estrada et al., 1997).

Similarly, phytic acid poses an issue with cereals and pseudocereals. Phytic acid (myoinositol hexaphosphoric acid) reduces the availability of many minerals like iron, zinc, calcium and magnesium (Cheryan & Rackis, 1980). The formation of iron-phytate complexes of low solubility, in the small intestine is the basis for the interference of phytate with iron absorption. Although the iron content in cereals is usually high, the iron absorption from them is often poor due to the presence of high amounts of phytates. They are also found to inhibit the proteases and amylases of the intestinal tract. Quinoa contains many essential minerals like calcium (874 mg/kg), phosphorus (5.3 g/kg),iron (5.3 g/kg) and zinc (36 mg/kg) and their availability is of importance in relation to its use as a basic source of nutrients in an infant food (Oatway et al., 2001). Soaking, sprouting and fermentation are some of the methods that can break down the phytic acid to increase the digestibility of the food as well as enhance mineral absorption (Egli et al., 2003).

Soaking and sprouting are an age-old technique however the benefits of these methods has not been completely exploited to increase the digestibility of food. Sprouted seeds are nutritionally superior to their respective seeds Germinated grains have higher levels of nutrients, lower levels of anti-nutrients such as phytic acid and flatulence-causing sugars, increased protein and starch digestibility, and increased bioavailability of some minerals (Carciochi et al., 2014). The inclusion of sprouted grains in the formulation of whole-grain-rich, fiber-rich, and gluten-free baked goods may provide a natural fortification and enrichment mechanism; it may improve nutritional quality because of the increased digestibility and bioavailability of some nutrients and the reduction of some anti-nutrients. Comprehensive reviews of the effects of germination on the nutrient composition of cereals and pseudocereals have been published (Carciochi et al., 2014) (Lombardi-Boccia, Lullo, Carnovale, & Agriculture, 1991) (Lorenz & D'Appolonia, 1980).

Sprouted grains have been consumed by people who have a strict diet schedule or by ones who have a rigorous exercise pattern. Not all people enjoy the flavour of a sprouted whole grain. Hence it is important to convert it to an interesting product before it reaches the consumer. Extrusion can play a major role by either converting it to an expanded snack or products such as noodles and pasta. Extrusion is a high-temperature and short-time process. During extrusion, moistened starch/protein-based food materials are cooked in a barrel. A combination of moisture content, pressure, temperature and mechanical shear in the barrel results in molecular transformation and chemical reactions (Muthukumarappan & Swamy, 2017). Extrusion cooking is a multi-step, multi-functional and thermal/mechanical process. It has numerous food applications such as ready-to-eat cereals, salty and sweet snacks, co-extruded snacks, indirect expanded products, croutons for soups and salads, dry pet foods and fish foods, textured meat analogues from defatted high-protein flour, infant foods and confectionery products (Paucar-Menacho et al., 2018). The impact of extrusion cooking on nutritional quality is unclear. Advantageous effects include removal of anti-nutritional factors, gelatinisation of starch, increased soluble dietary fiber and decrease of lipid oxidation (Bartnik & Szafrańska, 1987). Conversely, maillard reactions between protein and sugars decrease the nutritional value, depending on the raw material types, their composition and process conditions (Camire et al., 1990).

Extrusion processing is a commonly used processing technology in the food industry with a wide number of applications. It is a processing system that utilizes a single screw or a set of screws to force food materials through a die opening. The food ingredients are subjected to high shear, temperature, and pressure for a short period of time (Gopirajah & Muthukumarappan, 2018). The role of the screw is to assist in imparting shear to the ingredient mixture and forcing the dough of the mixed ingredients out from the extruder through the die (Muthukumarappan & Karunanithy, 2012). The screws are also responsible for the build-up of pressure that occurs at the end of the extruder as well as added mixing of the ingredients. This helps to transform the ingredients from solid powders to a melt state inside of the extruder (Guy, 2001). The melted ingredients are then forced through a die at the end of the extruder into the atmosphere. The melt coming out of the extruder encounters a sudden drop in pressure, resulting in rapid expansion as well as a decrease in temperature, helping it to transform into a cooked product. The final quality and texture of the extruded product depends on various factors, including the ingredient mixture and its properties, extrusion processing conditions, and post-processing conditions (Harper & Clark, 1979). Because of the great flexibility of extrusion processing, it has found very diverse applications in the food industry.

A single-screw extruder consists of only one screw housed in the barrel that often has a fluted or grooved design. Additionally, the screw in a single-screw extruder is usually designed with a decreasing pitch to create compression (Guy, 2001). The amount of decreasing pitch is referred to as the compression ratio. A twin-screw extruder has a pair of screws that are either intermeshing or non-intermeshing. The set of screws in the twin-screw extruder can be either co-rotating or counter-rotating. For any new material extrusion, a single screw extrusion is preferred because of the relatively low cost, straightforward design, ruggedness and favorable performance/cost ratio (Akdogan, 1999).

The effect of germination on the nutrition properties of grains has been studied by various authors (Donkor et al., 2012) (Hung et al., 2012) (Nelson et al., 2013). Similarly, the impact of extrusion variables on the quinoa extrudates have also been studied (Kowalski et al., 2016) (Doğan & Karwe, 2003). However, the method of producing highly digestible quinoa extrudates by removing the saponins and phytic acid has not been researched. The objective of this research is to understand the effect of germination on the changes in nutrients of quinoa and the impact of the single-screw extrusion variables such as moisture content of feed, temperature, screw speed of extruder and germination time on the physical and physico-chemical properties of extrudates made from the sprouted quinoa flour.

3.2 Materials and Methods

3.2.1 Raw materials

Quinoa seeds (white) were obtained from a national grain processor (Bob's Red Mill Natural Foods, Milwaukie, OR). For the proximate composition analysis of quinoa seeds and flour, the following AACC methods were used: Moisture -Oven drying at 135°C (method no. 44–19), Ash - calcination at 550°C (method no. 08–01), Lipids - defatting in a Soxhlet apparatus with petroleum ether (method no. 30–25), Protein - micro Kjeldahl (N 6.25) (method no. 46–13), Carbohydrate – sugars and starch (method no. 80–04, 76-13) and total fiber - (method no. 32–07). Saponin content and phytic acid content (hexaphosphate equivalents) were quantified using the method described by(Rathod & Annapure, 2017). All chemicals were purchased from Sigma–Aldrich, USA. All chemicals and reagents used were of analytical grade. All experiments were performed in triplicates.

3.2.2 Soaking and Germination

The quinoa (11 pounds) was soaked overnight (8 pm- 8 am) for 12 h in tap water (1:5 w/v) at room temperature. The soaked samples were then drained and washed. Excess water was drained, and the samples were wrapped in a moist cloth for 5 days (0 h – Day 0, 24 h - Day 1, 48 h - Day 2, 72 h – Day 3, 96 h – Day 4) at room temperature. All experiments were performed in triplicates.

3.2.3 Extrusion processing

The quinoa flour samples, each weighing around 200 g were randomly extruded using a 19.18 mm (0.755 in.) barrel inner diameter (i.d.), single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder® South Hackensack, NJ) as shown in Fig 3.1. It was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 200 rpm. The extruder had a barrel with length (L) to diameter (D) ratio of 20:1. A uniform 19.05 mm pitch screw having 381 mm (15.0 in.) screw length, a 19.05 mm (0.75 in.) constant outside (top of flight) diameter, a 3.81 mm (0.15 in.) initial screw feed depth, an 11.43 mm (0.45 in.) initial screw root diameter, and a screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiments. A die nozzle with 3 mm diameter was used. The extrudates were cooled to room temperature and sealed in polyethylene bags and stored at room temperature until measurements were taken.

3.2.4 Measurement of extrudate properties

3.2.4.1 Bulk Density

The bulk density (kg/m^3) was calculated as the ratio of the mass M (kg) to the volume V (m^3) of the measured and weighed extrudate sample. It was assumed that extrudates were of cylindrical shape. The bulk density of all 75 extrudates were measured and the measurements were done in triplicate.

$$Bulk\ Density = \frac{M}{V} \tag{3.1}$$

3.2.4.2 Expansion ratio (ER)

Expansion ratio was calculated by measuring the mean diameter of 10 randomly chosen locations on the extrudates with calipers for each extrusion process condition. Further it was divided by the die diameter of 3 mm.

$$Expansion\ Ratio = \frac{Mean\ diameter\ of\ the\ extrudate\ (mm)}{Die\ diameter\ (mm)} \tag{3.2}$$

3.2.4.3 Hardness

Hardness is defined as the maximum force per unit area of the quinoa extrudate that is necessary to breakdown the extrudate into two pieces. Hardness was tested by breaking the extrudate using a cylindrical probe (TA-4, Texture Technologies Corp, NY) attached to the crosshead of texture analyser. The sample was positioned in its natural position. A vertical force was applied at a speed of 50 mm/min. The force applied vs deformation was recorded and the maximum force recorded in the curve was considered as the hardness. The hardness of all 65 extrudates were measured and the measurements were done in triplicate.

3.2.4.4 Water Solubility Index and Water Absorption Index

The water absorption index and water solubility index of all 75 extrudates were measured and the measurements were done in triplicate. The quinoa extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was mixed in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was mixed occasionally and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminium cup and dried at 135°C for 2h (AACC, 2000). The weight of the gel in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by:

$$Water\ Solubility\ Index\ (\%) = \frac{W_{ss}}{W_{ds}} * 100 \tag{3.3}$$

$$Water\ Absorption\ Index = \frac{W_g}{W_{ds}} \tag{3.4}$$

where, W_{ss} is the weight of dry solids of supernatant (g), W_{ds} is the weight of dry sample (g), and W_g is the weight of gel (g)

3.2.4.5 Color

The color of quinoa extrudate samples was measured using colorimeter (LabScan XE, HunterLab, Reston, VA) in triplicate in terms of Hunter Lab values (L^{*}, a^{*}, b^{*}). The Hunter Lab value ‘L^{*}’ signifies lightness with 0 for dark and 100 for bright. The value ‘a^{*}’ measures the degree of green color in the range from -100 to 0 and red in the range 0 to 100. The ‘b^{*}’ value represents blue color in the range from -100 to 0 and yellow in the range from 0 to 100.The total color change (ΔE) is calculated as

$$Total\ Color\ Difference\ \Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \tag{3.5}$$

where $\Delta L = L^{*} - L_0$, $\Delta a = a^{*} - a_0$, and $\Delta b = b^{*} - b^{*}_0$; the subscript ‘‘0’’ represents initial color values before processing. All readings were taken in triplicate.

3.2.4.6 Starch and Protein Digestibility

The starch digestibility and protein digestibility of all 75 extrudates were measured and the measurements were done in triplicate. The quinoa extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The in-vitro starch digestibility was determined in the extrudate powder (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20 °C for 2 h according to the method of (Rathod & Annapure, 2017). At the end of the incubation period, 2 ml of 3,5-dini-trosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard.

A system consisting, 100 mg (0.1 g) dry and defatted samples were incubated at 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 °C for 3 h. Further it was neutralized with 7.5 ml 0.2N NaOH. Then 4 mg pancreatin in 7.5 ml phosphate buffer (pH 8) was added and incubated for 37 °C for 24 h, further 5 mg of TCA was added, centrifugated at 5000 rpm for 10 min. After that the supernatant dry residue at 40–50 °C was discarded and the micro Kjeldahl process for protein estimation was carried out. The percent in vitro protein digestibility was calculated using the following equation (Rathod & Annapure, 2017):

$$Protein\ digestibility\ (\%) = \frac{Total\ protein - Residual\ protein}{Total\ protein} * 100 \qquad (3.6)$$

3.2.5 Experimental Design and Statistical Analysis

Experiments were conducted using the Box Benhken design (BBD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA), consisting four factors namely moisture content (X_1), temperature (X_2), screw speed (X_3) and germination time (X_4). Three replicates were taken at the design center and the total number of observations were 75 [60 (not center points) and 15 (center points)]. Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN). The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA). The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

3.3 Results and Discussion

3.3.1 Effect of Soaking and Germination on the quinoa flour

The nutritional properties of the sprouted quinoa are shown in Table 3.1. After soaking for 12 h, the white color hypocotyls appeared in the quinoa seeds on Day 1. The germination ratio was more than 90%. The protein content decreased significantly on Day 4 of germination and a simultaneous increase in the hypocotyl length was observed. The change in color of the sprouted flour indicates the breakdown of phytates. The optimised soaking time was 12 h and germination time was 96 h.

In general, protein content of quinoa seeds ranges between 13.1% and 14.0% (James, 2009). The total protein content of quinoa is higher than that of grains such as rice, barley, corn, rye, sorghum and wheat (Navruz-Varli & Sanlier, 2016). The chief carbohydrate constituent of quinoa is starch, and it ranges from 62-64%. The total fiber was close to other grain products (7.0%-9.7%). Quinoa contains sugar by about 3%. It mostly contains maltose, D-galactose, and D-ribose in addition to low levels of fructose and glucose (Navruz-Varli & Sanlier, 2016).

The phytic acid concentration in quinoa seeds is comparable to whole grain rye flour (7.7 mg/g), whole grain wheat flour (8.7 mg/g), lentils (8.4 mg/g) and fava beans (8.0 mg/g). Phytic acid is not only present in the outer layers of quinoa, as in the case of rye and wheat but is also evenly distributed within the endosperm (Ahamed et al., 1998). Phytic acid binds minerals, such as Fe, Zn, Ca and Mg, and can make the mineral content of a cereal inadequate, especially for children (Khattak et al., 2007). Soaking and sprouting processes reduce the 'binding' effect of the phytic acid by activating the enzyme phytase (James, 2009). The amounts of phytic acid of the quinoa seeds obtained in this study are in concordance with the values for Cherry vanilla quinoa (Kowalski et al., 2016). A significant reduction in phytic acid content is observed with increase in germination time. Day4 had more than 50% reduction in phytic acid. Extrusion further decreased the phytic acid content of pea flour (Alonso, Orue, & Marzo, 1998), bean flour (Anton, Fulcher, & Arntfield, 2009) and other legumes (Lombardi-Boccia et al., 1991).

Saponins are the principal anti-nutritional factor present in the seed coat of quinoa. Most saponins are nitrogen-free glycosides, each consisting of a sapogenin and a sugar. Saponins affect quinoa's taste and digestibility, therefore should be removed before consumption (Ruales & Nair, 1992). Traditionally, saponin has been removed by laboriously hand scrubbing the quinoa with alkaline water. The saponin content is checked by placing the grain in a tube, adding water and vigorously shaking for 30 s. If no foaming occurs, all saponins are assumed to have been removed. The saponin content was significantly reduced by soaking and germination as shown in Table 3.1. Additionally, saponin content is expected to be reduced through either extrusion (Anuonye, Onuh, Egwim, & Adeyemo, 2010).

3.3.2 Effect of independent variables on the extrusion process

The results of the single screw extrusion process of sprouted quinoa flour along with the experimental design is presented in Appendix A.

3.3.2.1 Bulk Density

The bulk density of the extrudates varied between 116 and 154 kg/m^3. Low bulk density, which is a required characteristic of the extruded product, was achieved with low feed moisture content, average temperature and high screw speed combination. Fig 3.2 shows that feed moisture content had a significant effect on bulk density followed by screw speed, whereas temperature seemed to have a minor effect. Low bulk density of 116 kg/m^3 was obtained at feed moisture content of 15%, 115°C temperature, 220 rpm screw speed and Day 4 germinated samples.

3.3.2.2 Expansion ratio

The measured expansion ratio of quinoa extrudates ranged from 2.36 to 3.75. The expansion ratio was significantly affected by temperature and feed moisture content. High expansion ratio at low feed moisture content for expanded products is distinctive for cereals. However, Fig 3.3 shows that the temperature and screw speed also have a significant effect on the expansion ratio of the quinoa extrudates. Similar trends have been obtained for corn (Diaz et al., 2013), wheat (Pitts et al., 2014) and oats (Gopirajah & Muthukumarappan, 2018).

3.3.2.3 Hardness

Hardness, the maximum force to break the sample, ranged between 1.05 and 1.8 N. The coefficient of determination for hardness was high (R^2 = 0.93) and the moisture, temperature and germination period were found to have significant contributions to the hardness of the extrudates. At all feed moisture content levels, decrease in die temperature decreased the product hardness. Screw speed did not have a significant effect on the extrudate hardness. Fig 3.4 shows the effect of moisture content and screw speed on the product hardness. Previous studies have also reported similar trends for extrudates based on rice (Ding et al., 2006), wheat (Ding et al., 2006), oat bran and soy flour (Anuonye et al., 2010) and chickpea (Khattak et al., 2007). The hardness was high of 1.8 N at about 140°C, 150 rpm screw speed, 25% feed moisture content and Day 0 sprouted samples. Chemical changes occurring during extrusion cooking affect the development of textural and mechanical properties such as hardness in extrudates. The elastic swell effect and bubble growth effect equally contribute to the structural changes of starch during extrusion. Water performs as a plasticizer to the starch-based material decreasing its viscosity and the mechanical energy dissipation in the extruder. Hence the product becomes dense and bubble growth is compressed. The reduced starch conversion and compressed bubble growth result in a dense product and wetness of extrudate.

3.3.2.4 Water Solubility Index and Water Absorption Index

Water solubility index is related to the quantity of soluble solids, which is related to dextrinization. The coefficient of determination for water solubility index was highly significant as revealed by a high value of R^2 (0.98). The linear effects of feed moisture content, screw speed and temperature had significant effect on water solubility index. In general, water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. Fig 3.5 shows the significant effect of temperature and screw speed on water solubility index. The increase in water solubility index with increasing temperature was consistent with the results reported for oat extrudates (Gutkoski & El-Dash, 1999). The water solubility index decreased with the increase in moisture content. Similar trends have been reported earlier for starch, maize grits, wheat and pea (Ding et al., 2006) (Singh et al., 2007). The poor correlation between water solubility index and most of the process and product responses is explained by the fact that water solubility index includes the opposing effects of starch dextrinization and the molecular level interactions between degraded components, which may not be favoured at the same condition (Lei, Ruan, Fulcher, van Lengerich, & Engineering, 2008). An increase in the amount of dextrinized starch during extrusion cooking results in an increase in water solubility index. However, molecular interactions between degraded starch, protein, and lipid components, which in turn lead to an increase in molecular weight, may decrease the solubility, thus water solubility index (Camire et al., 1990).

Water absorption index depends on the availability of hydrophilic groups and on the gel formation capacity of the macromolecules. It is a measure of damaged starch together with protein denaturation and new macromolecular complex formations. Water absorption index of extrudates ranged between 2.36 and 3.51. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. The interaction effects except for temperature-screw speed were significant as shown in Fig 3.6. Similar responses were observed in rice flour (Hagenimana, Ding, & Fang, 2006), oats (Gutkoski & El-Dash, 1999), finger millet (Sawant, Thakor, Swami, Divate, & Vidyapeet, 2012) and cassava starch (Hashimoto, Grossmann, & technology, 2003). Water absorption index had poor correlations with almost all process and product responses except bulk density. This is an expected result since it includes the effect of starch gelatinization, protein denaturation and molecular level cross-linking reactions which are not always favored at the same conditions (Doğan & Karwe, 2003).

3.3.2.5 Color

Color is an important quality parameter since it reflects the extent of chemical reactions and degree of cooking or degradation that take place during extrusion cooking. In this study, ΔE represents the total color difference compared to the color of unsoaked and unsprouted quinoa. Higher color difference ΔE means darker products with more intense yellow and red color. The total color change in extruded products ranged between 15.5 and 23.7. Linear effects of temperature and moisture content were found to have the highest contribution to total color change. As depicted in Fig 3.7, low feed moisture content and high temperature increased the total color change possibly due to increased extent of browning reactions under this condition. Although, the screw speed was not a significant parameter, at low screw speeds a slight increase in color change observed due to longer residence times which might increase the extent of chemical reactions.

3.3.2.6 Starch Digestibility (SD) and Protein Digestibility (PD)

The results show that both protein digestibility and starch digestibility of the quinoa extrudates were increased significantly by soaking, germination and extrusion. Fig 3.8 and Fig 3.9 illustrate the effect of the independent variables on the protein and starch digestibility respectively. The linear effects of all the variables and interaction effect of moisture and screw speed had a significant effect on the protein and starch digestibility. Germination produced a more significant improvement of protein digestibility and starch digestibility in quinoa. The increases of digestibility produced by the different treatments were higher in protein than in starch. Improvement of protein digestibility after processing could be attributable to the reduction or elimination of different anti-nutrients. Phytic acid, as well as condensed tannins and polyphenols, are known to interact with protein to form complexes. This interaction increases the degree of cross-linking, decreasing the solubility of proteins and making protein complexes less susceptible to proteolytic attack than the same protein alone (Alonso et al., 1998). Extrusion produced a higher increase in protein digestibility than the other processing methods. This can be related to the higher efficiency of this thermal treatment in reduction of anti-nutrient activities in addition to soaking and germination.

Enhancement of starch digestibility during soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility (Linko, Linko, & Olkku, 1983). The degree of starch gelatinisation of germinated samples Day 4 is higher than in Day 0 and it is thus more readily hydrolysed. The rupture of starch granules of sprouted quinoa, making the substrate more accessible, facilitated the amylolysis. On the other hand, reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility.

3.3.3 Box-Behnken design and analysis

This response surface methodology is an empirical modeling technique used to evaluate the relationship between the experimental and the predicted results (Muthukumarappan & Swamy, 2018). In the present study, the Box Behnken Design was used to obtain a proper model for the optimization of the extrusion process variables. Experiments were carried out according to the experimental design in order to explore the optimum conditions and study the influence of process variables on the physical and physico-chemical properties of the extrudates. The predicted values were attained by the model fitting technique in Design Expert software version 8.0.7.1 and the values correlated well with the experimental values. Fitting of the data to various models such as the linear, interactive, quadratic and cubic models was performed to acquire the regression equations. Sequential model sum of squares and model summary statistics tests were carried out to decide the adequacy of models in the present study and the results are presented in Table 3.2. From Table 3.2, it is observed that the quadratic model is highly significant for the extrusion of sprouted quinoa. The model summary statistics demonstrated that the quadratic model had maximum R-Squared, Adjusted R-Squared and Predicted R-Squared values. The cubic model was not suggested for the extrusion process since the BBD matrix had enough data to understand the outcome of the system. Hence, quadratic model was chosen for further analysis of data.

3.3.4 Fitting of polynomial equations and statistical analysis

Based on the Box-Behnken experimental design model, the empirical relationship between the input variables and experimental results obtained were expressed by a second-order polynomial equation with interaction terms (Swamy, Sangamithra, & Chandrasekar, 2014). The equations generated in coded factors are presented in Table 3.3.

The investigation and optimization of a fitted response surface might generate poor or disingenuous results. Therefore, it was essential to ensure the fitness of the model. Regression analysis and Pareto analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. The results pointed out that the equation sufficiently presented the actual relationship between the input parameters and responses. ANOVA is a statistical technique that subdivides the total variation in a dataset into component parts allied with sources of variation for testing hypotheses on the variables of the model (Swamy & Muthukumarappan, 2017).

ANOVA followed by Fisher’s statistical test (F-test) was conducted to analyse the significance of each independent variable. The F-value is the ratio of the regression mean square and the real error mean. It indicates the influence of each controlled factor on the tested model. The ANOVA results in Table 3.4 shows the F-value for the responses, implying that the model is highly significant. The large Fisher value signifies that the variation in the responses can be explicated by the regression equation (Asokapandian, Venkatachalam, Swamy, & Kuppusamy, 2016). The associated p value estimates if F value is large enough to show statistical significance. The p values that are lower than 0.05 indicate that the model and the associated terms are statistically significant.

Coefficient of determination (R^2) and adjusted-R^2 were computed to verify the sufficiency and fitness of the model. The R^2 signifies the proportion of the total variation in the response expected by the model, thus indicating the ratio of the regression sum of squares (SSR) and total sum of squares (SST) (Sangamithra, Sivakumar, Kannan, & John, 2015). The values of R^2 imply that 95% of experimental data was well-suited. The high R^2 coefficient gives a satisfactory adjustment of the quadratic model to the experimental data. The purpose of the adjusted-R^2 is to analyse the model adequacy and fitness. The adjusted-R^2 value adjusts the R^2 value for the sample size and for the number of terms in the model (Albarracín et al., 2015). The high value of adjusted-R^2 supports a high correlation between the experimental and the predicted values. The high coefficient of determination and very small p-value (<0.0001) demonstrate that the quadratic polynomial model is significant and adequate to characterize the actual relationship between the response and input.

The coefficient of variation (CV) shows the scattering of the experimental points from the predicted values of the second order polynomial model (Swamy & Muthukumarappan, 2017). A high coefficient of variation points out that there is extreme variation in the mean value and does not adequately develop a sufficient model. The very low coefficient of variation value undoubtedly represents a high degree of precision and reliability of experiments conducted.

3.3.5 Selection of optimum conditions

Optimum conditions for the extrusion process were deduced to get desired extrudate properties. Second order polynomial models attained in the study were used for each response to acquire specified optimum conditions. To optimize the independent variables, the Derringer's desirability function method was utilized. After satisfying the necessities for each response in the design, the Derringer's desirability function seeks for a combination of factor levels that together optimize a set of responses. By the conversion of each into a dimensionless desirability scale which defines a partial desirability function, combining the discrete desirability values to attain the global desirability function and then maximizing the global function and recognizing the optimal factor settings, the optimization process is accomplished. The desirability function scale operates between 0 and 1 with 0 signifying a completely undesirable response and 1 representing a fully desired response. The individual desirability for each response is achieved by stating the goals, i.e., minimize, maximize or target the response, and boundaries essential for each response. A weight factor (defines the shape of the desirability function for each response) is then allocated. The weights range between 0.1 and 10 with the larger ones relating to more significant responses. A weight factor of 1 was selected for the individual desirability in this research. The "importance" of the goals can be arranged sequentially according to increasing importance. It can vary from 1 (least importance) to 5 (most important). To optimize the process the following parameters (1) Moisture (15-25%) (2) Temperature (90-140°C) (3) Screw speed (80-220 rpm) and (4) Germination period (Day 0- Day 4) respectively, were set for maximum desirability. By applying the methodology of the desired function, the optimized process variables were obtained. It indicated that a moisture 15%, temperature 115°C, screw speed 185 rpm and Day 4 germinated flour input will produce an extrudate with bulk density 118.8 kg/m^3, hardness 1.13 N, water solubility index 16.5, water absorption index 3.4, total color difference ΔE=20.6, expansion ratio=3.75, protein digestibility=86.3% and starch digestibility=85.7% respectively with overall desirability value of 0.999.

3.3.6 Verification of optimized conditions and predictive model

The fitness of the model equations for predicting optimum response values was investigated under the conditions: moisture 15%, temperature 115°C, screw speed 185 rpm and Day 4 germinated flour. These conditions were optimum by the RSM optimization approach. To verify the validity of the optimized conditions, experiments were performed to evaluate the experimental results vs predicted values of the output using the model equation. The experiments were carried out thrice and the average values were presented in Table 3.5. The results expected from the optimum solution was bulk density=118.8 kg/m^3, hardness=1.13 N, water solubility index=16.5, water absorption index =3.4, total color difference ΔE=20.6, expansion ratio=3.75, protein digestibility=86.3% and starch digestibility=85.7% and the experimental value was bulk density=118.8±0.07 kg/m^3, hardness=1.13±0.09 N, water solubility index=16.5±0.06, water absorption index =3.4±0.07, total color difference ΔE=20.6±0.12, expansion ratio=3.75±0.05, protein digestibility=86.3±0.04% and starch digestibility=85.7±0.09%. The mean values obtained through the experiments were compared with the predicted values. The values obtained through confirmation experiments are within 95% of predicted values. This shows that the developed quadratic models are well-suited. It is also evident that the optimal values are valid within the specified range of process variables.

3.4 Conclusions

As quinoa offers an excellent nutritional quality and has a high commercial value, this study was needed to increase individuals' awareness of this pseudo-grain's nutritional content and consumption, to reveal its nutritional benefits and to investigate its effects on health. In this study, soaking, germination and extrusion procedures were used to reduce anti-nutrient levels and improve the protein and starch digestibility. The influence of feed moisture (15-25% w.b.), temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of quinoa extrudates were studied. Both feed moisture and extruder temperature had significant effects on the extrudate characteristics. Changing the level of moisture content and temperature had significant effect ($p<0.01$) on all physical properties. The total color change in extruded products ranged between 15.5 and 23.7. Water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. Hardness ranged between 1.05 and 1.8 N and the coefficient of determination for hardness was high ($R^2 = 0.93$). Extruder screw speed had minimal effect on the extrudate properties. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility. By applying the methodology of the desired function, the optimized process variables were moisture 15%, temperature 115°C, screw speed 185 rpm and Day 4 germinated flour with bulk density=118.8 kg/m^3, hardness=1.13 N, water solubility index=16.5, water absorption index =3.4, total color difference ΔE=20.6, expansion ratio=3.75, protein digestibility=86.3% and starch digestibility=85.7% respectively with overall desirability value of 0.999. Overall, the quinoa studied in the range extrusion conditions showed moderate expansion characteristics as compared to widely used cereal grains, suggesting that quinoa is not well suited for making direct expanded products as compared to corn. However, this may be more useful in the products where direct expansion is not an important textural quality.

Table 3.1: Impact of soaking and germination on the nutritional properties of quinoa

	Raw quinoa	Day 0	Day 1	Day 2	Day 3	Day 4
Total Protein [a]	13.1±0.5	13.1±0.15	13.2±0.03	13.3±0.05	13.4±0.01	13.1±0.07
Carbohydrates [a]	63.2±0.6	63.2±0.16	63.3±0.03	63.4±0.07	63.4±0.09	63.6±0.02
Moisture [a]	11.1±0.2	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid [b]	1.10±0.07	0.69±0.05	0.55±0.05	0.46±0.02	0.33±0.04	0.21±0.02
Saponin [b]	0.80±0.04	0.40±0.11	0.40±0.06	0.30±0.04	0.20±0.02	0.10±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 3.2: Results of model adequacy tested in the Box-Behnken design

Source	Sum of squares	DF	Mean square	F value	Prob > F	Remarks
Sequential model sum of squares for ***Bulk Density***						
Quadratic	23.08	3	7.69	8.07	0.0002	Suggested
Sequential model sum of squares for ***Hardness***						
Quadratic	0.09	3	0.031	8.14	0.0002	Suggested
Sequential model sum of squares for ***Water Solubility Index***						
Quadratic	2.96	3	0.99	21.61	< 0.0001	Suggested
Sequential model sum of squares for ***Water Absorption Index***						
Quadratic	0.44	3	0.15	63.22	< 0.0001	Suggested
Sequential model sum of squares for ***Total Color Difference ΔE***						
Quadratic	10.95	3	3.65	10.67	< 0.0001	Suggested
Sequential model sum of squares for ***Expansion Ratio***						
Quadratic	0.32	3	0.11	20.17	< 0.0001	Suggested
Sequential model sum of squares for ***Protein Digestibility***						
Quadratic	2.56	3	0.85	13.10	< 0.0001	Suggested
Sequential model sum of squares for ***Starch Digestibility***						
Quadratic	3.19	3	1.06	15.43	< 0.0001	Suggested

Source	Std. Dev.	R-squared	Adjusted R-squared	Predicted R-squared	PRESS	
Model summary statistics for ***Bulk Density***						
Quadratic	0.26	0.9769	0.9652	0.9354	9.45	Suggested
Model summary statistics for ***Hardness***						
Quadratic	0.06	0.9318	0.8970	0.8090	0.52	Suggested
Model summary statistics for ***Water Solubility Index***						

Quadratic	0.21	0.9856	0.9782	0.9597	6.25	Suggested
*Model summary statistics for **Water Absorption Index***						
Quadratic	0.05	0.9830	0.9744	0.9524	0.32	Suggested
*Model summary statistics for **Total Color Difference ΔE***						
Quadratic	0.58	0.9398	0.9090	0.8312	46.99	Suggested
*Model summary statistics for **Expansion Ratio***						
Quadratic	0.07	0.9702	0.9550	0.9164	0.72	Suggested
*Model summary statistics for **Protein Digestibility***						
Quadratic	0.26	0.9774	0.9658	0.9411	8.32	Suggested
*Model summary statistics for **Starch Digestibility***						
Quadratic	0.26	0.9769	0.9652	0.9354	9.45	Suggested

+ case(s) with leverage of 1.0000: PRESS statistic not defined

Table 3.3: Second order polynomial equations representing the relationship between independent and dependent variables

Bulk Density	$136.40 + 8.85A - 3.50B + 1.85C + 3.77D_1 + 1.84D_2 + 0.11D_3 - 1.76D_4 + 2.40AB + 0.90AC - 0.10AD_1 - 0.47AD_2 - 0.47AD_3 + 0.025AD_4 + 2.60BC + 0.87BD_1 + 0.38BD_2 + 0.13BD_3 - 0.25BD_4 - 0.23CD_1 + 0.15CD_2 - 0.1CD_3 - 0.22CD_4 - 0.35A^2 + 0.45B^2 + 0.95C^2$
Hardness	$1.31 + 0.22A + 0.036B + 0.086C + 0.044D_1 + 0.055D_2 - 0.01D_3 - 0.031D_4 - 0.020AB + 0.045AC + 0.030AD_1 - 0.049AD_2 + 0.013AD_3 + 6.000E-003AD_4 + 0.023BC - 0.029BD_1 + 0.052BD_2 - 7.750E-003BD_3 - 6.500E-003BD_4 + 3.750E-003CD_1 + 6.250E-003CD_2 + 3.750E-003CD_3 - 5.000E-003CD_4 + 0.067A^2 + 0.013B^2 - 0.016C^2$
Water Solubility Index	$13.90 - 1.73A + 0.79B + 0.23C - 0.26D_1 - 0.10D_2 + 0.025D_3 + 0.11D_4 + 0.16AB + 0.075AC - 0.048AD_1 - 0.022AD_2 + 2.500E-003AD_3 + 0.028AD_4 - 0.010BC + 0.042BD_1 + 5.000E-003BD_2 - 7.500E-003BD_3 - 7.500E-003BD_4 + 0.030CD_1 - 7.500E-003CD_2 - 0.020CD_3 + 5.000E-003CD_4 + 0.39A^2 - 0.047B^2 + 0.087C^2$
Water Absorption Index	$2.74 - 0.34A + 0.15B + 0.057C - 0.029D_1 - 0.016D_2 - 0.003067D_3 + 0.015D_4 - 0.11AB - 0.056AC + 0.021AD_1 + 0.010AD_2 - 1.000E-003AD_3 - 0.012AD_4 + 0.0075BC + 0.001BD_1 + 0.001BD_2 + 0.001BD_3 - 0.0015BD_4 - 0.003CD_1 + 0.00325CD_2 + 0.002CD_3 - 0.00175CD_4 + 0.12A^2 - 0.061B^2 - 0.058C^2$
Total Color	$19.34 - 2.16A + 0.29B + 1.11C + 0.40D_1 + 0.20D_2 + 0.011D_3 - 0.18D_4$

Difference ΔE	$+0.33\ AB +0.095AC +0.050AD_1 +0.025AD_2 +0.013AD_3 -0.037AD_4 +0.32BC -0.043\ BD_1 +7.500E\text{-}003BD_2 -5.000E\text{-}003BD_3 +7.500E\text{-}003BD_4 -0.022CD_1 +2.500E\text{-}003CD_2 +2.500E\text{-}003CD_3 +0.015CD_4 -0.58A^2 +0.25B^2 -0.43C^2$
Expansion Ratio	$3.37 -0.41A +0.14B +0.11C -0.040D_1 -0.018D_2 +2.667E\text{-}003D_3 +0.017D_4 +0.063AB +3.500E\text{-}003AC -0.013AD_1 -1.500E\text{-}003AD_2 +3.500E\text{-}003\ AD_3 +4.750E\text{-}003AD_4 +0.018BC +0.022BD_1 +0.012BD_2 +2.000E\text{-}003BD_3 -0.014\ BD_4 +0.016CD_1 +7.000E\text{-}003CD_2 -5.000E\text{-}004CD_3 -5.500E\text{-}003CD_4 -0.060\ A^2 -0.10B^2 -0.076C^2$
Protein Digestibility	$85.00 -0.69A +0.37B +0.30C -2.34D_1 +0.39D_2 +0.51D_3 +0.66D_4 +0.28\ AB +0.11AC -0.29AD_1 +0.075AD_2 +0.038AD_3 +0.063AD_4 -0.12BC -0.19\ BD_1 +0.065BD_2 +0.028BD_3 +0.065BD_4 -0.19CD_1 +0.050CD_2 +0.025\ CD_3 +0.013CD_4 -5.000E\text{-}003A^2 -0.31B^2 -0.22C^2$
Starch Digestibility	$84.12 -0.48A +0.41B +0.42C -2.47D_1 +0.40D_2 +0.54D_3 +0.68D_4 -0.15\ AB +0.035AC +0.062AD_1 -0.025AD_3 -0.025AD_4 +0.040BC -0.095BD_1 -0.045\ BD_2 +0.055BD_3 +0.030BD_4 -0.17CD_1 -0.030CD_2 +0.045CD_3 +0.070CD_4 +0.030A^2 -0.29B^2 -0.31C^2$

A – Moisture Content (%w.b.); B – Screw Speed (rpm); C – Die Temperature; D_1, D_2, D_3, D_4 – Germination time (Days)

Table 3.4: Pareto analysis of variance (ANOVA) analysis and significant statistical parameters of the model

Source	Sum of squares	DF	Mean Square	F-value	p-value
Bulk Density					
Model	4628.14	25	185.13	194.09	< 0.0001
A-moisture content	3132.90	1	3132.90	3284.62	< 0.0001
B-screw speed	490.00	1	490.00	513.73	< 0.0001
C-die temperature	136.90	1	136.90	143.53	< 0.0001
D – germ period	546.21	4	136.55	143.17	< 0.0001
AB	115.20	1	115.20	120.78	< 0.0001
BC	135.20	1	135.20	141.75	< 0.0001
C.V. %	0.71				
Hardness					
Model	2.55	25	0.10	26.77	< 0.0001
A-moisture content	1.85	1	1.85	485.52	< 0.0001
C-die temperature	0.30	1	0.30	77.95	< 0.0001
D – germ period	0.14	4	0.035	9.16	< 0.0001
A^2	0.083	1	0.083	21.87	< 0.0001
C.V. %	4.60				
Water Solubility Index					
Model	152.64	25	6.11	133.85	< 0.0001
A-moisture content	119.37	1	119.37	2616.91	< 0.0001
B-screw speed	25.28	1	25.28	554.23	< 0.0001
C-die temperature	2.16	1	2.16	47.40	< 0.0001

D – germ period	2.16	4	0.54	11.85	< 0.0001
A^2	2.77	1	2.77	60.77	< 0.0001
C.V. %	1.51				
Water Absorption Index					
Model	6.54	25	0.26	113.49	< 0.0001
A-moisture content	4.73	1	4.73	2054.67	< 0.0001
B-screw speed	0.87	1	0.87	379.04	< 0.0001
C-die temperature	0.13	1	0.13	55.92	< 0.0001
AB	0.26	1	0.26	110.85	< 0.0001
AC	0.063	1	0.063	27.23	< 0.0001
A^2	0.27	1	0.27	118.79	< 0.0001
B^2	0.068	1	0.068	29.58	< 0.0001
C^2	0.062	1	0.062	26.73	< 0.0001
C.V. %	1.75				
Total Color Difference ΔE					
Model	261.57	25	10.46	30.58	< 0.0001
A-moisture content	187.06	1	187.06	546.76	< 0.0001
A^2	6.32	1	6.32	18.47	< 0.0001
C.V. %	3.09				
Expansion Ratio					
Model	8.38	25	0.34	63.87	< 0.0001
A-moisture content	6.67	1	6.67	1271.67	< 0.0001
B-screw speed	0.78	1	0.78	147.77	< 0.0001
C-die temperature	0.46	1	0.46	86.84	< 0.0001

B^2	0.19	1	0.19	35.88	< 0.0001
C^2	0.11	1	0.11	20.32	< 0.0001
C.V. %	2.24				
Protein Digestibility					
Model	138.02	25	5.52	84.63	< 0.0001
A-moisture content	18.91	1	18.91	289.81	< 0.0001
B-screw speed	5.55	1	5.55	85.08	< 0.0001
C-die temperature	3.60	1	3.60	55.18	< 0.0001
D – germ period	103.76	4	25.94	397.62	< 0.0001
AB	1.57	1	1.57	24.04	< 0.0001
B^2	1.83	1	1.83	28.08	< 0.0001
C.V. %	0.30				
Starch Digestibility					
Model	142.81	25	5.71	83.00	< 0.0001
A-moisture content	9.03	1	9.03	131.13	< 0.0001
B-screw speed	6.64	1	6.64	96.51	< 0.0001
C-die temperature	6.97	4	6.97	101.31	< 0.0001
D – germ period	115.92	1	28.98	421.07	< 0.0001
AB	0.48	1	0.48	6.98	< 0.0001
B^2	1.61	1	1.61	23.34	< 0.0001
C^2	1.72		1.72	24.95	< 0.0001
C.V. %	0.31				

Table 3.5: Predicted and experimental values of the responses at optimum conditions

Moisture Content (%w.b.) = 15; Die Temperature (°C) = 115; Screw Speed (rpm) = 185; Germination = Day 4

	Optimized values[a] (predicted values)	Experimental values[b]
Bulk density(kg/m^3)	118.8	118.8±0.07
Hardness (N)	1.13	1.13±0.09
Water Solubility Index (%)	16.5	16.5±0.06
Water absorption Index	3.4	3.4±0.07
Total Color Difference ΔE	20.6	20.6±0.12
Expansion Ratio	3.75	3.75±0.05
Protein Digestibility (%)	86.3	86.3±0.04
Starch Digestibility (%)	85.7	85.7±0.09

a Predicted using response surface quadratic model

b Mean ± standard deviation of triplicate determinations from experiments

Figure 3.1: Single-screw laboratory extruder

(Brabender Intelli-Torque Plasti-Corder® South Hackensack, NJ)

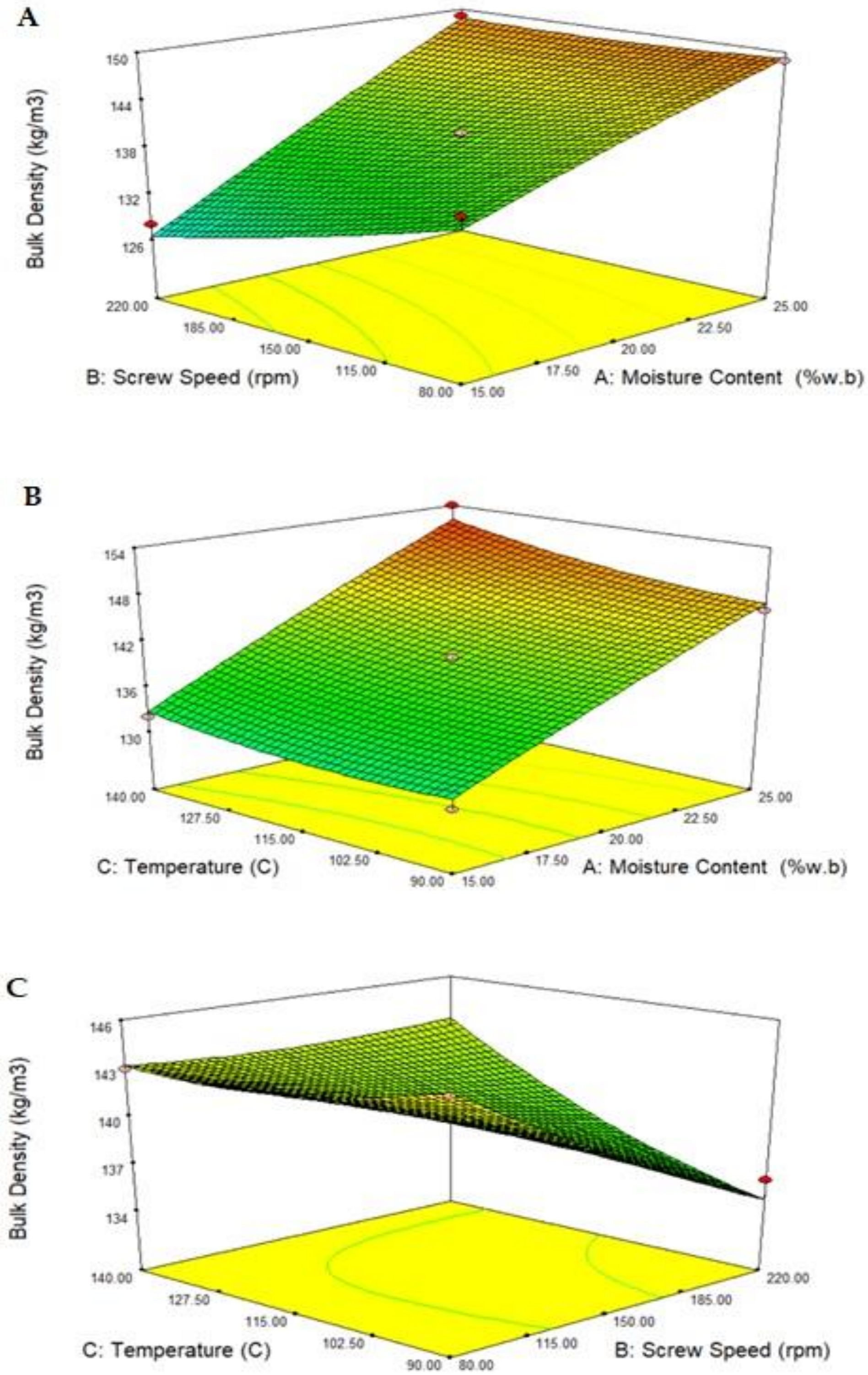


Figure 3.2: Response surface plots (3D) showing the effects on the Bulk Density (A) Moisture Content and Screw Speed (B) Temperature and Moisture Content (C) Temperature and Screw Speed

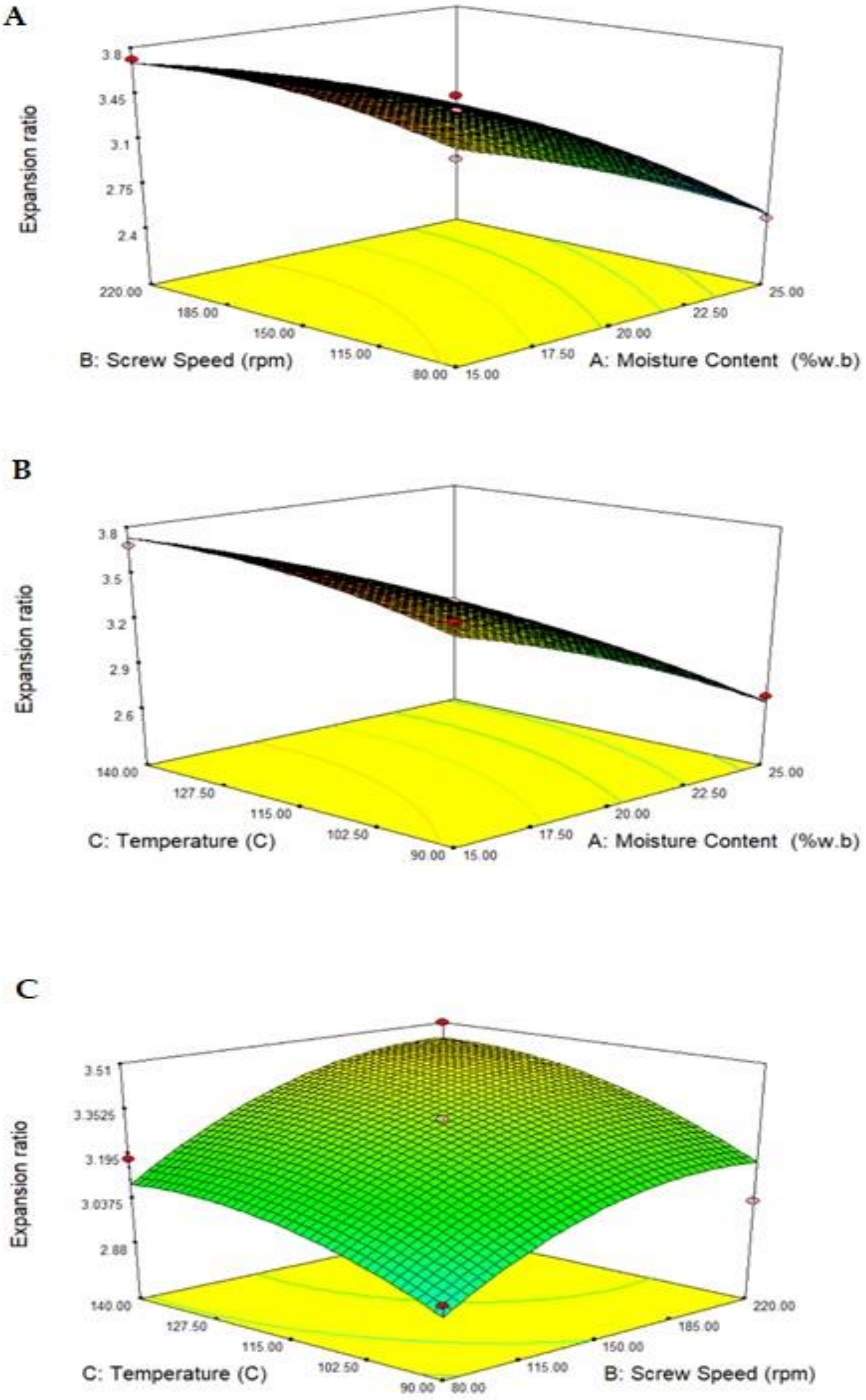


Figure 3.3: Response surface plots (3D) showing the effects on the Expansion Ratio (A) Moisture Content and Screw speed (B) Temperature and Moisture Content (C) Temperature and Screw speed

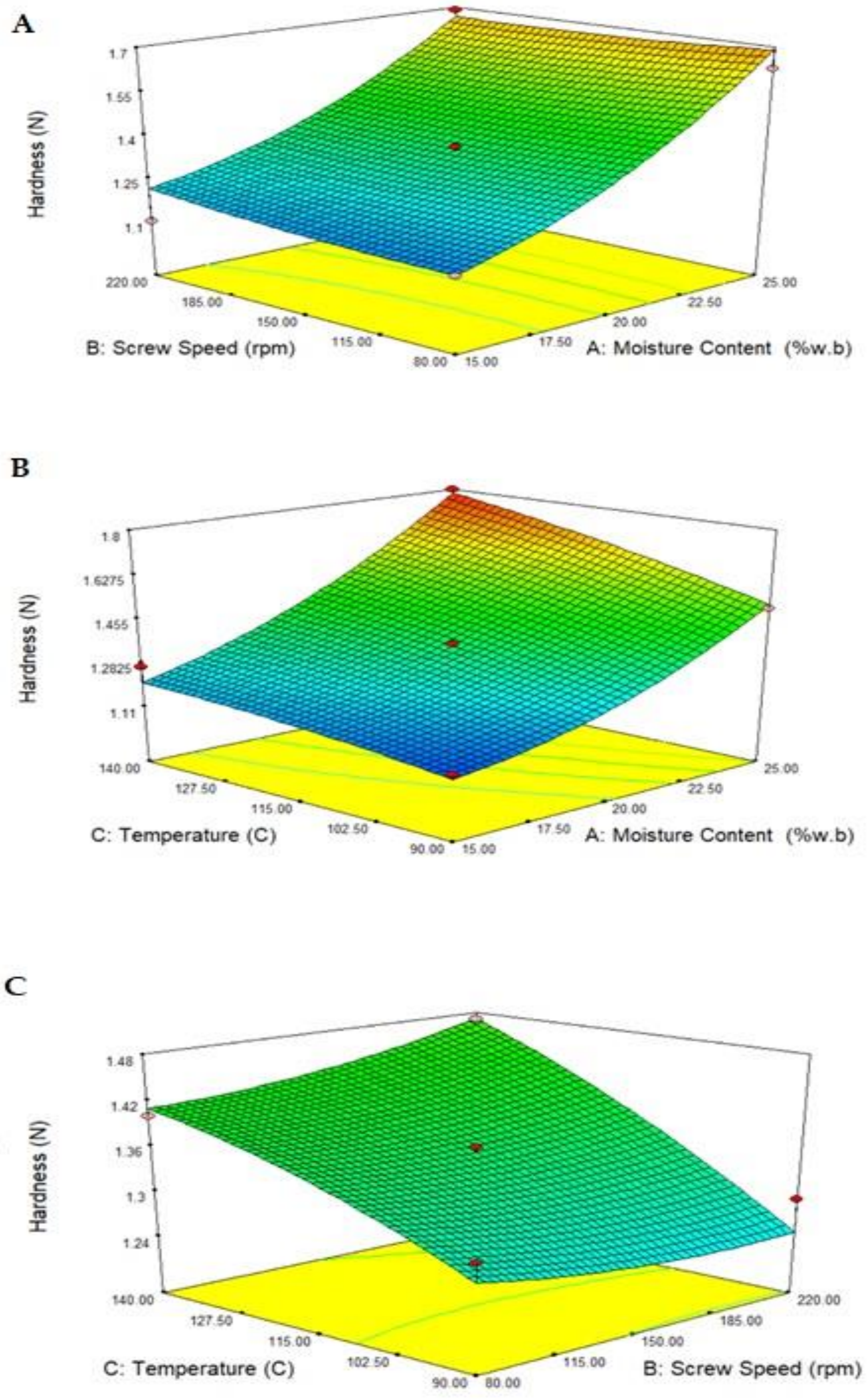


Figure 3.4: Response surface plots (3D) showing the effects on the Hardness (A) Moisture Content and Screw Speed (B) Temperature and Moisture Content (C) Temperature and Screw Speed

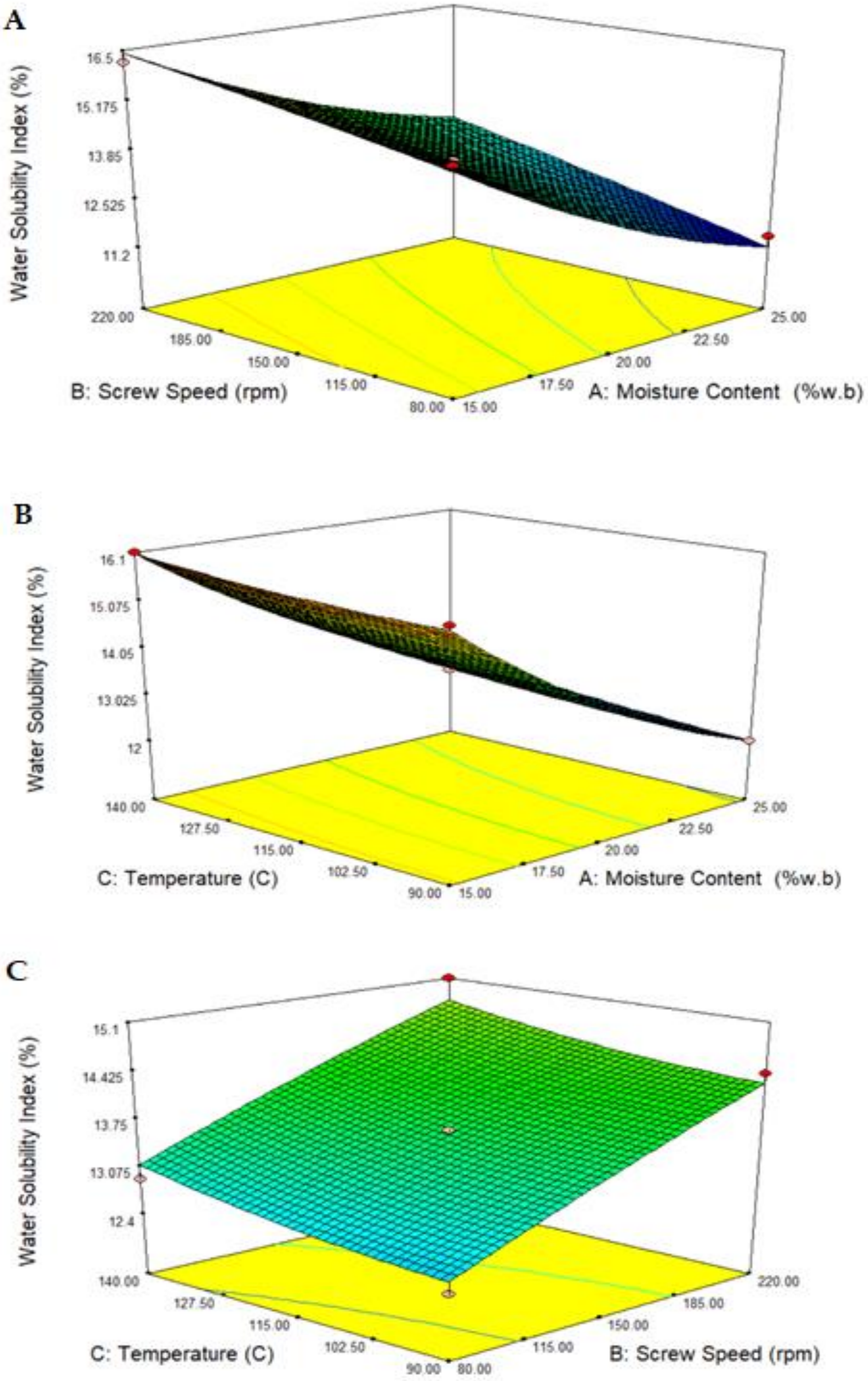


Figure 3.5: Response surface plots (3D) showing the effects on the Water Solubility Index (A) Moisture Content and Screw Speed (B) Temperature and Moisture Content (C) Temperature and Screw Speed

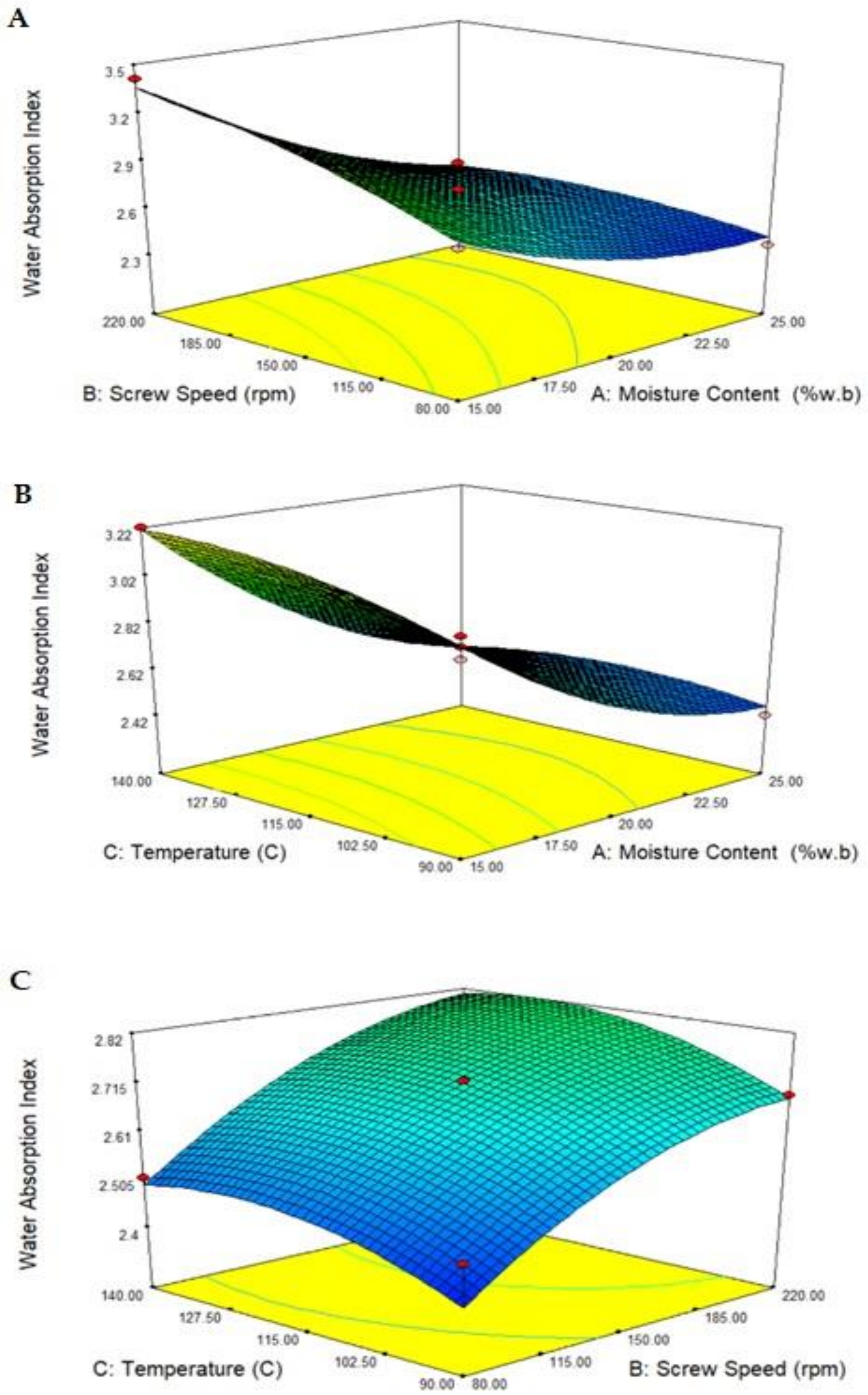


Figure 3.6: Response surface plots (3D) showing the effects on the Water Absorption Index (A) Moisture Content and Screw Speed (B) Temperature and Moisture Content (C) Temperature and Screw Speed

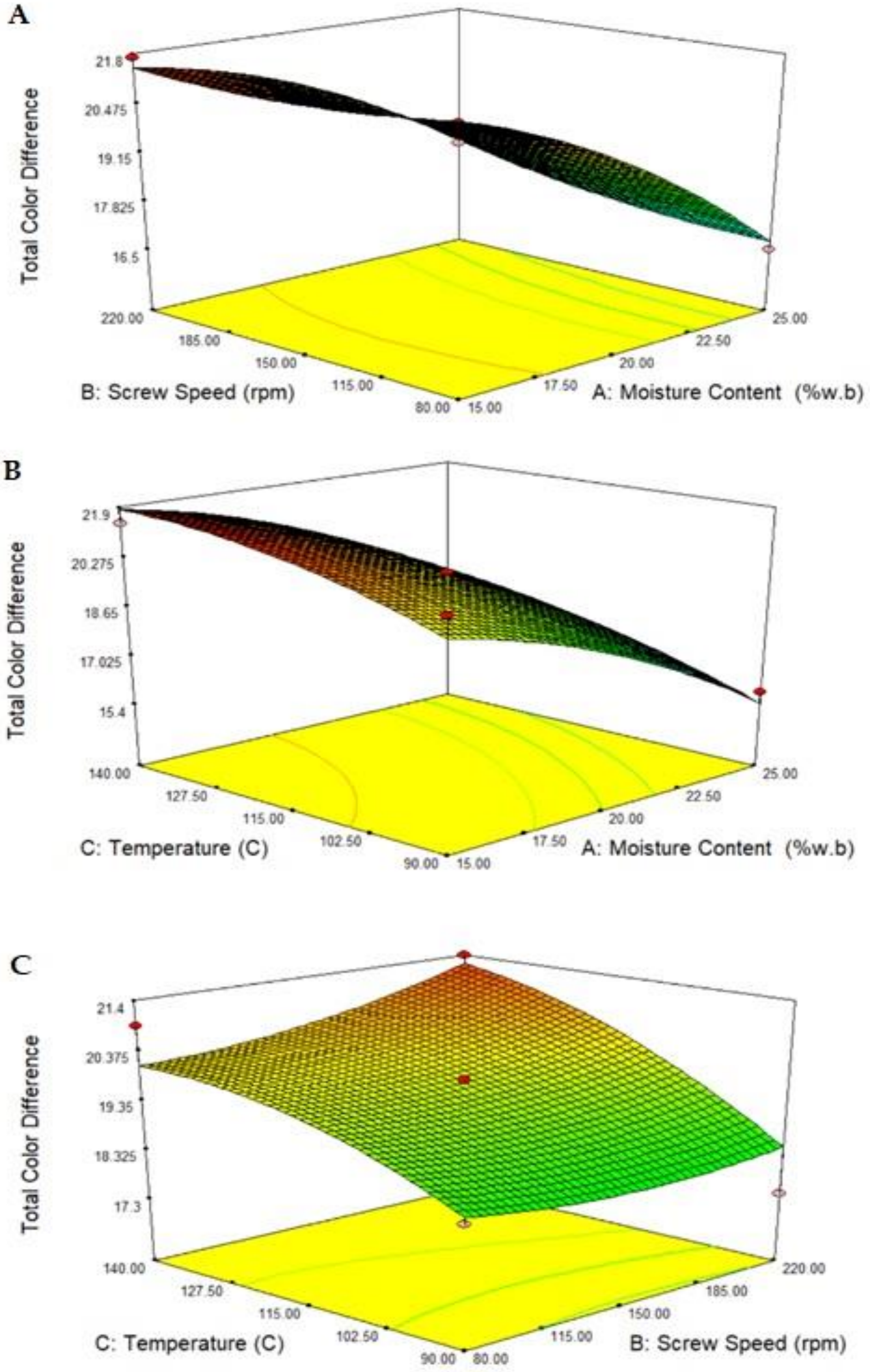


Figure 3.7: Response surface plots (3D) showing the effects on the Total Color Difference (A) Moisture Content and Screw Speed (B) Temperature and Moisture Content (C) Temperature and Screw Speed

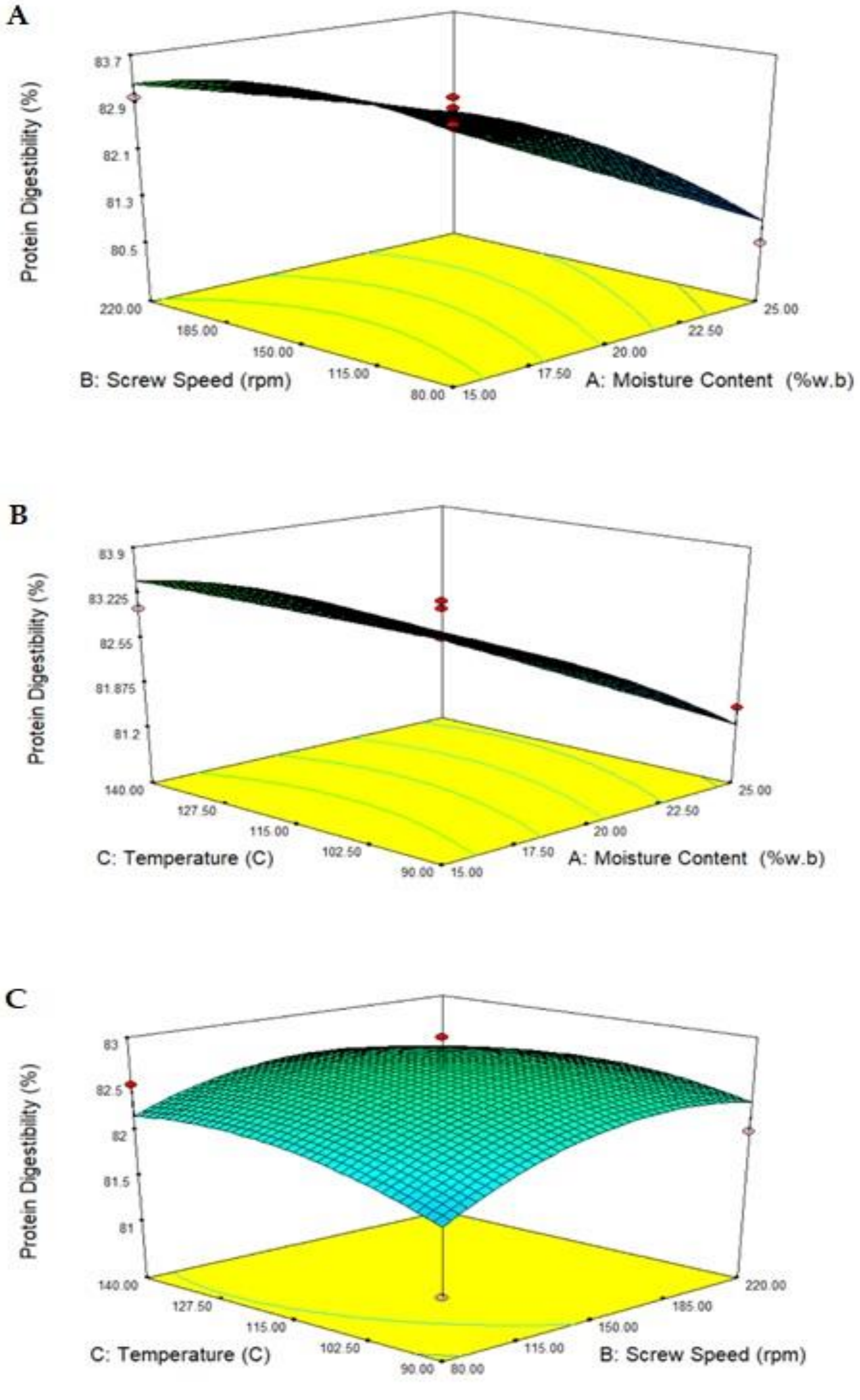


Figure 3.8: Response surface plots (3D) showing the effects on the Protein Digestibility (A) Moisture Content and Screw Speed (B) Temperature and Moisture Content (C) Temperature and Screw Speed

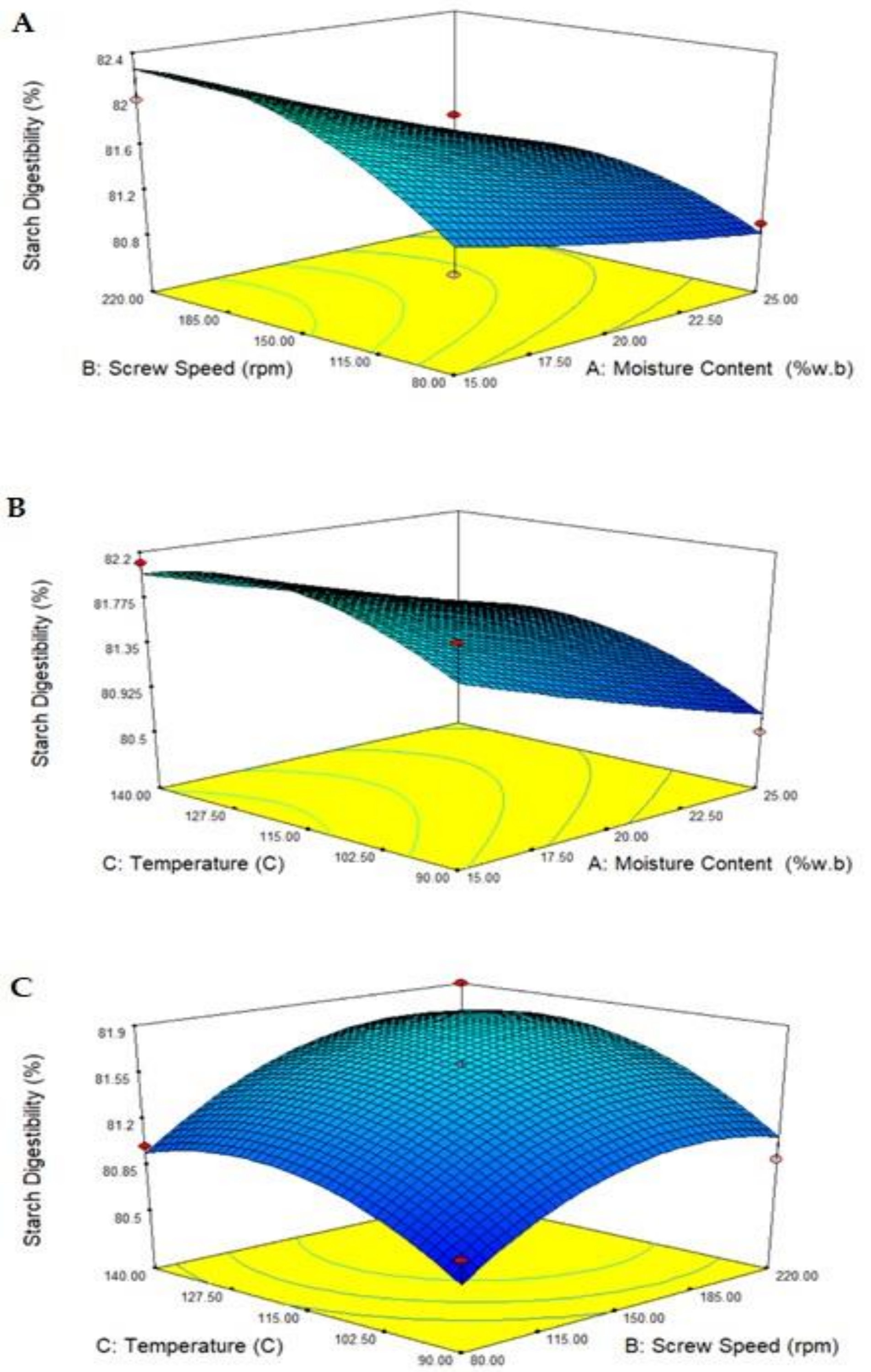


Figure 3.9: Response surface plots (3D) showing the effects on the Starch Digestibility (A) Moisture Content and Screw Speed (B) Temperature and Moisture Content (C) Temperature and Screw Speed

CHAPTER 4: EFFECT OF SINGLE-SCREW EXTRUSION PROCESS PARAMETERS ON THE PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF SPROUTED QUINOA-CORN MEAL EXTRUDATES

Abstract

Single-screw extrusion processing of sprouted quinoa-corn meal blend was studied using a response surface design to understand the influence of feed moisture (15-25% w.b.), die temperature (80-140°C), screw speed (90-220 rpm), corn meal ratio (0-30%) and quinoa germination time (Days 0-4) on the physical and physico-chemical properties of sprouted quinoa- corn meal blend extrudates. The following responses of the extrudates were measured and the values ranged from: bulk density (102-145 kg/m^3), hardness (1.03-1.62 N), water solubility index (12.3-19.9%), water absorption index (2.44-3.79), total color difference ΔE (14.1-21.4), expansion ratio (2.75-3.97), protein digestibility (78.4-85.8%) and starch digestibility (77.5-85.6%). As the germination time increased, the starch and protein digestibility of the extrudates increased however, the addition of corn meal (30%) reduced the starch and protein digestibility. The addition of corn meal (30%) increased the expansion ratio and reduced the total color difference caused by the germination of the sprouted quinoa flour. In short, the addition of corn meal (30%) helped in creating a puffed extruded product with a lighter color, however the starch and protein digestibility were significantly reduced.

Keywords: Quinoa, Corn Meal, Saponin, Gluten-Free, Soaking, Germination, Digestibility

4.1. Introduction

Extrusion processing is a commonly used processing technology in the food industry with a wide number of applications. Starch-based food materials, like corn, rice, and semolina, are ideal candidates for extrusion processing due to their expansion ability (Yu, Ramaswamy, Boye, & Technology, 2012). It is a processing system that utilizes a single screw or a twin screw to force food materials through a die opening. The food ingredients are subjected to high shear, temperature, and pressure for a short period of time (Gopirajah & Muthukumarappan, 2018). A single-screw extruder consists of only one screw housed in the barrel that often has a fluted or grooved design. Additionally, the screw in a single-screw extruder is usually designed with a decreasing pitch to create compression (Guy, 2001). The amount of decreasing pitch is referred to as the compression ratio. The role of the screw is to assist in imparting shear to the ingredient mixture and forcing the dough of the mixed ingredients out from the extruder through the die (Muthukumarappan & Karunanithy, 2012). The screws are also responsible for the build-up of pressure that occurs at the end of the extruder as well as added mixing of the ingredients. This helps to transform the ingredients from solid powders to a melt state inside of the extruder A twin-screw extruder has a pair of screws that are either intermeshing or non-intermeshing. The set of screws in the twin-screw extruder can be either co-rotating or counter-rotating. For any new material extrusion, a single screw extrusion is preferred because of the relatively low cost, straightforward design, ruggedness and favorable performance/cost ratio (Akdogan, 1999; Guy, 2001) The melted ingredients are then forced through a die at the end of the extruder into the atmosphere. The melt coming out of the extruder encounters a sudden drop in pressure, resulting in rapid expansion as well as a decrease in temperature, helping it to transform into a cooked and expanded product. The final quality and texture of the extruded product depends on various factors, including the ingredient mixture and its properties, extrusion processing conditions, and post-processing conditions (Harper & Clark, 1979).

Because of the great flexibility of extrusion processing, it has found very diverse applications in the food industry.

Quinoa is a pseudo cereal and has been cultivated in the Andean region. It has outstanding protein quality and a wide range of minerals and vitamins, which makes it popular for consumption. In addition, it is rich in amino acids like lysine and methionine that are deficient in cereals. Quinoa is an excellent source of fiber, iron, calcium, magnesium, and B vitamins (Navruz-Varli & Sanlier, 2016). Because of their nutritive properties, edible seeds other than the gluten-containing cereal grains and pseudo-cereals are viable candidates to formulate gluten-free diets. One of the factors which limit the widespread utilization of quinoa is its bitter taste caused by the presence of saponins. Saponins are triterpene glucosides that consist of a linear arrangement of one to six hexose or pentose glycoside units joined to the sapogenin aglycone, which can be a steroidal or a triterpenoid aglycone (Kuljanabhagavad, Thongphasuk, Chamulitrat, & Wink, 2008). Quinoa saponins are soluble in methanol or water. They produce stable foams in aqueous solutions. They may form chemical complexes with iron and reduce its absorption. Saponins are located on the outer layers of the seeds and can be removed by polishing and washing with water (Estrada, Redmond, & Laarveld, 1997). Similarly, phytic acid poses an issue with cereals and pseudo-cereals. Phytic acid (myoinositol hexaphosphoric acid) reduces the availability of many minerals like iron, zinc, calcium and magnesium (Cheryan & Rackis, 1980). The formation of iron-phytate complexes of low solubility, in the small intestine is the basis for the interference of phytate with iron absorption. Although the iron content in cereals is usually high, the iron absorption from them is often poor due to the presence of high amounts of phytates. They are also found to inhibit the proteases and amylases of the intestinal tract.

Sprouted seeds are nutritionally superior to their respective seeds Germinated grains have higher levels of nutrients, lower levels of anti-nutrients such as phytic acid and flatulence-causing sugars, increased protein and starch digestibility, and increased bioavailability of some minerals (Carciochi et al., 2014). The inclusion of sprouted grains in the formulation of whole-grain-rich, fiber-rich, and gluten-free baked goods may provide a natural fortification and enrichment mechanism; it may improve nutritional quality because of the increased digestibility and bioavailability of some nutrients and the reduction of some anti-nutrients. Comprehensive reviews of the effects of germination on the nutrient composition of cereals and pseudocereals have been published (Carciochi et al., 2014; Lombardi-Boccia et al., 1991; Lorenz & D'Appolonia, 1980).

Extrusion can play a major role by either converting it to an expanded snack or products such as noodles and pasta. Extrusion is a high-temperature and short-time process. During extrusion, moistened starch/protein-based food materials are cooked in a barrel. A combination of moisture, pressure, temperature and mechanical shear in the barrel results in molecular transformation and chemical reactions (Muthukumarappan & Swamy, 2017). Since quinoa high protein, quinoa is more suited to produce noodles or paste. However, it may be blended with starch rich grains to produce a puffed cereal. Wheat and corn meal flours are the most blended materials for food extrusion. Other materials like rice flour, soy, potato, rye, barley, oats, sorghum, cassava, tapioca, buckwheat, pea flour and other related materials can also be used.

Maize or corn is the third most important crop next to rice and wheat worldwide and commonly has been used as a base ingredient in the snack industry. In general, there are several uses of corn in food as well as feed industry such as the production of flour, corn meal, grits, sweeteners, starches, alcoholic beverages, cooking oil, tortilla, snacks, breakfast foods and other products. Dry-milled corn meal is the most common primary ingredient used in corn-based extruded snacks. Because of its composition, ratio of vitreous to floury endosperm, and particle size, under optimal extruding conditions corn meal makes for a light, highly expanded, crunchy and soft product. The basic recipe may vary widely; however, the main ingredient components which affect the characteristics of the final product are starch, water, protein, fiber, oil, additives and particle size. Corn has been blended with various cereals and legumes such as soybean (Yu et al., 2012), milk protein (Peri, Barbieri, Casiraghi, & Technology, 1983), teff and soy (Forsido, Ramaswamy, & Technology, 2011), and pea (Sobota & Rzedzicki, 2009) to create puffed snacks. The objective of this research is to understand the impact of the single-screw extrusion variables such as moisture content of feed, die temperature, screw speed of extruder and germination time on the physical and physico-chemical properties of extrudates made from the sprouted quinoa flour-corn meal blend.

4.2 Materials and Methods

4.2.1 Raw materials

Quinoa seeds (white) and corn meal were obtained from a national grain processor (Bob's Red Mill Natural Foods, Milwaukie, OR). For the proximate composition analysis of quinoa and corn meal, the following AACC methods were used: Moisture -Oven drying at 135°C (method no. 44–19), Ash - calcination at 550°C (method no. 08–01), Lipids - defatting in a Soxhlet apparatus with petroleum ether (method no. 30–25), Protein - micro Kjeldahl (N 6.25) (method no. 46–13), Carbohydrate – sugars and starch (method no. 80–04, 76-13) and total fiber - (method no. 32–07). Saponin content and phytic acid content (hexaphosphate equivalents) were quantified using the method described by (Rathod & Annapure, 2017). All chemicals were purchased from Sigma–Aldrich, USA. All chemicals and reagents used were of analytical grade. All experiments were performed in triplicates.

4.2.2 Soaking and Germination of quinoa

The quinoa (11 pounds) was soaked overnight (8 pm- 8 am) for 12 h in tap water (1:5 w/v) at room temperature (~25°C). The soaked samples were then drained and washed. Excess water was drained, and the samples were wrapped in a moist cloth for 5 days (0 h – Day 0, 24 h - Day 1, 48 h - Day 2, 72 h – Day 3, 96 h – Day 4) at room temperature. All experiments were performed in triplicates.

4.2.3 Extrusion processing

The sprouted quinoa-corn meal blend, each weighing around 200 g were randomly extruded using a 19.18 mm (0.755 in.) barrel inner diameter (i.d.), single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder® South Hackensack, NJ) as shown in Fig 4.1. It was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 200 rpm. The extruder had a barrel with length (L) to diameter (D) ratio of 20:1. A uniform 19.05 mm pitch screw having 381 mm (15.0 in.) screw length, a 19.05 mm (0.75 in.) constant outside (top of flight) diameter, a 3.81 mm (0.15 in.) initial screw feed depth, an 11.43 mm (0.45 in.) initial screw root diameter, and a screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiments. A die nozzle with 3 mm diameter was used. The extrudates were cooled to room temperature and sealed in polyethylene bags and stored at room temperature until measurements were taken.

4.2.4 Measurement of extrudate properties

4.2.4.1 Bulk Density

The bulk density (kg/m^3) was calculated as the ratio of the mass M (kg) to the volume V (m^3) of the measured and weighed extrudate sample. It was assumed that extrudates were of cylindrical shape. The bulk density of all extrudates were measured and the measurements were done in triplicate.

$$Bulk\ Density = \frac{M}{V} \tag{4.1}$$

4.2.4.2 Expansion ratio (ER)

Expansion ratio was calculated by measuring the mean diameter of 10 randomly chosen locations on the extrudates with calipers for each extrusion process condition. Further it was divided by the die diameter of 3 mm.

$$Expansion\ Ratio = \frac{Mean\ diameter\ of\ the\ extrudate\ (mm)}{Die\ diameter\ (mm)} \tag{4.2}$$

4.2.4.3 Hardness

Hardness is defined as the maximum force per unit area of the sprouted quinoa-corn meal extrudate that is necessary to breakdown the extrudate into two pieces. Hardness was tested by breaking the extrudate using a cylindrical probe (TA-4, Texture Technologies Corp, NY) attached to the crosshead of texture analyser. The sample was positioned in its natural position. A vertical force was applied at a speed of 50 mm/min. The force applied vs deformation was recorded and the maximum force recorded in the curve was considered as the hardness. The hardness of all extrudates were measured and the measurements were done in triplicate.

4.2.4.4 Water Solubility Index and Water Absorption Index

The water absorption index and water solubility index of all extrudates were measured and the measurements were done in triplicate. The quinoa-corn meal extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was mixed in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was mixed occasionally and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminium cup and dried at 135°C for 2h (AACC, 2000). The weight of the gel in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by:

$$Water\ Solubility\ Index\ (\%) = \frac{W_{ss}}{W_{ds}} * 100 \tag{4.3}$$

$$Water\ Absorption\ Index = \frac{W_g}{W_{ds}} \tag{4.4}$$

where, W_{ss} is the weight of dry solids of supernatant (g), W_{ds} is the weight of dry sample (g), and W_g is the weight of gel (g)

4.2.4.5 Color

The color of quinoa-corn meal extrudate samples was measured using colorimeter (LabScan XE, HunterLab, Reston, VA) in triplicate in terms of Hunter Lab values (L^*, a^*, b^*). The Hunter Lab value 'L^*' signifies lightness with 0 for dark and 100 for bright. The value 'a^*' measures the degree of green color in the range from -100 to 0 and red in the range 0 to 100. The 'b^*' value represents blue color in the range from -100 to 0 and yellow in the range from 0 to 100.The total color change (ΔE) is calculated as

$$Total\ Color\ Difference\ \Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \tag{4.5}$$

where $\Delta L = L^* - L_0$, $\Delta a = a^* - a_0$, and $\Delta b = b^* - b^*_0$; the subscript ‘‘0’’ represents initial color values before processing. All readings were taken in triplicate.

4.2.4.6 Starch and Protein Digestibility

The starch digestibility and protein digestibility of all extrudates were measured and the measurements were done in triplicate. The quinoa-corn meal extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The in-vitro starch digestibility was determined in the extrudate powder (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20 °C for 2 h according to the method of (Rathod & Annapure, 2017). At the end of the incubation period, 2 ml of 3,5-dini-trosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard.

A system consisting, 100 mg (0.1 g) dry and defatted samples were incubated at 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 °C for 3 h. Further it was neutralized with 7.5 ml 0.2N NaOH. Then 4 mg pancreatin in 7.5 ml phosphate buffer (pH 8) was added and incubated for 37 °C for 24 h, further 5 mg of TCA was added, centrifugated at 5000 rpm for 10 min. After that the supernatant dry residue at 40–50 °C was discarded and the micro Kjeldahl process for protein estimation was carried out. The percent in vitro protein digestibility was calculated using the following equation (Rathod & Annapure, 2017):

$$Protein\ digestibility\ (\%) = \frac{Total\ protein - Residual\ protein}{Total\ protein} * 100 \tag{4.6}$$

4.2.5 Experimental Design and Statistical Analysis

Experiments were conducted using the Box Benhken design (BBD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA), consisting four factors namely moisture content (X_1), temperature (X_2), screw speed (X_3), corn meal ratio (X_4) and germination time (X_5). Three replicates were taken at the design center and the total number of observations were 135. Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN). The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA). The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

4.3 Results and Discussion

4.3.1 Effect of Soaking and Germination on the quinoa flour

The nutritional properties of the sprouted quinoa-corn meal blend are shown in Table 4.1. The addition of corn meal increased the protein content of the blend. In general, protein content of quinoa seeds ranges between 13.1% and 14.0% (James, 2009). The total protein content of quinoa is higher than that of grains such as rice, barley, corn, rye, sorghum and wheat (Navruz-Varli & Sanlier, 2016). The chief carbohydrate constituent of quinoa and corn meal is It ranges from 62-64% in quinoa and 68-74% in corn meal. The total fiber was close to other grain products (7.0%-9.7%). Quinoa contains sugar by about 3%. It mostly contains maltose, D-galactose, and D-ribose in addition to low levels of fructose and glucose (Navruz-Varli & Sanlier, 2016). Corn meal is nutritious, providing fiber, which aids in digestion, plus folate, thiamin, phosphorus, vitamin C, and magnesium.

Saponins are the principal anti-nutritional factor present in the seed coat of quinoa. Most saponins are nitrogen-free glycosides, each consisting of a sapogenin and a sugar. Saponins affect quinoa's taste and digestibility, therefore should be removed before consumption (Ruales & Nair, 1992). Traditionally, saponin has been removed by laboriously hand scrubbing the quinoa with alkaline water. The saponin content is checked by placing the grain in a tube, adding water and vigorously shaking for 30 s. If no foaming occurs, all saponins are assumed to have been removed. The saponin content was significantly reduced by soaking and germination as shown in Table 4.1. Additionally, saponin content is expected to be reduced through extrusion (Anuonye et al., 2010).

The phytic acid concentration in quinoa seeds is comparable to whole grain rye flour (7.7 mg/g), whole grain wheat flour (8.7 mg/g), lentils (8.4 mg/g) and fava beans (8.0 mg/g). Phytic acid is not only present in the outer layers of quinoa, as in the case of rye and wheat but is also evenly distributed within the endosperm (Ahamed et al., 1998). Phytic acid binds minerals, such as Fe, Zn, Ca and Mg, and can make the mineral content of a cereal inadequate, especially for children (Khattak et al., 2007). Soaking and sprouting processes reduce the 'binding' effect of the phytic acid by activating the enzyme phytase (James, 2009). The amounts of phytic acid of the quinoa seeds obtained in this study are in concordance with the values for Cherry vanilla quinoa (Kowalski et al., 2016). A significant reduction in phytic acid content is observed with increase in germination time. Day4 germinated quinoa samples had more than 50% reduction in phytic acid. Extrusion further decreased the phytic acid content of pea flour (Alonso et al., 1998), bean flour (Anton et al., 2009) and other legumes (Lombardi-Boccia et al., 1991).

4.3.2 Effect of independent variables on the single screw extrusion process

The results of the single screw extrusion process along with the experimental design is presented in Appendix B.

4.3.2.1 Bulk Density

The bulk density of the extrudates varied between 102 and 145 kg/m^3. Low bulk density of 102 kg/m^3, which is a required characteristic of the extruded product, was achieved with low feed moisture content (15%), average temperature (115°C) and high screw speed combination (220 rpm). The addition of corn meal (15-30%) significantly reduced the bulk density creating a less dense and high expansion ratio. Fig 4.2 shows that feed moisture content had a significant effect on bulk density followed by screw speed, whereas temperature seemed to have a minor effect. Similar results were reported in oat-corn meal extrudates (Liu, Hsieh, Heymann, & Huff, 2000) and soy-corn meal extrudates (Yu et al., 2012).

4.3.2.2 Expansion ratio

The measured expansion ratio of quinoa-corn meal extrudates ranged from 2.75 to 3.97. The expansion ratio was significantly affected by the higher starch content of corn meal. As the corn meal content was increased from 15% to 30%, the expansion ratio increased from 3.11-3.57 to 3.30 – 3.97. In addition, temperature and feed moisture content played a significant role in increasing the expansion ratio. High expansion ratio at low feed moisture content for expanded products is distinctive for cereals. However, Fig 4.3 shows that the temperature and corn meal ratio have a significant effect on the expansion ratio of the extrudates. Similar trends have been obtained for corn (Diaz et al., 2013), wheat (Pitts et al., 2014) and oats (Gopirajah & Muthukumarappan, 2018).

4.3.2.3 Hardness

Hardness, the maximum force to break the sample, ranged between 1.03 and 1.62 N. The coefficient of determination for hardness was high ($R^2 = 0.8$) and the corn meal ratio, moisture, temperature and germination period were found to have significant contributions to the hardness of the extrudates. At 30% corn meal ratio, the hardness of the extrudate was 1.03 N whereas the control sample (only quinoa and no corn meal) had a hardness of 1.62 N. Chemical changes occurring during extrusion cooking affect the development of textural and mechanical properties such as hardness in extrudates. The elastic swell effect and bubble growth effect equally contribute to the structural changes of starch during extrusion. Water performs as a plasticizer to the starch-based material decreasing its viscosity and the mechanical energy dissipation in the extruder. Hence the product becomes dense and bubble growth is compressed. The reduced starch conversion and compressed bubble growth result in a dense product and wetness of extrudate. At all feed moisture content levels, decrease in die temperature decreased the product hardness. Screw speed did not have a significant effect on the extrudate hardness. Fig 4.4 shows the effect of moisture and screw speed on the product hardness. Previous studies have also reported similar trends for extrudates based on rice (Ding et al., 2006), wheat (Ding et al., 2006), oat bran and soy flour (Anuonye et al., 2010) and chickpea (Khattak et al., 2007). The hardness was high of 1.8 N at about 140°C, 150 rpm screw speed, 25% feed moisture content, 0% corn meal ratio and Day 0 sprouted samples.

4.3.2.4 Water Solubility Index and Water Absorption Index

Water solubility index is related to the quantity of soluble solids, which is related to dextrinization. The coefficient of determination for water solubility index was highly significant as revealed by a high value of R^2 (0.9). Higher content of corn meal in the blend increased the water solubility of the extrudates. The linear effects of feed moisture, screw speed and temperature had significant effect on water solubility index. In general, water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy favors starch dextrinization. The poor correlation between water solubility index and most of the process and product responses is explained by the fact that water solubility index includes the opposing effects of starch dextrinization and the molecular level interactions between degraded components, which may not be favoured at the same condition (Lei et al., 2008). An increase in the amount of dextrinized starch during extrusion cooking results in an increase in water solubility index. However, molecular interactions between degraded starch, protein, and lipid components, which in turn lead to an increase in molecular weight, may decrease the solubility, thus water solubility index (Camire et al., 1990). Fig 4.5 shows the significant effect of corn meal ratio, temperature and screw speed on water solubility index. The increase in water solubility index with increasing temperature was consistent with the results reported for oat extrudates (Gutkoski & El-Dash, 1999). The water solubility index decreased with the increase in moisture. Similar trends have been reported earlier for starch, maize grits, wheat and pea (Ding et al., 2006; Singh et al., 2007)

Water absorption index depends on the availability of hydrophilic groups and on the gel formation capacity of the macromolecules. It is a measure of damaged starch together with protein denaturation and new macromolecular complex formations. Water absorption index of extrudates ranged between 2.44 and 3.79. The increased corn meal ratio in the blend significantly increased the water absorption ratio. This is an expected result since it includes the effect of starch gelatinization, protein denaturation and molecular level cross-linking reactions which are always favoured at the same conditions (Doğan & Karwe, 2003) The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. The interaction effects except for temperature-screw speed were significant as shown in Fig 4.6. Similar responses were observed in rice flour (Hagenimana et al., 2006), oats (Gutkoski & El-Dash, 1999), finger millet (Sawant et al., 2012) and cassava starch (Hashimoto et al., 2003). Water absorption index had poor correlations with almost all process and product responses.

4.3.2.5 Color

Color is an important quality parameter since it reflects the extent of chemical reactions and degree of cooking or degradation that take place during extrusion cooking. Higher color difference ΔE means darker products with more intense yellow and red color. The total color change in extruded products ranged between 14.1 and 21.4. Germination increased the color of the quinoa flour by breaking down the reducing sugars. The addition of corn meal, which is typically a yellow colored meal masked the effects of germination on color. Hence lighter colored products were obtained when 30% corn meal was added. Linear effects of temperature and moisture content were found to have the highest contribution to total color change. As depicted in Fig 4.7, low feed moisture content and high temperature increased the total color change possibly due to increased extent of browning reactions under this condition. Although, the screw speed was not a significant parameter, at low screw speeds a slight increase in color change observed due to longer residence times which might increase the extent of chemical reactions.

4.3.2.6 Starch Digestibility (SD) and Protein Digestibility (PD)

The results show that both protein digestibility and starch digestibility of corn meal-free extrudates were increased significantly by soaking, germination and extrusion. Fig 4.8 and Fig 4.9 illustrate the effect of the independent variables on the protein and starch digestibility respectively. The addition of corn meal decreased the starch and protein digestibility. This is attributed to the reason that the complex starch and protein were not broken down into simpler substances. However, extrusion was the reason which partially increased the digestibility by breaking down the starch and protein. This can be related to the higher efficiency of this thermal treatment in reduction of anti-nutrient activities in addition to soaking and germination. The linear effects of temperature and interaction effect of moisture and screw speed had a significant effect on the protein and starch digestibility. Enhancement of starch digestibility during soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility (Linko et al., 1983). The rupture of starch granules of sprouted quinoa, making the substrate more accessible, facilitated the amylolysis. On the other hand, reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility.

4.3.3 Box-Behnken design and analysis

This response surface methodology is an empirical modeling technique used to evaluate the relationship between the experimental and the predicted results (Muthukumarappan & Swamy, 2018). In the present study, the Box Behnken Design was used to obtain a proper model for the optimization of the extrusion process variables. Experiments were carried out according to the experimental design in order to explore the optimum conditions and study the influence of process variables on the physical and physico-chemical properties of the extrudates. The predicted values were attained by the model fitting technique in Design Expert software version 8.0.7.1 and the values correlated well with the experimental values. Fitting of the data to various models such as the linear, interactive, quadratic and cubic models was performed to acquire the regression equations. Sequential model sum of squares and model summary statistics tests were carried out to decide the adequacy of models in the present study and the results are presented in Table 4.2. From Table 4.2, it is observed that the quadratic model is highly significant for the extrusion of the blend. The model summary statistics demonstrated that the quadratic model had maximum R- Squared, Adjusted R-Squared and Predicted R-Squared values. The cubic model was not suggested for the extrusion process since the BBD matrix had enough data to understand the outcome of the system. Hence, quadratic model was chosen for further analysis of data.

4.3.4 Fitting of polynomial equations and statistical analysis

Based on the Box-Behnken experimental design model, the empirical relationship between the input variables and experimental results obtained were expressed by a second-order polynomial equation with interaction terms (Swamy et al., 2014). The equations generated in coded factors are presented in Table 4.3.

The investigation and optimization of a fitted response surface might generate poor or disingenuous results. Therefore, it was essential to ensure the fitness of the model. Regression analysis and Pareto analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. The results pointed out that the equation sufficiently presented the actual relationship between the input parameters and responses. ANOVA is a statistical technique that subdivides the total variation in a dataset into component parts allied with sources of variation for testing hypotheses on the variables of the model (Swamy & Muthukumarappan, 2017).

ANOVA followed by Fisher's statistical test (F-test) was conducted to analyse the significance of each independent variable. The F-value is the ratio of the regression mean square and the real error mean. It indicates the influence of each controlled factor on the tested model. The ANOVA results in Table 4.4 shows the F-value for the responses, implying that the model is highly significant. The large Fisher value signifies that the variation in the responses can be explicated by the regression equation (Asokapandian et al., 2016). The associated p value estimates if F value is large enough to show statistical significance. The p values that are lower than 0.0001 indicate that the model and the associated terms are statistically significant.

Coefficient of determination (R^2) and adjusted-R^2 were computed to verify the sufficiency and fitness of the model. The R^2 signifies the proportion of the total variation in the response expected by the model, thus indicating the ratio of the regression sum of squares (SSR) and total sum of squares (SST) (Sangamithra et al., 2015). The values of R^2 imply that 95% of experimental data was well-suited. The high R^2 coefficient gives a satisfactory adjustment of the quadratic model to the experimental data. The purpose of the adjusted-R^2 is to analyse the model adequacy and fitness. The adjusted-R^2 value adjusts the R^2 value for the sample size and for the number of terms in the model (Albarracín et al., 2015). The high value of adjusted-R^2 supports a high correlation between the experimental and the predicted values. The high coefficient of determination and very small p-value (<0.0001) demonstrate that the quadratic polynomial model is significant and adequate to characterize the actual relationship between the response and input.

The coefficient of variation (CV) shows the scattering of the experimental points from the predicted values of the second order polynomial model (Swamy & Muthukumarappan, 2017). A high coefficient of variation points out that there is extreme variation in the mean value and does not adequately develop a sufficient model. The very low coefficient of variation value undoubtedly represents a high degree of precision and reliability of experiments conducted.

4.3.5 Selection of optimum conditions

Optimum conditions for the extrusion process were deduced to get desired extrudate properties. Second order polynomial models attained in the study were used for each response to acquire specified optimum conditions. To optimize the independent variables, the Derringer’s desirability function method was utilized. After satisfying the necessities for each response in the design, the Derringer’s desirability function seeks for a combination of factor levels that together optimize a set of responses. By the conversion of each into a dimensionless desirability scale which defines a partial desirability function, combining the discrete desirability values to attain the global desirability function and then maximizing the global function and recognizing the optimal factor settings, the optimization process is accomplished. The desirability function scale operates between 0 and 1 with 0 signifying a completely undesirable response and 1 representing a fully desired response. The individual desirability for each response is achieved by stating the goals, i.e., minimize, maximize or target the response, and boundaries essential for each response. A weight factor (defines the shape of the desirability function for each response) is then allocated. The weights range between 0.1 and 10 with the larger ones relating to more significant responses.

A weight factor of 1 was selected for the individual desirability in this research. The “importance” of the goals can be arranged sequentially according to increasing importance. It can vary from 1 (least importance) to 5 (most important). To optimize the process the following parameters (1) Moisture (15-25% w.b.) (2) Temperature (90-140°C) (3) Screw speed (80-220 rpm) (4) Corn meal ratio (0-30%) and (5) Germination period (Day 0- Day 4) respectively, were set for maximum desirability. By applying the methodology of the desired function, the optimized process variables were obtained. It indicated that a moisture 15%, temperature 115°C, screw speed 185 rpm and Day 4 germinated flour input will produce an extrudate with bulk density 110.5 kg/m^3, hardness 1.07 N, water solubility index 18.2, water absorption index 3.5, total color difference ΔE=17.6, expansion ratio=3.78, protein digestibility=84.7% and starch digestibility=85.6% respectively with overall desirability value of 0.729.

4.3.6 Verification of optimized conditions and predictive model

The fitness of the model equations for predicting optimum response values was investigated under the conditions: moisture 15%, temperature 140°C, screw speed 219 rpm, corn meal ratio 6% and Day 4 germinated quinoa flour. These conditions were optimum by the RSM optimization approach. To verify the validity of the optimized conditions, experiments were performed to evaluate the experimental results vs predicted values of the output using the model equation. The experiments were carried out thrice and the average values were presented in Table 4.5. The results expected from the optimum solution was with bulk density 110.5 kg/m^3, hardness 1.07 N, water solubility index 18.2, water absorption index 3.5, total color difference ΔE=17.6, expansion ratio=3.78, protein digestibility=84.7% and starch digestibility=85.6% and the experimental value was bulk density=110.5±0.07 kg/m^3, hardness=1.07±0.09N, water solubility index=18.2±0.06, water absorption index=3.5±0.07, total color difference ΔE=17.62±0.12, expansion ratio=3.78±0.05, protein digestibility=84.7±0.04 and starch digestibility=85.6±0.09. The mean values obtained through the experiments were compared with the predicted values. The values obtained through confirmation experiments are within 95% of predicted values. This shows that the developed quadratic models are well-suited. It is also evident that the optimal values are valid within the specified range of process variables.

4.4 Conclusions

In this study, the effect of corn meal addition to the sprouted quinoa flour was studied. The influence of feed moisture (15-25% w.b.), temperature (80-140°C), screw speed (90-220 rpm), corn meal ratio (0-30%) and germination time (Days 0-4) on the physical and physico-chemical properties of sprouted quinoa-corn meal extrudates were studied. The levels of corn meal ratio significantly improved the color and expansion ratio of the extrudates. The total color change in extruded products ranged between 14.1 and 21.4. Both feed moisture and extruder temperature had significant effects on the extrudate characteristics. Changing the level of moisture content and temperature had significant effect ($p<0.01$) on all physical properties. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. Hardness ranged between 1.03 and 1.62 N and the coefficient of determination for hardness was high ($R^2 = 0.8$). Extruder screw speed had minimal effect on the extrudate properties. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility. The addition of corn meal however significantly decreased the starch and protein digestibility of the extrudates. By applying the methodology of the desired function, the optimized process variables were moisture 15%, temperature 115°C, screw speed 185 rpm and Day 4 germinated flour with bulk density=118.8 kg/m^3, hardness=1.13 N, water solubility index=16.5, water absorption index =3.4, total color difference ΔE=20.6, expansion ratio=3.75, protein digestibility=86.3% and starch digestibility=85.7% respectively with overall desirability value of 0.999. The results therefore clearly showed that highly expanded products can be produced by blending corn meal with quinoa. However, the starch and protein digestibility of the extrudates is significantly reduced.

Table 4.1: Nutritional properties of sprouted quinoa-corn meal blend

	Raw quinoa + corn meal	Day 0 + corn meal	Day 1 + corn meal	Day 2 + corn meal	Day 3 + corn meal	Day 4 + corn meal
Total Protein[a]	15.1±0.5	15.1±0.15	15.2±0.03	15.3±0.05	15.4±0.01	15.1±0.07
Carbohydrates[a]	69.2±0.6	69.2±0.16	69.3±0.03	69.4±0.07	69.4±0.09	69.9±0.02
Moisture[a]	11.1±0.2	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid[b]	1.01±0.07	0.61±0.05	0.45±0.05	0.36±0.02	0.23±0.04	0.11±0.02
Saponin[b]	0.60±0.02	0.40±0.11	0.40±0.06	0.30±0.04	0.20±0.02	0.10±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 4.2: Results of model adequacy tested in the Box-Behnken design

Source	**Sum of squares**	**DF**	**Mean square**	**F value**	**Prob > F**	**Remarks**
Sequential model sum of squares for ***Bulk Density***						
Quadratic	541.33	4	135.33	30.10	< 0.0001	Suggested
Sequential model sum of squares for ***Hardness***						
Quadratic	0.15	4	0.037	3.20	0.0162	Suggested
Sequential model sum of squares for ***Water Solubility Index***						
Quadratic	155.09	4	38.77	79.71	< 0.0001	Suggested
Sequential model sum of squares for ***Water Absorption Index***						
Quadratic	1.93	4	0.48	13.46	< 0.0001	Suggested
Sequential model sum of squares for ***Total Color Difference ΔE***						
Quadratic	10.95	4	2.46	5.65	0.0004	Suggested
Sequential model sum of squares for ***Expansion Ratio***						
Quadratic	0.29	4	0.074	22.42	< 0.0001	Suggested
Sequential model sum of squares for ***Protein Digestibility***						
Quadratic	133.42	4	33.36	38.71	< 0.0001	Suggested
Sequential model sum of squares for ***Starch Digestibility***						
Quadratic	26.64	4	6.66	14.87	< 0.0001	Suggested

Source	**Std. Dev.**	**R-squared**	**Adjusted R-squared**	**Predicted R-squared**	**PRESS**	
Model summary statistics for ***Bulk Density***						
Quadratic	2.12	0.9613	0.9481	0.9254	866.14	Suggested
Model summary statistics for ***Hardness***						
Quadratic	0.11	0.7637	0.6834	0.5450	2.24	Suggested
Model summary statistics for ***Water Solubility Index***						
Quadratic	0.70	0.8925	0.8560	0.7929	93.73	Suggested
Model summary statistics for ***Water Absorption Index***						
Quadratic	0.19	0.8713	0.8275	0.7520	6.92	Suggested
Model summary statistics for ***Total Color Difference ΔE***						
Quadratic	0.66	0.8351	0.7791	0.6836	83.53	Suggested
Model summary statistics for ***Expansion Ratio***						
Quadratic	0.057	0.9629	0.9502	0.9284	0.63	Suggested
Model summary statistics for ***Protein Digestibility***						
Quadratic	0.93	0.8918	0.8550	0.7916	165.95	Suggested
Model summary statistics for ***Starch Digestibility***						
Quadratic	0.67	0.9269	0.9020	0.8596	85.92	Suggested

+ case(s) with leverage of 1.0000: PRESS statistic not defined

Table 4.3: Second order polynomial equations representing the relationship between independent and dependent variables

Bulk Density (kg/m^3)	122.00 +8.97A -4.17B -2.57C -7.90D +2.28E1 +0.95E2 -0.052E3 -1.05E4 -2.25AB +1.55AC +1.20AD -0.050AE1 -0.22AE2 +0.033AE3 +0.033AE4 -2.00BC +1.25BD -0.45CD -0.10CE1 -0.017CE2 +0.067CE3 +0.067CE4 -0.52DE1 -0.100DE2 +0.23DE3 +0.23DE4 -0.30A^2 +3.00B^2 -0.75C^2 +2.75D^2
Hardness (N)	1.15 +0.18A -0.079B -0.045C -0.12D +0.030E1 +0.016E2 -0.027E3 -5.037E-003E4 +5.000E-004AB -0.075AC -9.500E-003AD -0.014AE1 -0.015AE[2] +0.060AE3 -0.014AE4 -0.020BC -0.017BD +0.016BE1 +0.015BE2 -0.060BE3 +0.015BE4 -0.042CD +5.000E-004CE1 +5.000E-004CE2 -3.333E-004CE3 -3.333E-004CE4 -9.167E-003DE1 -8.333E-004DE2 +1.667E-003DE3 +4.167E-003DE4 +0.034A^2 +1.000E-003B^2 +0.033C^2 -0.040D^2
Water Solubility Index (%)	13.60 -1.89A +0.51B +0.23C +0.21D -0.22E1 -0.093E2 +7.407E-003E3 +0.10E4 -0.12AB -0.15AC -0.46AD -6.667E-003AE1 +1.667E-003AE2 +1.667E-003AE3 +1.667E-003AE4 -0.45BD +6.667E-003BE1 -1.667E-003BE2 -1.667E-003BE3 -1.667E-003BE4 +0.14CD +1.000E-002CE1 +1.667E-003CE2 +1.667E-003CE3 -6.667E-003CE4 +0.053DE1 -5.000E-003DE2 -5.000E-003DE3 -0.013DE4 +2.08A^2 -0.19B^2 -0.15C^2 +6.667E-003D^2
Water Absorption Index	3.54 -0.40A +0.045B +0.043C +0.43D -0.029E1 -8.296E-003E2 +2.444E-003E3 +0.012E4 -0.11AB -0.10AC +0.20AD -2.667E-003AE1 +6.667E-004AE2 +6.667E-004AE3 +6.667E-004AE4 -0.034BC -0.039BD +2.333E-003BE1 +6.667E-004 BE2 -1.000E -

	003BE3 -1.000E-003BE4 +0.063CD +1.667E-004CE1 -6.667E-004CE2 +1.667E-004CE3 +1.667E-004CE4 +1.500E-003DE1 -1.000E-003DE2 -1.667E-004DE3 -1.667E-004DE4 $-0.23A^2$ $-0.14B^2$ $-0.11C^2$ $-0.20D^2$
Total Color Difference ΔE	17.06 -0.41A +0.12B +0.11C -1.40D -0.046 * E1 +5.926E-003E2 +9.630E-003E3 +0.013E4 +0.62AB +0.41AC +1.35AD -0.12AE1 -0.045AE2 +5.000E-003AE3 +0.055AE4 -0.010BC +0.17BD +0.060BE1 +1.000E-002BE2 -6.667E-003BE3 -0.023BE4 +1.27CD -0.053CE1 -0.012CE2 +5.000E-003CE3 +0.022CE4 -0.22DE1 -0.070DE2 +0.013DE3 +0.097DE4 $+0.048A^2$ $-0.019B^2$ $+0.47C^2$ $-0.20D^2$
Expansion Ratio	3.54 -0.28A +0.063B +0.059C +0.22D -0.031E1 -8.815E-003E2 +2.296E-003E3 +0.013E4 -0.022AB -0.015AC +0.047AD +3.333E-003AE2 +8.333E-004AE3 +0.051BC -0.026BD -6.667E-004BE1 -6.667E-004BE2 +1.667E-004BE3 +1.667E-004BE4 -7.500E-003CD -3.167E-003CE1 +1.667E-004 * CE2 +1.000E-003CE3 +1.000E-003CE4 +4.167E-003DE1 -2.500E-003DE2 -1.667E-003DE3 -8.333E-004DE4 $-0.052A^2$ $-0.095B^2$ $-0.025C^2$ $-0.067D^2$
Protein Digestibility (%)	79.58 -0.74A +0.70B +0.38C -2.81D -0.48E1 -0.036 E2 +0.045E3 +0.19E4 +0.51AB +0.57AC -0.27AD +0.64AE1 -0.14AE2 -0.21AE3 -0.15AE4 -0.040BC +0.12BD -0.34BE1 +0.048BE2 +0.082BE3 +0.11BE4 +0.13CD +0.17CE1 -0.020CE2 -0.095CE3 -0.028CE4 +0.36DE1 -0.13DE2 -0.095DE3 -0.070DE4 $+1.54A^2$ $+0.37B^2$ $+0.18C^2$ $+1.83D^2$
Starch	81.20 -0.94A +0.78B +1.05C -2.44D -0.57E1 -6.667E-003E2 +0.10E3

Digestibility (%)	+0.20E4 -0.075AB +0.55AC +0.15AD +0.37AE1 -0.092AE2 -0.092AE3 -0.092AE4 -0.020BC -0.51BD +0.040BE1 -0.018BE2 -0.027BE3 -0.027BE4 +0.35CD +5.000E-003CE1 +0.013CE2 +0.013CE3 +0.013CE4 +0.20DE1 -0.057DE2 -0.048DE3 -0.048DE4 $+0.45A^2 -0.11B^2 -0.32C^2 +0.65D^2$

A – Moisture Content (%w.b.); B – Screw Speed (rpm); C – Die Temperature; D – Corn meal ratio; E$_1$, E$_2$, E$_3$, E$_4$ – Germination time (Days)

Table 4.4: Pareto analysis of variance (ANOVA) analysis and significant statistical parameters of the model

Source	Sum of squares	DF	Mean square	F-value	p-value
Bulk Density					
Model	11163.65	34	328.33	73.03	< 0.0001
A-moisture	4824.07	1	4824.07	1073.01	< 0.0001
B-screw speed	1041.67	1	1041.67	231.70	< 0.0001
C-temp	395.27	1	395.27	87.92	< 0.0001
D – corn meal ratio	3744.60	1	3744.60	832.91	
E – germ period	316.79	4	79.20	17.62	< 0.0001
AB	101.25	1	101.25	22.52	
BC	80.00	1	80.00	10.69	
B^2	240.00	1	240.00	6.41	
D^2	201.67	1	201.67	44.86	< 0.0001
C.V. %	1.71				
Hardness					
Model	2.55	34	0.10	26.77	< 0.0001
A-moisture	1.85	1	1.85	485.52	< 0.0001
C-temp	0.30	1	0.30	77.95	< 0.0001
D – corn meal ratio	0.14	1	0.035	9.16	< 0.0001
E – germ period	395.27	4	395.27	87.92	< 0.0001
A^2	0.083	1	0.083	21.87	< 0.0001
C.V. %	4.10				

Water Solubility Index					
Model	152.64	34	6.11	133.85	< 0.0001
A-moisture	119.37	1	119.37	2616.91	< 0.0001
B-screw speed	25.28	1	25.28	554.23	< 0.0001
C-temp	2.16	1	2.16	47.40	< 0.0001
D – corn meal ratio	2.16	1	0.54	11.85	< 0.0001
E – germ period	0.30	4	0.30	77.95	< 0.0001
A^2	2.77	1	2.77	60.77	< 0.0001
C.V. %	1.51				
Water Absorption Index					
Model	6.54	34	0.26	113.49	< 0.0001
A-moisture	4.73	1	4.73	2054.67	< 0.0001
B-screw speed	0.87	1	0.87	379.04	< 0.0001
C-temp	0.13	1	0.13	55.92	< 0.0001
D – corn meal ratio	187.06	1	187.06	546.76	< 0.0001
E – germ period	119.37	4	119.37	2616.91	< 0.0001
AB	0.26	1	0.26	110.85	< 0.0001
AC	0.063	1	0.063	27.23	< 0.0001
A^2	0.27	1	0.27	118.79	< 0.0001
B^2	0.068	1	0.068	29.58	< 0.0001
C^2	0.062	1	0.062	26.73	< 0.0001
C.V. %	1.75				

Total Color Difference ΔE					
Model	261.57	34	10.46	30.58	< 0.0001
A-moisture	187.06	1	187.06	546.76	< 0.0001
D – corn meal ratio	0.87	1	0.87	379.04	< 0.0001
A^2	6.32	1	6.32	18.47	< 0.0001
C.V. %	3.09				
Expansion Ratio					
Model	8.38	34	0.34	63.87	< 0.0001
A-moisture	6.67	1	6.67	1271.67	< 0.0001
B-screw speed	0.78	1	0.78	147.77	< 0.0001
C-temp	0.46	1	0.46	86.84	< 0.0001
D- corn meal ratio	0.068	4	0.063	27.23	< 0.0001
B^2	0.19	1	0.19	35.88	< 0.0001
C^2	0.11	1	0.11	20.32	< 0.0001
C.V. %	2.24				
Protein Digestibility					
Model	138.02	34	5.52	84.63	< 0.0001
A-moisture	18.91	1	18.91	289.81	< 0.0001
B-screw speed	5.55	1	5.55	85.08	< 0.0001
C-temp	3.60	1	3.60	55.18	< 0.0001
D – corn meal ratio	103.76	1	25.94	397.62	< 0.0001
E- germ period	0.78	4	0.78	147.77	< 0.0001
AB	1.57	1	1.57	24.04	< 0.0001
B^2	1.83	1	1.83	28.08	< 0.0001
C.V. %	0.30				

Starch Digestibility					
Model	142.81	34	5.71	83.00	< 0.0001
A-moisture	9.03	1	9.03	131.13	< 0.0001
B-screw speed	6.64	1	6.64	96.51	< 0.0001
C-temp	6.97	1	6.97	101.31	< 0.0001
D – corn meal ratio	115.92	1	28.98	421.07	< 0.0001
E – germ period	1.57	4	1.57	24.04	< 0.0001
AB	0.48	1	0.48	6.98	< 0.0001
B^2	1.61	1	1.61	23.34	< 0.0001
C^2	1.72		1.72	24.95	< 0.0001
C.V. %	0.31				

Table 4.5: Predicted and experimental values of the responses at optimum conditions

Moisture (%) = 15; Temperature (°C) = 140; Screw Speed (rpm) = 219; Corn meal ratio = 6%; Germination = Day 4

	Optimized values[a] (predicted values)	Experimental values[b]
Bulk density(kg/m^3)	110.5	110.5±0.07
Hardness (N)	1.07	1.07±0.09
Water Solubility Index (%)	18.2	18.2±0.06
Water absorption Index	3.5	3.5±0.07
Total Color Difference ΔE	17.62	17.62±0.12
Expansion Ratio	3.78	3.78±0.05
Protein Digestibility (%)	84.7	84.7±0.04
Starch Digestibility (%)	85.6	85.6±0.09

a Predicted using response surface quadratic model

b Mean ± standard deviation of triplicate determinations from experiments

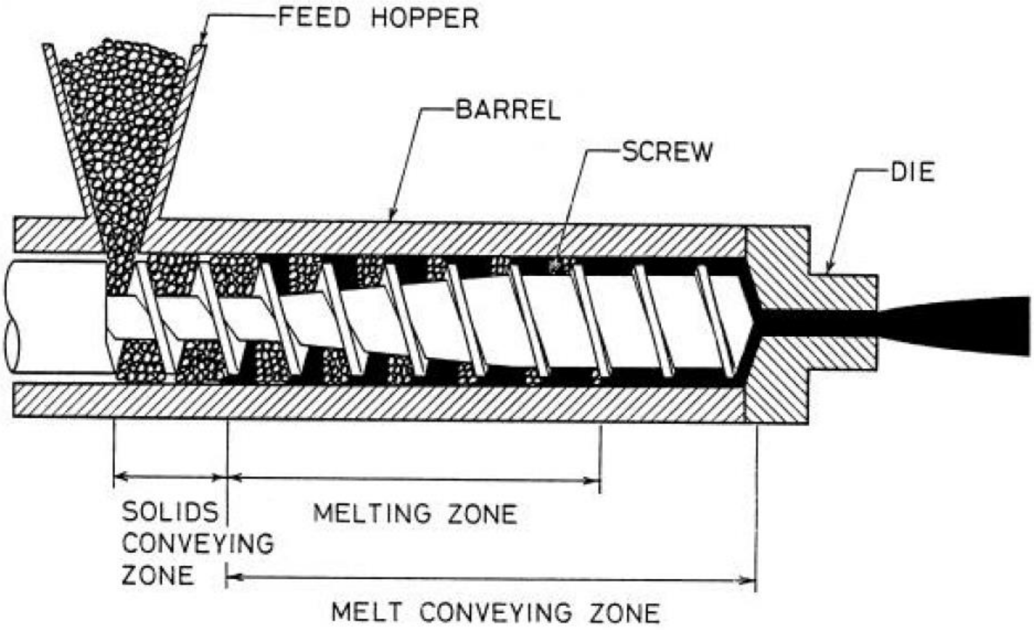


Figure 4.1: Single-screw extruder zones

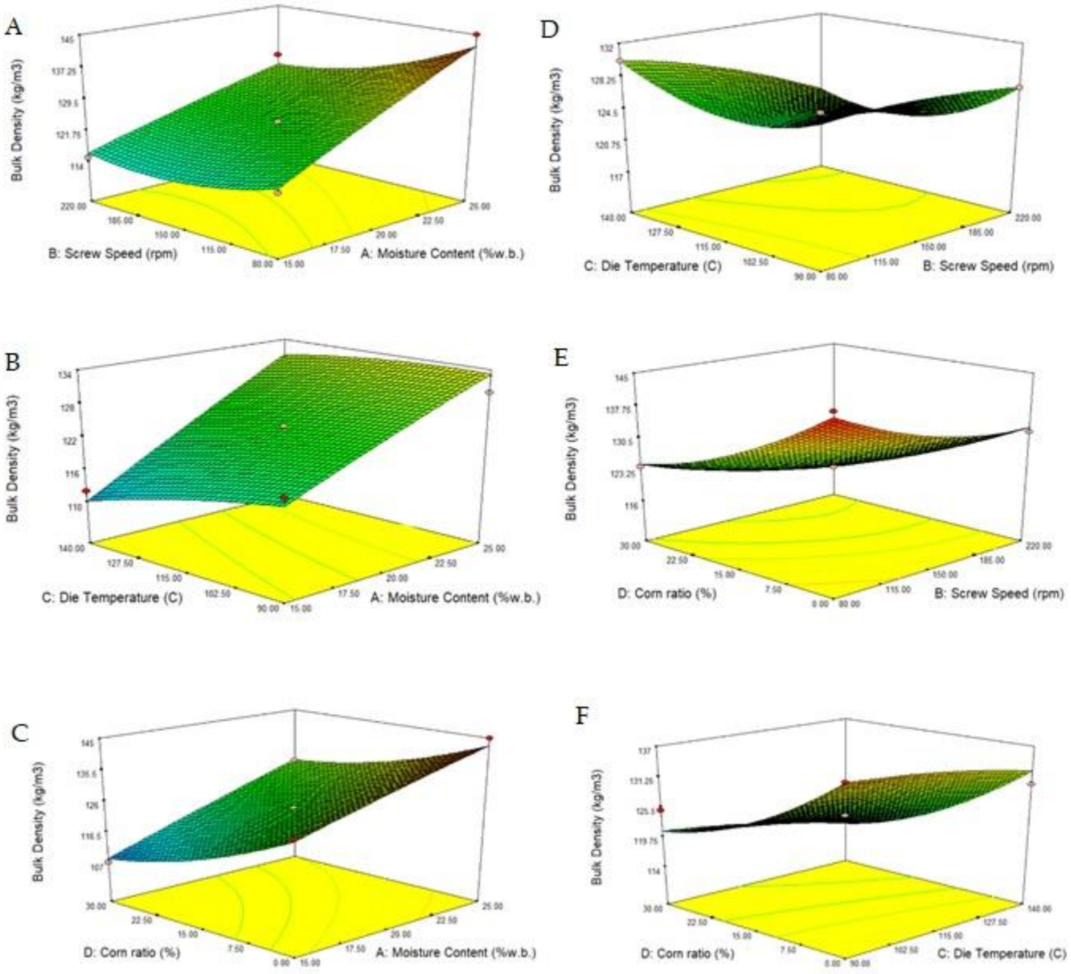


Figure 4.2: Response surface plots (3D) showing the effects on the Bulk Density (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and Die Temperature

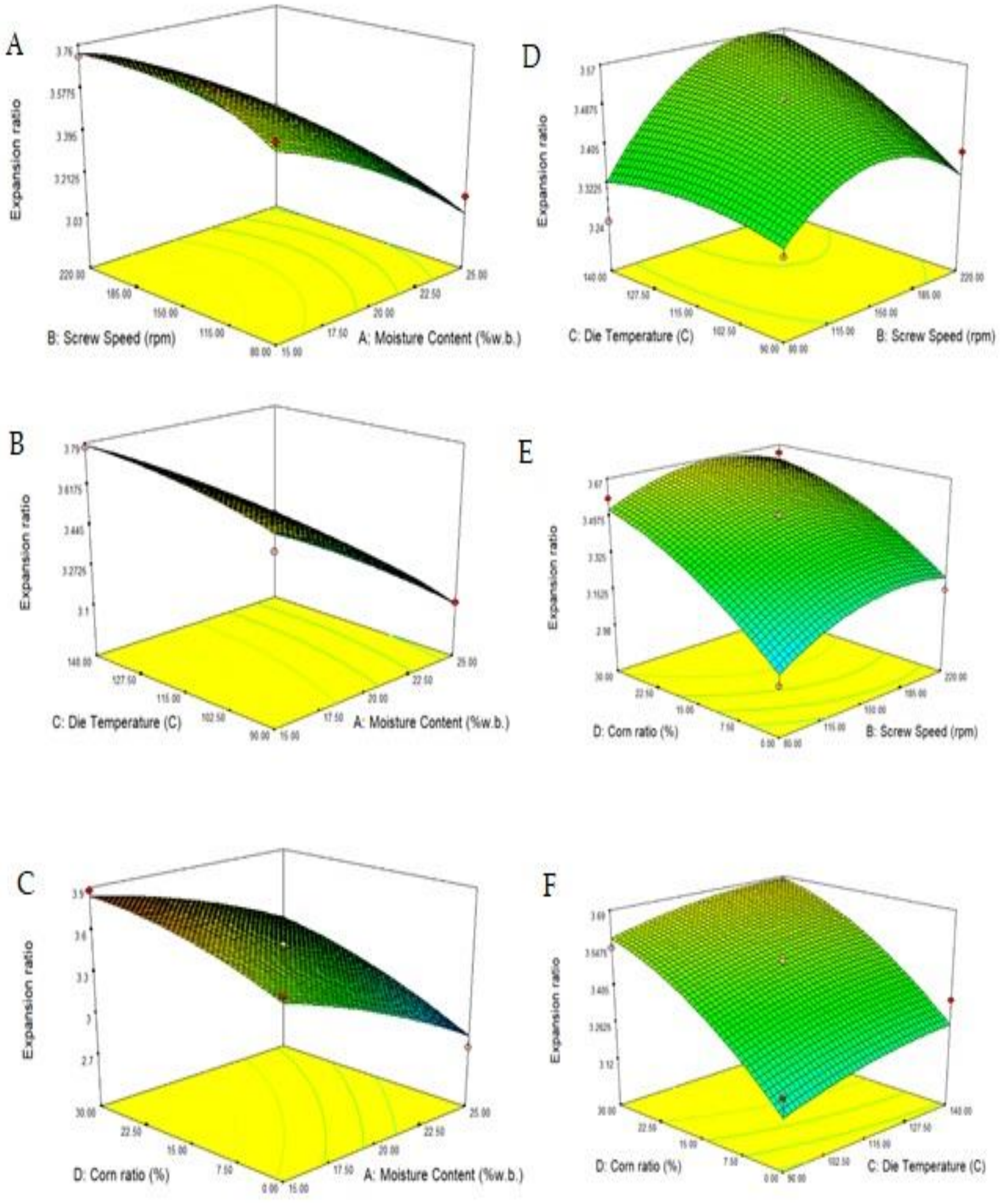


Figure 4.3: Response surface plots (3D) showing the effects on the expansion ratio (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and Die temperature

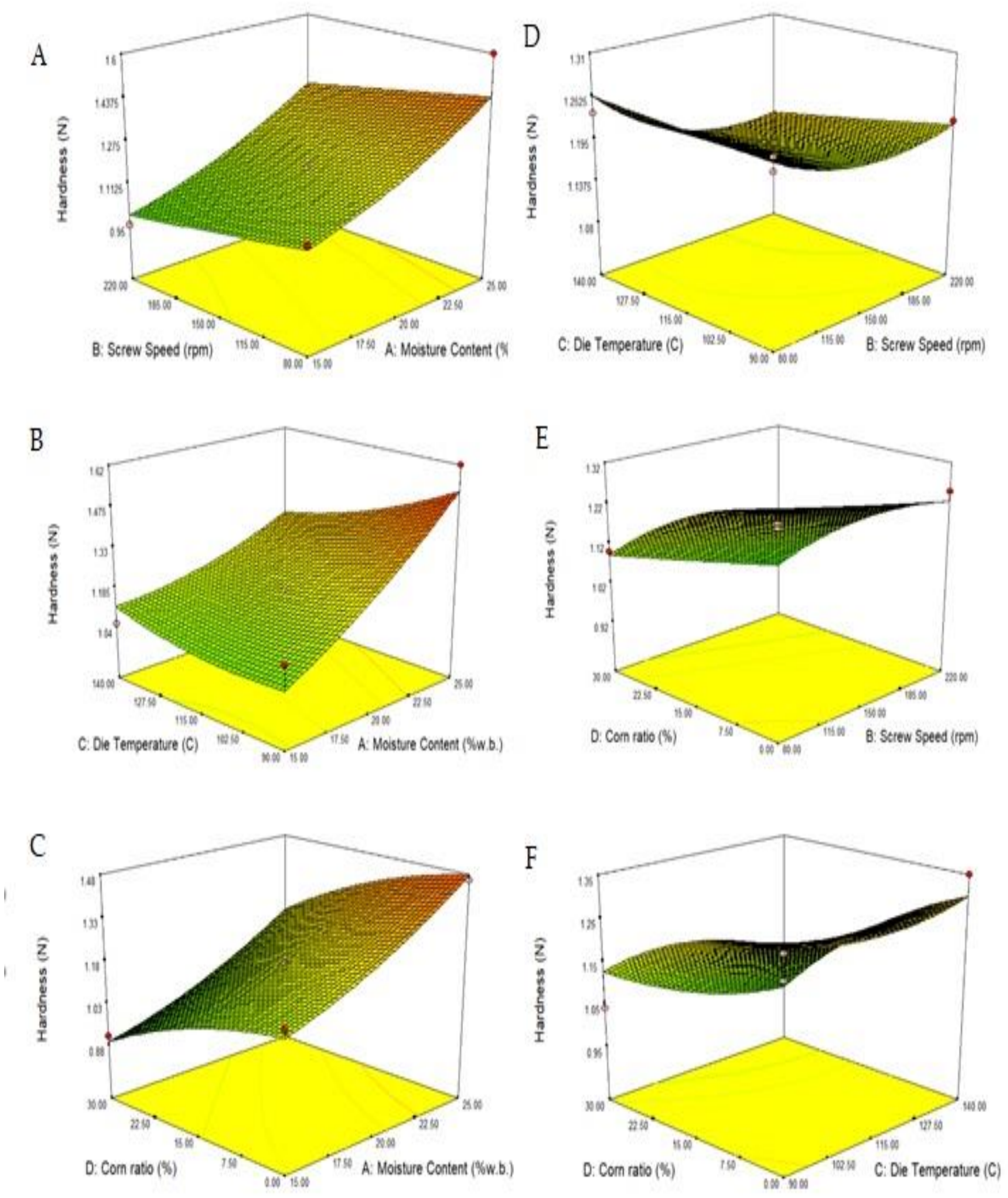


Figure 4.4: Response surface plots (3D) showing the effects on the hardness (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and Die temperature

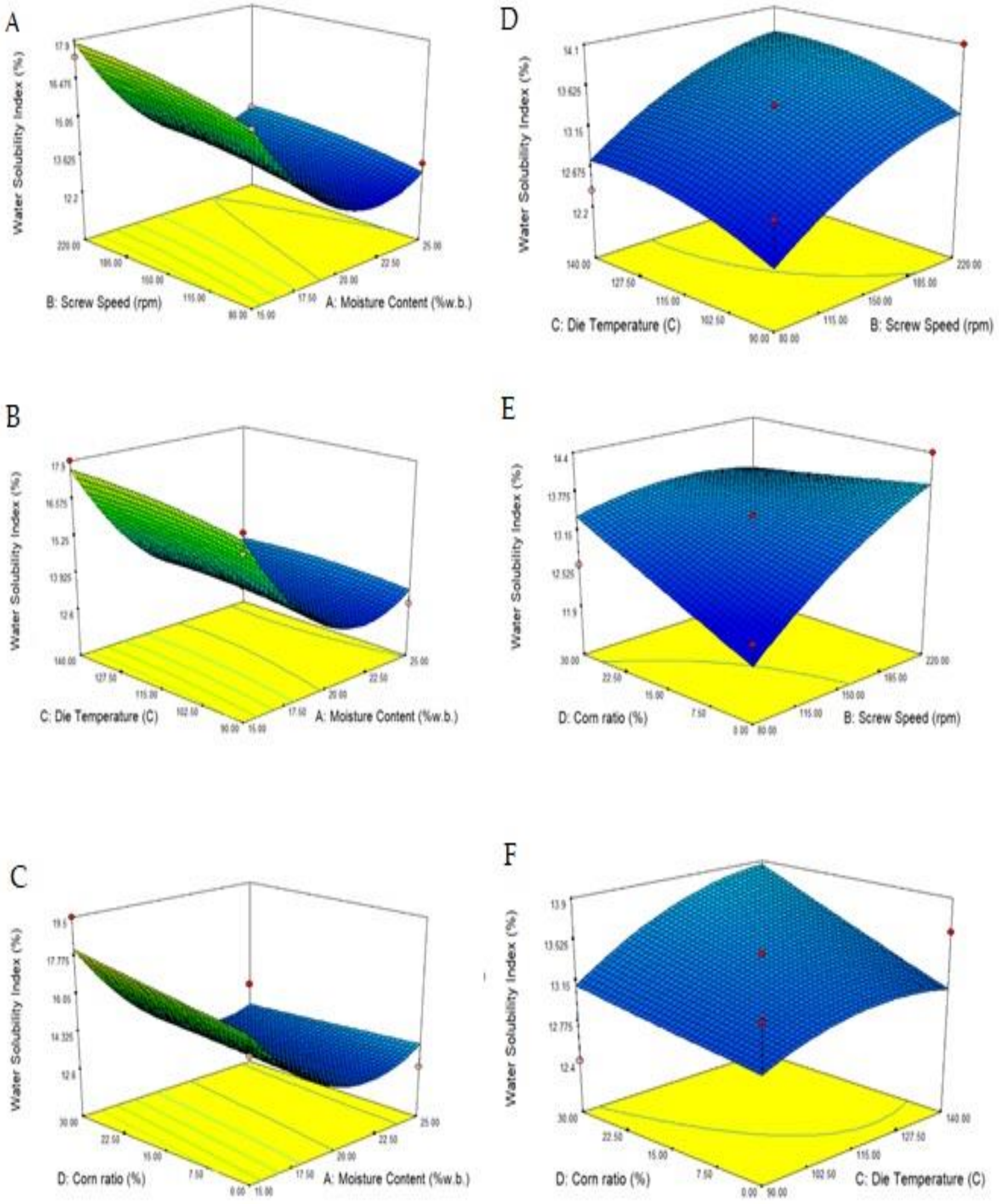


Figure 4.5: Response surface plots (3D) showing the effects on the water solubility index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and Die temperature

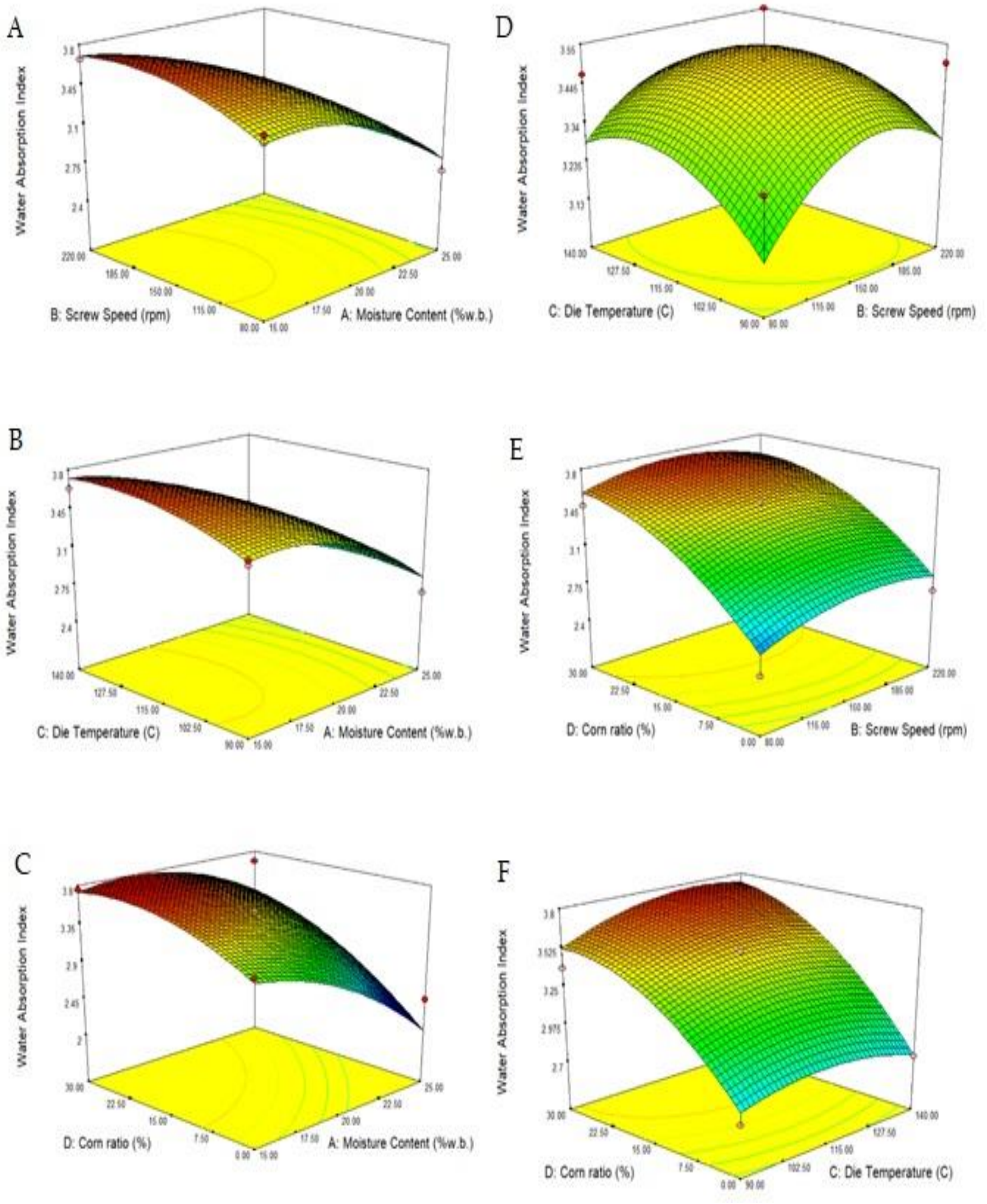


Figure 4.6: Response surface plots (3D) showing the effects on the water absorption index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and die temperature

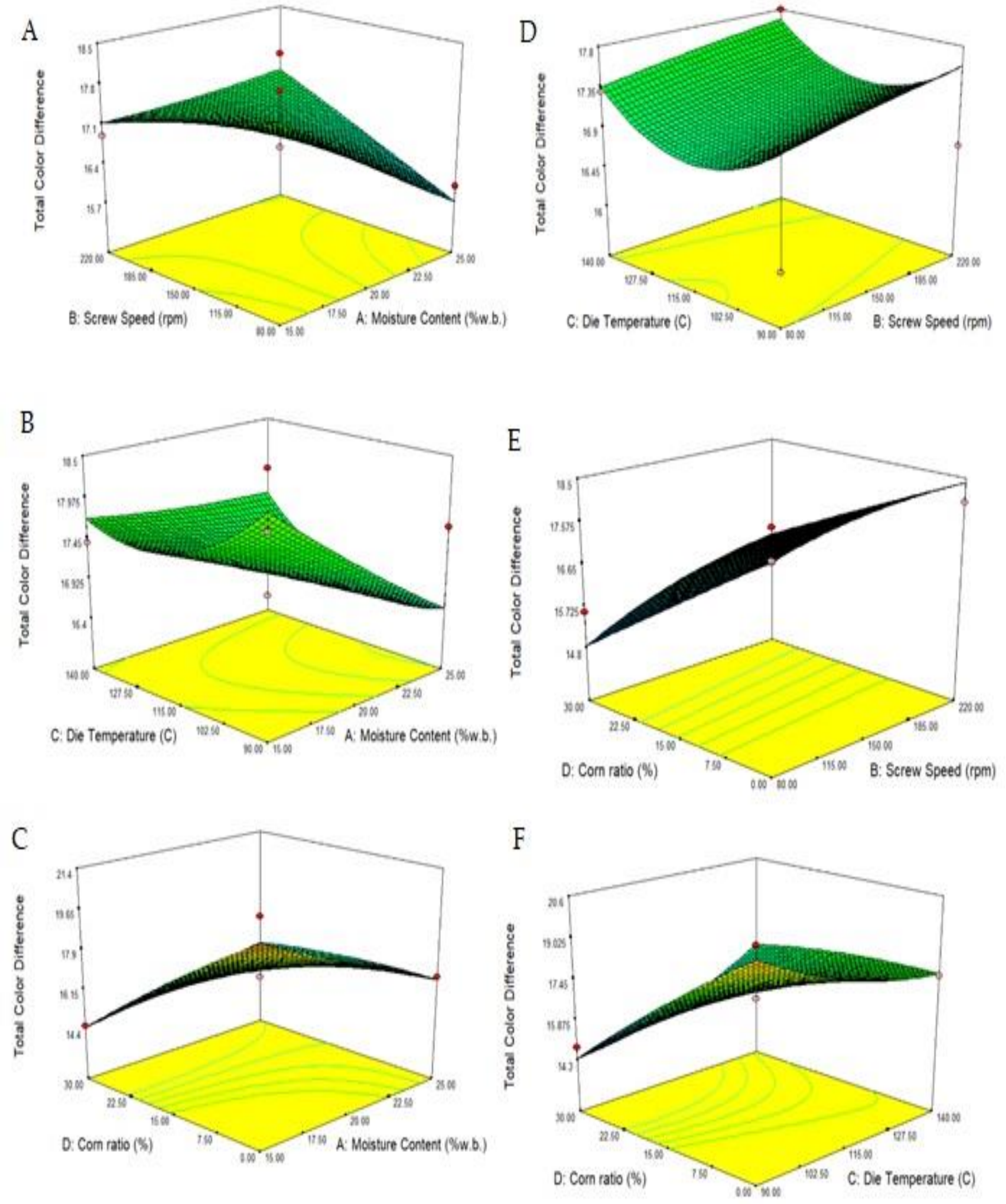


Figure 4.7: Response surface plots (3D) showing the effects on the total color difference (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and Die Temperature

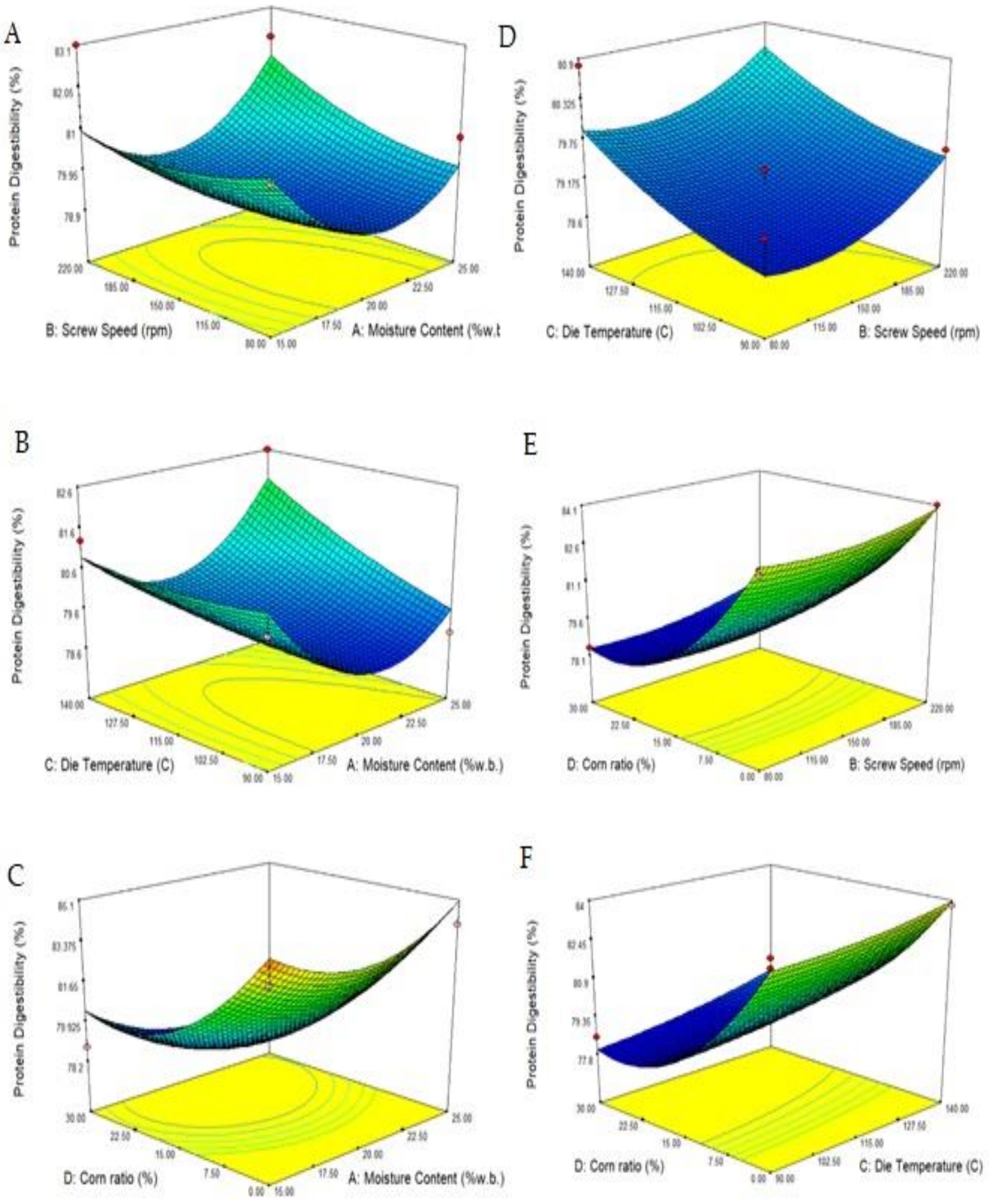


Figure 4.8: Response surface plots (3D) showing the effects on the protein digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and die temperature

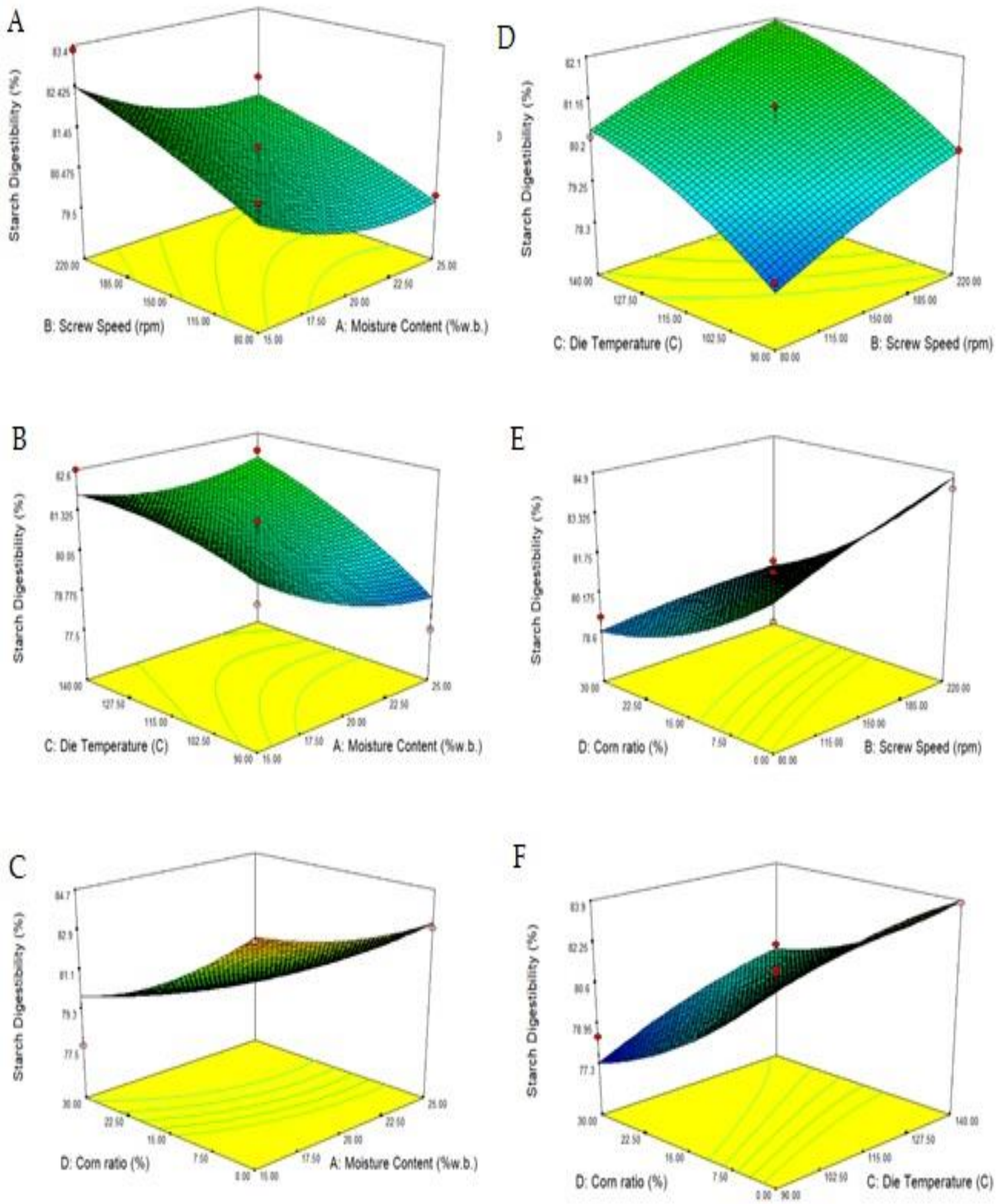


Figure 4.9: Response surface plots (3D) showing the effects on the starch digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Corn Meal Ratio and Moisture Content (D) Die temperature and Screw Speed (E) Corn Meal Ratio and Screw Speed (F) Corn Meal Ratio and Die Temperature

CHAPTER 5: EFFECT OF TWIN-SCREW EXTRUSION PROCESS PARAMETERS ON THE PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF SPROUTED QUINOA EXTRUDATES

Abstract

Twin-screw extrusion processing of sprouted quinoa flour was studied using a response surface design to understand the influence of feed moisture content (15-25% w.b.), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of quinoa extrudates. Soaking and germination pre-treatments of quinoa were carried out to reduce the saponin and phytic acid content and therefore increase protein and starch digestibility. The following responses of the extrudates were measured and the values ranged from: bulk density (132-175 kg/m^3), hardness (1.56-2.14 N), water solubility index (14.4-18.5%), water absorption index (2.93-3.41), ΔE (16.7-20.8), expansion ratio (2.28-2.83), protein digestibility (78.4-84.1%) and starch digestibility (77.1-83.4%). All independent parameters had statistically significant effects on all the extrudate characteristics. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility.

Keywords: Quinoa, Saponin, Gluten-Free, Soaking, Germination, Digestibility

5.1. Introduction

Twin-Screw extrusion processing is a commonly used processing technology in the food industry with a wide number of applications. It is a processing system that utilizes a twin screw or a set of screws to force food materials through a die opening. The food ingredients are subjected to high shear, temperature, and pressure for a short period of time (Gopirajah & Muthukumarappan, 2018). The role of the screw is to assist in imparting shear to the ingredient mixture and forcing the dough of the mixed ingredients out from the extruder through the die (Muthukumarappan & Karunanithy, 2012). The screws are also responsible for the build-up of pressure that occurs at the end of the extruder as well as added mixing of the ingredients. This helps to transform the ingredients from solid powders to a melt state inside of the extruder (Guy, 2001). The melted ingredients are then forced through a die at the end of the extruder into the atmosphere. The melt coming out of the extruder encounters a sudden drop in pressure, resulting in rapid expansion as well as a decrease in temperature, helping it to transform into a cooked product. The final quality and texture of the extruded product depends on various factors, including the ingredient mixture and its properties, extrusion processing conditions, and post-processing conditions (Harper & Clark, 1979). Because of the great flexibility of extrusion processing, it has found very diverse applications in the food industry.

In general extruders are divided into single screw and twin-screw extruders. Single screw extruders are most popular for their low cost, simple designs, ruggedness, and reliability. Co-rotating and counter-rotating twin-screw extruders are commonly used in the food industry for profile extrusion to compounding and mixing (Van Zuilichem, Kuiper, Stolp, & Jager, 1999). Counter-rotating twin-screw extruders, which are like gear pumps, provide the maximum positive displacement. They are the machine of choice for profile extrusion, whereas co-rotating twin-screw extruders are more suitable for other applications such as compounding and mixing (Van Zuilichem, Stolp, & Janssen, 1983). The main reason for suitability of twin-screw extruders for these applications is the complexity of the flow in the intermeshing region, which provides them good mixing and compounding characteristics (Yang & Williams, 1990). Compared to single screw extruders, twin screw extruders are more efficient in providing homogeneous mixing of different ingredients. Larger variety of twin-screw extruders are available that may vary in construction, could have parallel or conical screws that may rotate in the same direction (corotating) or opposite direction (counter-rotating), and have different degree of intermeshing. The complexities of the flow make it difficult to predict the performance of a twin-screw extruder and also difficult to design a extruder given the performance requirements.

Coeliac disease, also known as gluten-sensitive enteropathy, is one of the common food intolerances on a global scale. Tiny hair-like projections in the small intestine called villi are injured through an immune-mediated response which leads to mal-absorption of vital nutrients like iron, folic acid, calcium and fat-soluble vitamins, when exposed to gluten (Thompson, 2009). Due to lack of any treatment, food allergic and food intolerant people need to avoid the foods to which they are allergic or intolerant. However, individuals who follow an allergy-free diet may be at danger of several nutrient deficiencies. Instead of wheat, rye, barley, or other gluten-containing cereal grains, there are numerous other edible seeds that can be consumed, including amaranth, buckwheat, quinoa, teff and wild rice, as well as cereal grains that do not contain gluten, including corn, millet, rice, and sorghum (Thompson, 2009). For example, quinoa is a pseudo cereal and has been cultivated in the Andean region. It is an annual plant, 1–2 m tall with deep penetrating roots. It can be grown from sea level up to an altitude of 3800 m (Alandia, Rodriguez, Jacobsen, Bazile, & Condori, 2016). The plant has tolerance to frost, salinity and drought, and be cultivated on marginal soils (Jacobsen, 2003). Quinoa has outstanding protein quality and a wide range of minerals and vitamins, which makes it popular for consumption. In addition, it is rich in amino acids like lysine and methionine that are deficient in cereals. Quinoa is an excellent source of fiber, iron, calcium, magnesium, and B vitamins (Navruz-Varli & Sanlier, 2016). Because of their nutritive properties, edible seeds other than the gluten-containing cereal grains and pseudocereals are viable candidates to formulate gluten-free diets.

One of the factors which limit the widespread utilization of quinoa is its bitter taste caused by the presence of saponins. Saponins are triterpene glucosides that consist of a linear arrangement of one to six hexose or pentose glycoside units joined to the sapogenin aglycone, which can be a steroidal or a triterpenoid aglycone (Kuljanabhagavad, Thongphasuk, Chamulitrat, & Wink, 2008). Quinoa saponins are soluble in methanol or water. They produce stable foams in aqueous solutions. They may form chemical complexes with iron and reduce its absorption. Saponins are located on the outer layers of the seeds and can be removed by polishing and washing with water (Estrada, Redmond, & Laarveld, 1997). Similarly, phytic acid poses an issue with cereals and pseudocereals. Phytic acid (myoinositol hexaphosphoric acid) reduces the availability of many minerals like iron, zinc, calcium and magnesium (Cheryan, Rackis, & Nutrition, 1980). The formation of iron-phytate complexes of low solubility, in the small intestine is the basis for the interference of phytate with iron absorption. Although the iron content in cereals is usually high, the iron absorption from them is often poor due to the presence of high amounts of phytates. They are also found to inhibit the proteases and amylases of the intestinal tract. Quinoa contains many essential minerals like calcium (874 mg/kg), phosphorus (5.3 g/kg),iron (5.3 g/kg) and zinc (36 mg/kg) and their availability is of importance in relation to its use as a basic source of nutrients in an infant food (Oatway, Vasanthan, & Helm, 2001). Soaking, sprouting and fermentation are some of the methods that can break down the phytic acid to increase the digestibility of the food as well as enhance mineral absorption (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2003).

Sprouted grains have been consumed by people who have a strict diet schedule or by ones who have a rigorous exercise pattern. Soaking and sprouting are an age-old technique however the benefits of these methods has not been completely exploited to increase the digestibility of food. Sprouted seeds are nutritionally superior to their respective seeds Germinated grains have higher levels of nutrients, lower levels of anti-nutrients such as phytic acid and flatulence-causing sugars, increased protein and starch digestibility, and increased bioavailability of some minerals (Carciochi, Manrique, & Dimitrov, 2014). The inclusion of sprouted grains in the formulation of whole-grain-rich, fiber-rich, and gluten-free baked goods may provide a natural fortification and enrichment mechanism; it may improve nutritional quality because of the increased digestibility and bioavailability of some nutrients and the reduction of some anti-nutrients. Comprehensive reviews of the effects of germination on the nutrient composition of cereals and pseudocereals have been published (Carciochi et al., 2014) (Lombardi-Boccia, Lullo, Carnovale, & Agriculture, 1991) (Lorenz & D'Appolonia, 1980). Not all people enjoy the flavour of a sprouted whole grain. Hence it is important to convert it to an interesting product before it reaches the consumer.

The effect of germination on the nutrition properties of grains has been studied by various authors (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012) (Hung, Maeda, Yamamoto, & Morita, 2012) (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013). Similarly, the impact of extrusion variables on the quinoa extrudates have also been studied (Kowalski, Medina-Meza, Thapa, Murphy, & Ganjyal, 2016) (Doğan & Karwe, 2003). However, the method of producing highly digestible quinoa extrudates by removing the saponins and phytic acid has not been researched. The objective of this research is to study the effect of germination on the changes in nutrients of quinoa and the impact of the twin-screw extrusion variables such as moisture content of feed, temperature and screw speed of extruder on the physical and physico-chemical properties of extrudates made from the sprouted quinoa flour.

5.2 Materials and Methods

5.2.1 Raw materials

Quinoa seeds (white) were obtained from a national grain processor (Bob's Red Mill Natural Foods, Milwaukie, OR). For the proximate composition analysis of quinoa seeds and flour, the following AACC methods were used: Moisture -Oven drying at 135°C (method no. 44–19), Ash - calcination at 550°C (method no. 08–01), Lipids - defatting in a Soxhlet apparatus with petroleum ether (method no. 30–25), Protein - micro Kjeldahl (N 6.25) (method no. 46–13), Carbohydrate – sugars and starch (method no. 80–04, 76-13) and total fiber - (method no. 32–07). Saponin content and phytic acid content (hexaphosphate equivalents) were quantified using the method described by (Rathod & Annapure, 2017). All chemicals were purchased from Sigma–Aldrich, USA. All chemicals and reagents used were of analytical grade. All experiments were performed in triplicates.

5.2.2 Soaking and Germination

The quinoa (11 pounds) was soaked overnight (8 pm- 8 am) for 12 h in tap water (1:5 w/v) at room temperature. The soaked samples were then drained and washed. Excess water was drained, and the samples were wrapped in a moist cloth for 5 days (0 h – Day 0, 24 h - Day 1, 48 h - Day 2, 72 h – Day 3, 96 h – Day 4) at room temperature. All experiments were performed in triplicates.

5.2.3 Extrusion processing

The quinoa flour samples, each weighing around 200 g were randomly extruded using a counter rotating conical twin screw laboratory extruder (C. W. Brabender, Inc., S. Hackensack, NJ, USA) as shown in Fig 5.1. The conical screws had diameters decreasing from 43mm to 28mm along their length of 365mm from the feed end to the exit end. The compression screws were universally single flighted counter rotating intermeshing screws with interrupted flight mixing zones. The mixing screws had a mixing section, in which small portions of the screw flights were cut away. The mixing section enhanced the mixing action and increased the residence time of the sample in the barrel. A die nozzle with 3 mm diameter was used. Data was recorded by a computer for subsequent analyses. Extrusion conditions selected were based on the preliminary studies and previous experiments. The extrudates were cooled to room temperature and sealed in polyethylene bags and stored at room temperature until measurements were taken.

5.2.4 Measurement of extrudate properties

5.2.4.1 Bulk Density

The bulk density (kg/m^3) was calculated as the ratio of the mass M (kg) to the volume V (m^3) of the measured and weighed extrudate sample. It was assumed that extrudates were of cylindrical shape. The bulk density of all 65 extrudates were measured and the measurements were done in triplicate.

$$Bulk\ Density = \frac{M}{V} \tag{5.1}$$

5.2.4.2 Expansion ratio (ER)

Expansion ratio was calculated by measuring the mean diameter of 10 randomly chosen locations on the extrudates with calipers for each extrusion process condition. Further it was divided by the die diameter of 3 mm.

$$Expansion\ ratio = \frac{Mean\ diameter\ of\ the\ extrudate\ (mm)}{Die\ diameter\ (mm)} \tag{5.2}$$

5.2.4.3 Hardness

Hardness is defined as the maximum force per unit area of the quinoa extrudate that is necessary to breakdown the extrudate into two pieces. Hardness was tested by breaking the extrudate using a cylindrical probe (TA-4, Texture Technologies Corp, NY) attached to the crosshead of texture analyser. The sample was positioned in its natural position. A vertical force was applied at a speed of 50 mm/min. The force applied vs deformation was recorded and the maximum force recorded in the curve was considered as the hardness. The hardness of all 65 extrudates were measured and the measurements were done in triplicate.

5.2.4.4 Water Solubility Index and Water Absorption Index

The water absorption index and water solubility index of all extrudates were measured and the measurements were done in triplicate. The quinoa extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was mixed in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was mixed occasionally and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminium cup and dried at 135°C for 2h (AACC, 2000). The weight of the gel in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by:

$$Water\ Solubility\ Index(\%) = \frac{W_{ss}}{W_{ds}} * 100 \tag{5.3}$$

$$Water\ Absorption\ Index = \frac{W_g}{W_{ds}} \tag{5.4}$$

where, W_{ss} is the weight of dry solids of supernatant (g), W_{ds} is the weight of dry sample (g), and W_g is the weight of gel (g)

5.2.4.5 Color

The color of quinoa extrudate samples was measured using colorimeter (LabScan XE, HunterLab, Reston, VA) in triplicate in terms of Hunter Lab values (L^*, a^*, b^*). The Hunter Lab value 'L^*' signifies lightness with 0 for dark and 100 for bright. The value 'a^*' measures the degree of green color in the range from -100 to 0 and red in the range 0 to 100. The 'b^*' value represents blue color in the range from -100 to 0 and yellow in the range from 0 to 100.The total color change (ΔE) is calculated as

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \tag{5.5}$$

where $\Delta L = L^* - L_0$, $\Delta a = a^* - a_0$, and $\Delta b = b^* - b^*_0$; the subscript ''0'' represents initial color values before processing. All readings were taken in triplicate.

5.2.4.6 Starch and Protein Digestibility

The starch digestibility and protein digestibility of all 65 extrudates were measured and the measurements were done in triplicate. The quinoa extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The in-vitro starch digestibility was determined in the extrudate powder (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20 °C for 2 h according to the method of (Rathod & Annapure, 2017). At the end of the incubation period, 2 ml of 3,5-dini-trosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard.

A system consisting, 100 mg (0.1 g) dry and defatted samples were incubated at 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 °C for 3 h. Further it was neutralized with 7.5 ml 0.2N NaOH. Then 4 mg pancreatin in 7.5 ml phosphate buffer (pH 8) was added and incubated for 37 °C for 24 h, further 5 mg of TCA was added, centrifuged at 5000 rpm for 10 min. After that the supernatant dry residue at 40–50 °C was discarded and the micro Kjeldahl process for protein estimation was carried out. The percent in vitro protein digestibility was calculated using the following equation (Rathod & Annapure, 2017):

$$Protein\ digestibility\ (\%) = \frac{Total\ protein - Residual\ protein}{Total\ protein} * 100 \tag{5.6}$$

5.2.5 Experimental Design and Statistical Analysis

Experiments were conducted using the Box Benhken design (BBD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA), consisting four factors namely moisture content (X_1), temperature (X_2), screw speed (X_3) and germination time (X_4). Three replicates were taken at the design center and the total number of observations were 75 [60 (not center points) and 15 (center points)]. Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN). The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA). The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

5.3 Results and Discussion

5.3.1 Effect of Soaking and Germination on the extrudates

The nutritional properties of the sprouted quinoa are shown in Table 5.1. After soaking for 12 h, the white color hypocotyls appeared in the quinoa seeds on Day 1. The germination ratio was more than 90%. The protein content decreased significantly on Day 4 of germination and a simultaneous increase in the hypocotyl length was observed. The change in color of the sprouted flour indicates the breakdown of phytates. The optimised soaking time was 12 h and germination time was 96 h.

In general, protein content of quinoa seeds ranges between 13.1% and 14.0% (James, 2009). The total protein content of quinoa is higher than that of grains such as rice, barley, corn, rye, sorghum and wheat (Navruz-Varli & Sanlier, 2016). The chief carbohydrate constituent of quinoa is starch, and it ranges from 62-64%. The total fiber was close to other grain products (7.0%-9.7%). Quinoa contains sugar by about 3%. It mostly contains maltose, D-galactose, and D-ribose in addition to low levels of fructose and glucose (Navruz-Varli & Sanlier, 2016).

The phytic acid concentration in quinoa seeds is comparable to whole grain rye flour (7.7 mg/g), whole grain wheat flour (8.7 mg/g), lentils (8.4 mg/g) and fava beans (8.0 mg/g). Phytic acid is not only present in the outer layers of quinoa, as in the case of rye and wheat but is also evenly distributed within the endosperm (Ahamed, Singhal, Kulkarni, Pal, & Bulletin, 1998). Phytic acid binds minerals, such as Fe, Zn, Ca and Mg, and can make the mineral content of a cereal inadequate, especially for children (Khattak, Zeb, Bibi, Khalil, & Khattak, 2007). Soaking and sprouting processes reduce the 'binding' effect of the phytic acid by activating the enzyme phytase (James, 2009). The amounts of phytic acid of the quinoa seeds obtained in this study are in concordance with the values for Cherry vanilla quinoa (Kowalski et al., 2016). A significant reduction in phytic acid content is observed with increase in germination time. Day4 had more than 50% reduction in phytic acid. Previous studies had shown that extrusion further decreased the phytic acid content of pea flour (Alonso, Orue, & Marzo, 1998), bean flour (Anton, Fulcher, & Arntfield, 2009) and other legumes (Lombardi-Boccia et al., 1991).

Saponins are the principal anti-nutritional factor present in the seed coat of quinoa. Most saponins are nitrogen-free glycosides, each consisting of a sapogenin and a sugar. Saponins affect quinoa's taste and digestibility, therefore should be removed before consumption (Ruales & Nair, 1992). Traditionally, saponin has been removed by laboriously hand scrubbing the quinoa with alkaline water. The saponin content is checked by placing the grain in a tube, adding water and vigorously shaking for 30 s. If no foaming occurs, all saponins are assumed to have been removed. The saponin content was significantly reduced by soaking and germination as shown in Table 5.1. Additionally, saponin content is expected to be reduced through either extrusion (Anuonye, Onuh, Egwim, & Adeyemo, 2010).

5.3.2 Effect of independent variables on the twin-screw extrusion process

The results of the twin-screw extrusion process along with the experimental design is presented in Appendix C.

5.3.2.1 Bulk Density

The bulk density of the extrudates varied between 132 and 175 kg/m^3. Low density, which is a required characteristic of the extruded product, was achieved with low feed moisture content, average temperature and high screw speed combination. Fig 5.2 shows that feed moisture content had a significant effect on bulk density followed by screw speed, whereas temperature seemed to have a minor effect. Low product bulk density of 132 kg/m^3 was obtained at feed moisture of 15%, 115°C temperature, 220 rpm screw speed and Day 4 germinated samples.

5.3.2.2 Expansion ratio

The measured expansion ratio of quinoa extrudates ranged from 2.28 to 2.83. The expansion ratio was significantly affected by temperature and feed moisture content. High expansion ratio at low feed moisture content for expanded products is distinctive for cereals. However, Fig 5.3 shows that the temperature and screw speed also have a significant effect on the expansion ratio of the quinoa extrudates. Similar trends have been obtained for corn (Diaz et al., 2013), wheat (Pitts, Favaro, Austin, & Day, 2014) and oats (Gopirajah & Muthukumarappan, 2018).

5.3.2.3 Hardness

Hardness, the maximum force to break the sample, ranged between 1.56 and 2.14 N. The coefficient of determination for hardness was high ($R^2 = 0.9$) and the moisture, temperature and germination period were found to have significant contributions to the hardness of the extrudates. At all feed moisture content levels, decrease in die temperature decreased the product hardness. Screw speed did not have a significant effect on the extrudate hardness. Fig 5.4 shows the effect of moisture and screw speed on the product hardness. Previous studies have also reported similar trends for extrudates based on rice (Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006), wheat (Ding et al., 2006), oat bran and soy flour (Anuonye et al., 2010) and chickpea (Khattak et al., 2007). The hardness was high of 1.8 N at about 140°C, 150 rpm screw speed, 25% feed moisture content and Day 0 sprouted samples. Chemical changes occurring during extrusion cooking affect the development of textural and mechanical properties such as hardness in extrudates. The elastic swell effect and bubble growth effect equally contribute to the structural changes of starch during extrusion. Water performs as a plasticizer to the starch-based material decreasing its viscosity and the mechanical energy dissipation in the extruder. Hence the product becomes dense and bubble growth is compressed. The reduced starch conversion and compressed bubble growth result in a dense product and wetness of extrudate.

5.3.2.4 Water Solubility Index and Water Absorption Index

Water solubility index is related to the quantity of soluble solids, which is related to dextrinization. The coefficient of determination for water solubility index was highly significant as revealed by a high value of R^2 (0.9). The linear effects of feed moisture, screw speed and temperature had significant effect on water solubility index. In general, water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. Fig 5.5 shows the significant effect of temperature and screw speed on water solubility index. The increase in water solubility index with increasing temperature was consistent with the results reported for oat extrudates (Gutkoski & El-Dash, 1999). The water solubility index decreased with the increase in moisture. Similar trends have been reported earlier for starch, maize grits, wheat and pea (Ding et al., 2006) (Singh, Sekhon, & Singh, 2007). The poor correlation between water solubility index and most of the process and product responses is explained by the fact that water solubility index includes the opposing effects of starch dextrinization and the molecular level interactions between degraded components, which may not be favoured at the same condition (Lei, Ruan, Fulcher, van Lengerich, & Engineering, 2008). An increase in the amount of dextrinized starch during extrusion cooking results in an increase in water solubility index. However, molecular interactions between degraded starch, protein, and lipid components, which in turn lead to an increase in molecular weight, may decrease the solubility, thus water solubility index (Camire, Camire, & Krumhar, 1990).

Water absorption index depends on the availability of hydrophilic groups and on the gel formation capacity of the macromolecules. It is a measure of damaged starch together with protein denaturation and new macromolecular complex formations. Water absorption index of extrudates ranged between 2.93 and 3.41. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. The interaction effects were insignificant as shown in Fig 5.6. Similar responses were observed in rice flour (Hagenimana, Ding, & Fang, 2006), oats (Gutkoski & El-Dash, 1999), finger millet (Sawant, Thakor, Swami, Divate, & Vidyapeet, 2012) and cassava starch (Hashimoto, Grossmann, & technology, 2003). Water absorption index had poor correlations with almost all process and product responses except product density. This is an expected result since it includes the effect of starch gelatinization, protein denaturation and molecular level cross-linking reactions which are not always favored at the same conditions (Doğan & Karwe, 2003).

5.3.2.5 Color

Color is an important quality parameter since it reflects the extent of chemical reactions and degree of cooking or degradation that take place during extrusion cooking. In this study, ΔE represents the total color difference compared to the color of unsoaked and unsprouted quinoa. Higher ΔE means darker products with more intense yellow and red color. The total color change in extruded products ranged between 16.7 and 20.8. Linear effects of temperature and moisture content were found to have the highest contribution to total color change. As depicted in Fig 5.7, low feed moisture content and high temperature increased the total color change possibly due to increased extent of browning reactions under this condition. At low screw speeds a slight increase in color change observed due to longer residence times which might increase the extent of chemical reactions.

5.3.2.6 Starch Digestibility (SD) and Protein Digestibility (PD)

The results show that both protein digestibility and starch digestibility of the quinoa extrudates were increased significantly by soaking, germination and extrusion. Fig 5.8 and Fig 5.9 illustrate the effect of the independent variables on the protein and starch digestibility respectively. The squared effects of all the variables had a significant effect on the protein and starch digestibility. The increases of digestibility produced by the different treatments were higher in protein than in starch. Improvement of protein digestibility after processing could be attributable to the reduction or elimination of different anti-nutrients. Phytic acid, as well as condensed tannins and polyphenols, are known to interact with protein to form complexes. This interaction increases the degree of cross-linking, decreasing the solubility of proteins and making protein complexes less susceptible to proteolytic attack than the same protein alone (Alonso et al., 1998). Extrusion produced a higher increase in protein digestibility than the other processing methods. This can be related to the higher efficiency of this thermal treatment in reduction of anti-nutrient activities in addition to soaking and germination.

Enhancement of starch digestibility during soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility (Linko, Linko, & Olkku, 1983). The degree of starch gelatinisation of germinated samples Day 4 is higher than in Day 0 and it is thus more readily hydrolysed. The rupture of starch granules of sprouted quinoa, making the substrate more accessible, facilitated the amylolysis. On the other hand, reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility.

5.3.3 Box-Behnken design and analysis

This response surface methodology is an empirical modeling technique used to evaluate the relationship between the experimental and the predicted results (Muthukumarappan & Swamy, 2018). In the present study, the Box Behnken Design was used to obtain a proper model for the optimization of the extrusion process variables. Experiments were carried out according to the experimental design in order to explore the optimum conditions and study the influence of process variables on the physical and physico-chemical properties of the extrudates. The predicted values were attained by the model fitting technique in Design Expert software version 8.0.7.1 and the values correlated well with the experimental values. Fitting of the data to various models such as the linear, interactive, quadratic and cubic models was performed to acquire the regression equations. Sequential model sum of squares and model summary statistics tests were carried out to decide the adequacy of models in the present study and the results are presented in Table 5.2. From Table 5.2, it is observed that the quadratic model is highly significant for the extrusion of sprouted quinoa. The model summary statistics demonstrated that the quadratic model had maximum R-Squared, Adjusted R-Squared and Predicted R-Squared values. The cubic model was not suggested for the extrusion process since the BBD matrix had enough data to understand the outcome of the system. Hence, quadratic model was chosen for further analysis of data.

5.3.4 Fitting of polynomial equations and statistical analysis

Based on the Box-Behnken experimental design model, the empirical relationship between the input variables and experimental results obtained were expressed by a second-order polynomial equation with interaction terms (Swamy, Sangamithra, & Chandrasekar, 2014). The equations generated in coded factors are presented in Table 5.3.

The investigation and optimization of a fitted response surface might generate poor or disingenuous results. Therefore, it was essential to ensure the fitness of the model. Regression analysis and Pareto analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. The results pointed out that the equation sufficiently presented the actual relationship between the input parameters and responses. ANOVA is a statistical technique that subdivides the total variation in a dataset into component parts allied with sources of variation for testing hypotheses on the variables of the model (Swamy & Muthukumarappan, 2017).

ANOVA followed by Fisher's statistical test (F-test) was conducted to analyse the significance of each independent variable. The F-value is the ratio of the regression mean square and the real error mean. It indicates the influence of each controlled factor on the tested model. The ANOVA results in Table 5.4 shows the F-value for the responses, implying that the model is highly significant. The large Fisher value signifies that the variation in the responses can be explicated by the regression equation (Asokapandian, Venkatachalam, Swamy, & Kuppusamy, 2016). The associated p value estimates if F value is large enough to show statistical significance. The p values that are lower than 0.05 indicate that the model and the associated terms are statistically significant.

Coefficient of determination (R^2) and adjusted-R^2 were computed to verify the sufficiency and fitness of the model. The R^2 signifies the proportion of the total variation in the response expected by the model, thus indicating the ratio of the regression sum of squares (SSR) and total sum of squares (SST) (Sangamithra, Sivakumar, Kannan, & John, 2015). The values of R^2 imply that 95% of experimental data was well-suited. The high R^2 coefficient gives a satisfactory adjustment of the quadratic model to the experimental data. The purpose of the adjusted-R^2 is to analyse the model adequacy and fitness. The adjusted-R^2 value adjusts the R^2 value for the sample size and for the number of terms in the model (Albarracín, De Greef, González, & Drago, 2015). The high value of adjusted-R^2 supports a high correlation between the experimental and the predicted values. The high coefficient of determination and very small p-value (<0.0001) demonstrate that the quadratic polynomial model is significant and adequate to characterize the actual relationship between the response and input.

The coefficient of variation (CV) shows the scattering of the experimental points from the predicted values of the second order polynomial model (Swamy & Muthukumarappan, 2017). A high coefficient of variation points out that there is extreme variation in the mean value and does not adequately develop a sufficient model. The very low coefficient of variation value undoubtedly represents a high degree of precision and reliability of experiments conducted.

5.3.5 Selection of optimum conditions

Optimum conditions for the extrusion process were deduced to get desired extrudate properties. Second order polynomial models attained in the study were used for each response to acquire specified optimum conditions. To optimize the independent variables, the Derringer’s desirability function method was utilized. After satisfying the necessities for each response in the design, the Derringer's desirability function seeks for a combination of factor levels that together optimize a set of responses. By the conversion of each into a dimensionless desirability scale which defines a partial desirability function, combining the discrete desirability values to attain the global desirability function and then maximizing the global function and recognizing the optimal factor settings, the optimization process is accomplished. The desirability function scale operates between 0 and 1 with 0 signifying a completely undesirable response and 1 representing a fully desired response. The individual desirability for each response is achieved by stating the goals, i.e., minimize, maximize or target the response, and boundaries essential for each response. A weight factor (defines the shape of the desirability function for each response) is then allocated. The weights range between 0.1 and 10 with the larger ones relating to more significant responses. A weight factor of 1 was selected for the individual desirability in this research. The "importance" of the goals can be arranged sequentially according to increasing importance. It can vary from 1 (least importance) to 5 (most important). To optimize the process the following parameters (1) Moisture (15-25%) (2) Temperature (90-140°C) (3) Screw speed (80-220 rpm) and (4) Germination period (Day 0- Day 4) respectively, were set for maximum desirability. By applying the methodology of the desired function, the optimized process variables were obtained. It indicated that a moisture 15%, temperature 125°C, screw speed 179 rpm and Day 3 germinated flour input will produce an extrudate with bulk density 133.6 kg/m^3, hardness 1.56 N, water solubility index 15.3, water absorption index 3.4, $\Delta E=16.8$, ER=2.8, protein digestibility=83.8% and starch digestibility=83.2% respectively with overall desirability value of 0.955.

5.3.6 Verification of optimized conditions and predictive model

The fitness of the model equations for predicting optimum response values was investigated under the conditions: moisture 15%, temperature 125°C, screw speed 179 rpm and Day 3 germinated flour. These conditions were optimum by the RSM optimization approach. To verify the validity of the optimized conditions, experiments were performed to evaluate the experimental results vs predicted values of the output using the model equation. The experiments were carried out thrice and the average values were presented in Table 5.5. The results expected from the optimum solution was bulk density 133.6 kg/m^3, hardness 1.56 N, water solubility index 15.3, water absorption index 3.4, ΔE=16.8, expansion ratio=2.8, protein digestibility =83.8% and starch digestibility=83.2% and the experimental value was bulk density=133.6±0.05 kg/m^3, hardness=1.56±0.08N, water solubility index =15.3±0.09, water absorption index=3.4±0.04, ΔE=16.8±0.08, expansion ratio=2.8±0.09, protein digestibility=83.8±0.07% and starch digestibility=83.2±0.11%. The mean values obtained through the experiments were compared with the predicted values. The values obtained through confirmation experiments are within 95% of predicted values. This shows that the developed quadratic models are well-suited. It is also evident that the optimal values are valid within the specified range of process variables.

5.4 Conclusions

In this study, soaking, germination and extrusion procedures were used to reduce anti-nutrient levels and improve the protein and starch digestibility. The influence of feed moisture (15-25% w.b.), temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of quinoa extrudates were studied. Both feed moisture and extruder temperature had significant effects on the extrudate characteristics. Changing the level of moisture content and temperature had significant effect ($p<0.01$) on all physical properties. The total color change in extruded products ranged between 16.7 and 20.8. water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. Hardness ranged between 1.56 and 2.14 N and the coefficient of determination for hardness was high ($R^2 = 0.9$). Extruder screw speed had minimal effect on the extrudate properties. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility. By applying the methodology of the desired function, the optimized process variables were moisture 15%, temperature 125°C, screw speed 179 rpm and Day 3 germinated flour with bulk density 133.6 kg/m^3, hardness 1.56 N, water solubility index 15.3, water absorption index 3.4, ΔE=16.8, expansion ratio=2.8, protein digestibility=83.8% and starch digestibility=83.2% respectively with overall desirability value of 0.999. Overall, the quinoa studied in the range extrusion conditions showed poor expansion characteristics as compared to widely used cereal grains, suggesting that quinoa is not well suited for making direct expanded products.

Table 5.1: Impact of soaking and germination on the nutritional properties of quinoa

	Raw quinoa	Day 0	Day 1	Day 2	Day 3	Day 4
Total Protein[a]	13.1±0.5	13.1±0.15	13.2±0.03	13.3±0.05	13.4±0.01	13.1±0.07
Carbohydrates[a]	63.2±0.6	63.2±0.16	63.3±0.03	63.4±0.07	63.4±0.09	63.6±0.02
Moisture[a]	11.1±0.2	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid[b]	1.1±0.07	0.69±0.05	0.55±0.05	0.46±0.02	0.33±0.04	0.21±0.02
Saponin[b]	0.80±0.04	0.40±0.11	0.40±0.06	0.30±0.04	0.20±0.02	0.10±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 5.2: Results of model adequacy tested in the Box-Behnken design

Source	Sum of squares	DF	Mean square	F value	Prob > F	Remarks
Sequential model sum of squares for ***Bulk Density***						
Quadratic	452.29	3	150.76	138.09	< 0.0001	Suggested
Sequential model sum of squares for ***Hardness***						
Quadratic	0.094	3	0.031	323.86	< 0.0001	Suggested
Sequential model sum of squares for ***Water Solubility Index***						
Quadratic	10.36	3	3.45	59.40	< 0.0001	Suggested
Sequential model sum of squares for ***Water Absorption Index***						
Quadratic	0.053	3	0.018	243.46	< 0.0001	Suggested
Sequential model sum of squares for ***Total Color Difference ΔE***						
Quadratic	2.69	3	0.90	35.27	< 0.0001	Suggested
Sequential model sum of squares for ***Expansion Ratio***						
Quadratic	0.079	3	0.026	329.20	< 0.0001	Suggested
Sequential model sum of squares for ***Protein Digestibility***						
Quadratic	2.11	3	0.70	51.87	< 0.0001	Suggested
Sequential model sum of squares for ***Starch Digestibility***						
Quadratic	5.28	3	1.76	8.74	< 0.0001	Suggested

Source	**Std. Dev.**	**R-squared**	**Adjusted R-squared**	**Predicted R-squared**	**PRESS**	
Model summary statistics for ***Bulk Density***						
Quadratic	1.08	0.9913	0.9877	0.9763	187.13	Suggested
Model summary statistics for ***Hardness***						
Quadratic	0.0098	0.9969	0.9956	0.9921	0.015	Suggested
Model summary statistics for ***Water Solubility Index***						
Quadratic	0.24	0.9534	0.9337	0.8737	9.30	Suggested
Model summary statistics for ***Water Absorption Index***						
Quadratic	0.0085	0.9962	0.9946	0.9897	0.012	Suggested
Model summary statistics for ***Total Color Difference*** ΔE						
Quadratic	0.16	0.9745	0.9636	0.9474	3.09	Suggested
Model summary statistics for ***Expansion Ratio***						
Quadratic	0.0089	0.9969	0.9956	0.9919	0.012	Suggested
Model summary statistics for ***Protein Digestibility***						
Quadratic	0.12	0.9933	0.9905	0.9856	1.73	Suggested
Model summary statistics for ***Starch Digestibility***						
Quadratic	0.45	0.9202	0.8864	0.7932	30.76	Suggested

+ case(s) with leverage of 1.0000: PRESS statistic not defined

Table 5.3: Second order polynomial equations representing the relationship between independent and dependent variables

Bulk Density	$145.00 + 11.93A - 5.05B - 2.57C + 3.00D_1 + 1.06D_2 - 0.059D_3 - 1.18D_4 - 1.55AB - 0.30AC + 0.20AD_1 - 0.050AD_2 + 0.075AD_3 - 0.050AD_4 - 0.050BC - 0.82BD_1 - 0.20BD_2 + 0.050BD_3 + 0.30BD_4 - 0.17CD_1 - 0.050CD_2 + 0.075CD_3 - 0.050CD_4 + 3.40A^2 + 2.45B^2 + 1.40C^2$
Hardness	$1.75 + 0.20A - 0.064B - 0.030C + 0.029D_1 + 0.011D_2 - 1.059E{-}003D_3 - 0.013D_4 - 9.000E{-}003AB - 4.000E{-}003AC + 2.000E{-}003AD_1 - 5.000E{-}004AD_2 - 5.000E{-}004AD_3 - 5.000E{-}004AD_4 + 5.000E{-}004BC - 1.000E{-}002BD_1 - 2.500E{-}003BD_2 + 1.250E{-}003BD_3 + 5.000E{-}003BD_4 - 1.500E{-}003CD_1 + 1.000E{-}003CD_2 - 2.500E{-}004CD_3 - 1.500E{-}003CD_4 + 0.045A^2 + 0.036B^2 + 0.026C^2$
Water Solubility Index	$15.60 + 1.10A - 0.25B - 0.16C + 0.12D_1 + 0.059D_2 - 0.059D_4 - 0.53AB - 0.15AC + 0.25AD_1 + 0.1AD_2 - 0.11AD_4 - 0.040BC - 0.020BD_1 - 7.500E{-}003BD_2 + 5.000E{-}003BD_3 + 5.000E{-}003BD_4 - 0.015CD_1 - 2.500E{-}003CD_2 + 1.000E{-}002CD_3 - 2.500E{-}003CD_4 + 0.56A^2 + 0.23B^2 + 0.28C^2$
Water Absorption Index	$3.25 - 0.15A + 0.054B + 0.025C - 0.028D_1 - 9.647E{-}003D_2 + 9.412E{-}004D_3 + 0.013D_4 + 4.000E{-}003AB + 2.000E{-}003AC - 2.500E{-}004AD_1 + 1.000E{-}003AD_2 - 2.500E{-}004AD_3 - 2.500E{-}004AD_4 + 3.500E{-}003BC + 0.010BD_1 + 1.250E{-}003BD_2 - 3.750E{-}003BD_4 + 2.750E{-}003CD_1 + 2.500E{-}004CD_2 + 2.500E{-}004CD_3 - 1.000E{-}003CD_4 - 0.030A^2 - 0.033B^2 - 0.017C^2$
Total Color Difference ΔE	$17.70 + 0.73A - 0.37B - 0.13C + 1.11D_1 - 0.20D_2 - 0.25D_3 - 0.30D_4 - 0.030AB - 0.030AC + 0.013AD_1 + 0.065BC - 0.27BD_1 + 0.042BD_2 + 0.055BD_3 + 0.067BD_4 - 0.12CD_1 + 7.500E{-}003CD_2 + 0.020CD_3 + 0.033CD_4 + 0.19A^2 + 0.22B^2 + 0.17C^2$

Expansion Ratio	$2.65 -0.17A +0.061B +0.027C -0.029D_1 -9.882E\text{-}003D_2 +1.294E\text{-}003D_3 +0.013D_4 +7.500E\text{-}003AB +2.500E\text{-}003AC +7.000E\text{-}003BC +5.500E\text{-}003BD_1 +1.750E\text{-}003BD_2 -7.500E\text{-}004BD_3 -2.000E\text{-}003BD_4 -5.000E\text{-}004CD_1 +7.500E\text{-}004CD_2 +7.500E\text{-}004CD_3 -5.000E\text{-}004CD_4 -0.035A^2 -0.040B^2 -0.024C^2$
Protein Digestibility	$82.18 -1.03A +0.47B +0.22C -1.70D_1 +0.26D_2 +0.37D_3 +0.48D_4 -5.000E\text{-}003AB -0.030AC +0.25AD_1 -0.060AD_2 -0.073AD_3 -0.060AD_4 +0.07BC +0.25BD_1 -0.035BD_2 -0.048BD_3 -0.073BD_4 +0.05CD_1 -0.012CD_4 -0.098A^2 -0.23B^2 -0.17C^2$
Starch Digestibility	$81.94 -1.16A +0.49B +0.19C -1.65D_1 +0.11D_2 +0.41D_3 +0.51D_4 +0.080AB +0.06AC -0.35AD_1 +0.085AD_2 +0.11AD_3 +0.072AD_4 -0.01BC -0.13BD_1 +0.33BD_2 -0.053BD_3 -0.065BD_4 +0.53CD_1 -0.39CD_2 -0.057CD_3 -0.033CD_4 -0.41A^2 -0.19B^2 -0.16C^2$

A – Moisture Content (%w.b.); B – Screw Speed (rpm); C – Die Temperature; D_1, D_2, D_3, D_4 – Germination time (Days)

Table 5.4: Pareto analysis of variance (ANOVA) analysis and significant statistical parameters of the model

Source	Sum of squares	DF	Mean square	F-value	p-value
Bulk Density					
Model	7818.36	25	312.73	270.45	< 0.0001
A-moisture	5688.23	1	5688.23	4919.20	< 0.0001
B- temp	1020.10	1	1020.10	882.19	< 0.0001
C- screw speed	265.23	1	265.23	229.37	< 0.0001
D – germ period	331.18	4	82.79	71.60	< 0.0001
AB	48.05	1	48.05	41.55	< 0.0001
A^2	243.37	1	243.37	210.47	< 0.0001
B^2	126.37	1	126.37	109.28	< 0.0001
C^2	41.26	1	41.26	35.68	< 0.0001
C.V. %	0.72				
Hardness					
Model	1.85	25	0.074	769.68	< 0.0001
A-moisture	1.53	1	1.53	15861.35	< 0.0001
B- temp	0.16	1	0.16	1686.58	< 0.0001
C- screw speed	0.035	1	0.035	367.3	< 0.0001
D – germ period	0.031	4	0.007	80.48	< 0.0001
A^2	0.042	1	0.042	437.40	< 0.0001
B^2	0.028	1	0.028	287.02	< 0.0001
C^2	0.015	1	0.015	150.51	< 0.0001
C.V. %	0.55				

Water Solubility Index					
Model	70.22	25	2.81	48.31	< 0.0001
A-moisture	48.40	1	48.40	832.47	< 0.0001
B- temp	2.60	1	2.60	44.74	< 0.0001
C- screw speed	1.02	1	1.02	17.61	< 0.0001
A^2	6.72	1	6.72	115.59	< 0.0001
B^2	1.16	1	1.16	20.00	< 0.0001
C^2	1.59	1	1.59	27.38	< 0.0001
C.V. %	1.50				
Water Absorption Index					
Model	1.14	25	0.045	622.14	< 0.0001
A-moisture	0.91	1	0.91	12488.41	< 0.0001
B- temp	0.12	1	0.12	1582.38	< 0.0001
C- screw speed	0.025	1	0.025	355.51	< 0.0001
D – germ period	0.028	4	0.007	96.88	< 0.0001
A^2	0.019	1	0.019	255.14	< 0.0001
B^2	0.023	1	0.023	318.70	< 0.0001
C^2	0.006	1	0.006	85.78	< 0.0001
C.V. %	0.27				

Total Color Difference ΔE					
Model	57.18	25	2.29	90.06	< 0.0001
A-moisture	21.03	1	21.03	827.78	< 0.0001
B- temp	5.40	1	5.40	212.69	< 0.0001
C- screw speed	0.70	1	0.70	27.65	< 0.0001
D – germ period	26.35	4	6.59	259.35	< 0.0001
BD	0.75	4	0.19	7.35	< 0.0001
A^2	0.74	1	0.74	29.14	< 0.0001
B^2	1.04	1	1.04	41.03	< 0.0001
C^2	0.63	1	0.63	24.66	< 0.0001
C.V. %	0.89				
Expansion Ratio					
Model	1.51	25	0.06	753.68	< 0.0001
A-moisture	1.22	1	1.22	15267.79	< 0.0001
B- temp	0.15	1	0.15	1839.89	< 0.0001
C- screw speed	0.029	1	0.029	356.74	< 0.0001
D – germ period	0.029	4	0.007	89.06	< 0.0001
A^2	0.026	1	0.026	321.43	< 0.0001
B^2	0.033	1	0.033	409.39	< 0.0001
C^2	0.013	1	0.013	157.50	< 0.0001
C.V. %	0.34				

Protein Digestibility					
Model	119.13	25	4.77	351.16	< 0.0001
A-moisture	42.23	1	42.23	3112.08	< 0.0001
B- temp	8.93	1	8.93	658.10	< 0.0001
C- screw speed	2.03	1	2.03	149.23	< 0.0001
D – germ period	62.39	4	15.60	1149.43	< 0.0001
AD	0.64	4	0.16	11.76	< 0.0001
BD	0.66	4	0.16	12.09	< 0.0001
B^2	1.09	1	1.09	80.30	< 0.0001
C^2	0.63	1	0.63	46.16	< 0.0001
C.V. %	0.14				
Starch Digestibility					
Model	136.87	25	5.47	27.21	< 0.0001
A-moisture	53.82	1	53.82	267.51	< 0.0001
B-temp	9.60	1	9.60	47.73	< 0.0001
D – germ period	60.50	1	15.13	75.17	< 0.0001
A^2	3.54	1	3.54	17.59	< 0.0001
C.V. %	0.55				

Table 5.5: Predicted and experimental values of the responses at optimum conditions

Moisture (%) = 15; Temperature (°C) = 125; Screw Speed (rpm) = 179; Germination = Day 3

	Optimized values[a] (predicted values)	Experimental values[b]
Bulk density(kg/m^3)	133.6	133.6±0.05
Hardness (N)	1.56	1.56±0.08
Water Solubility Index (%)	15.3	15.3±0.09
Water absorption Index	3.4	3.4±0.04
Total Color Difference ΔE	16.8	16.8±0.08
Expansion Ratio	2.8	2.8±0.09
Protein Digestibility (%)	83.8	83.8±0.07
Starch Digestibility (%)	83.2	83.2±0.11

a Predicted using response surface quadratic model

b Mean ± standard deviation of triplicate determinations from experiments

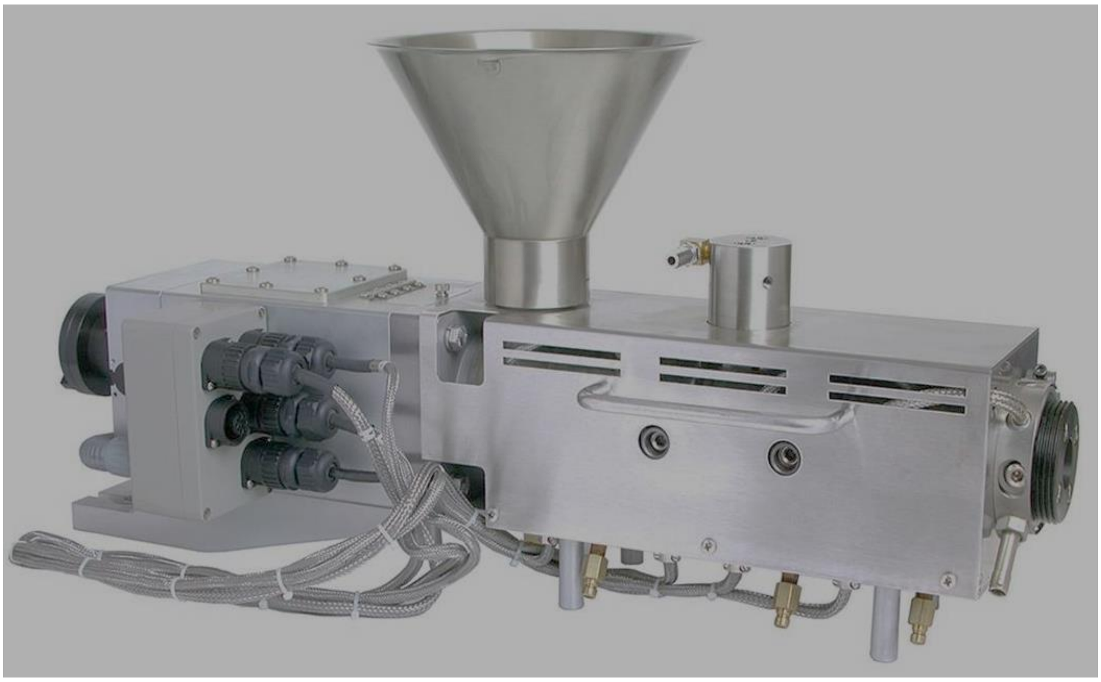


Figure 5.1: Counter rotating conical twin screw laboratory extruder

(Brabender CTSE, NJ)

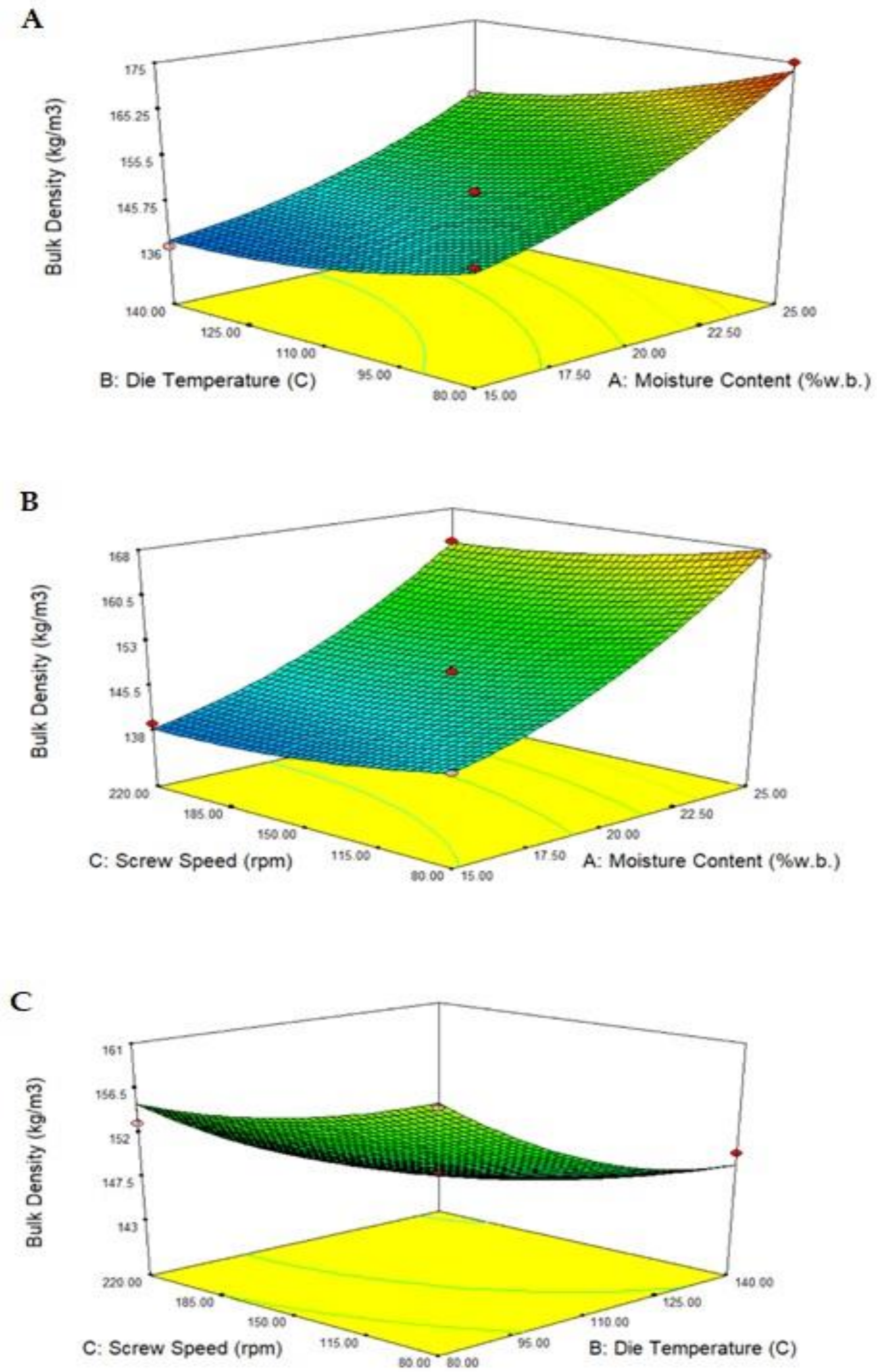


Figure 5.2: Response surface plots (3D) showing the effects on the Bulk Density (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

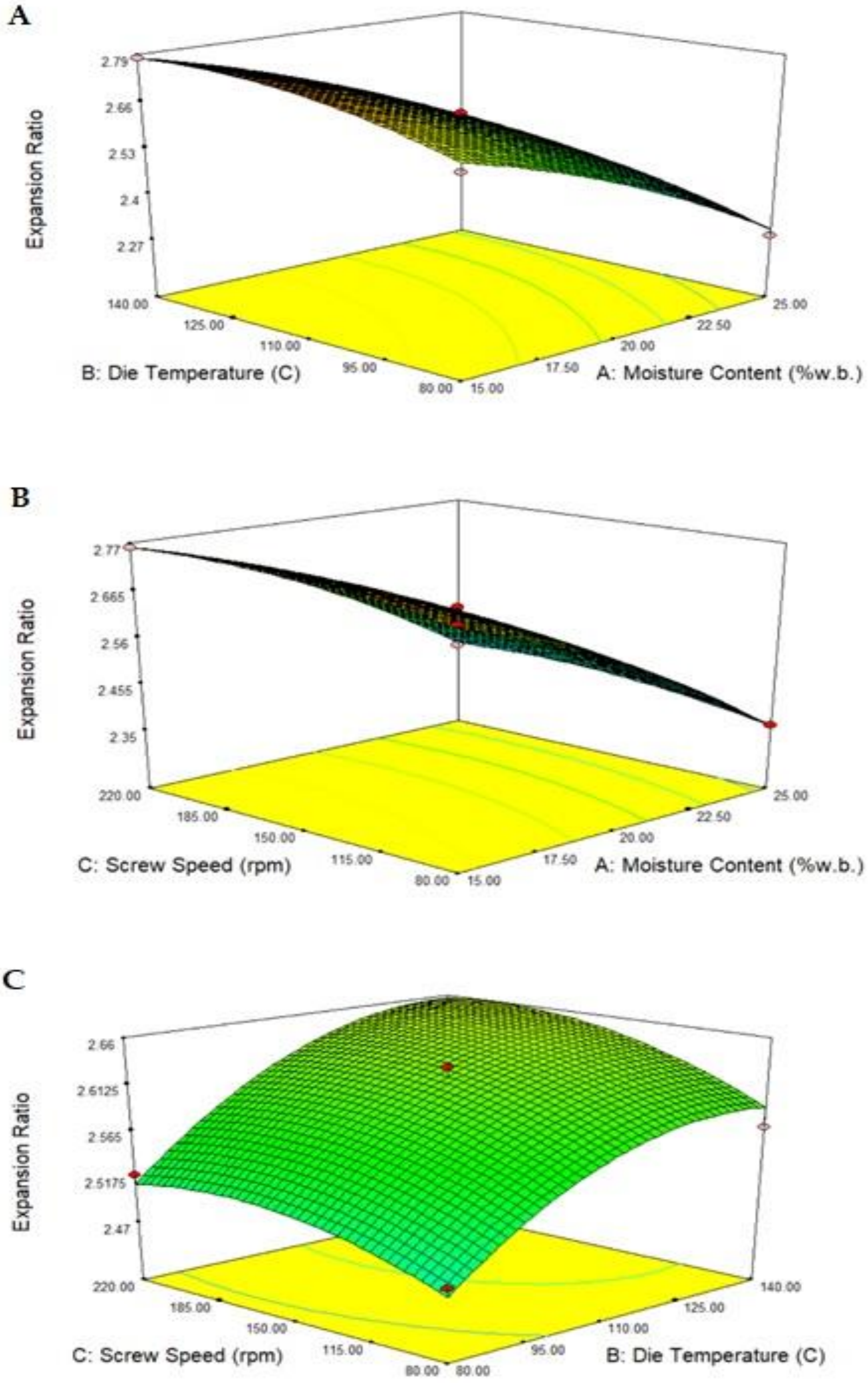


Figure 5.3: Response surface plots (3D) showing the effects on the Expansion Ratio (A) Moisture Content and Screw speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

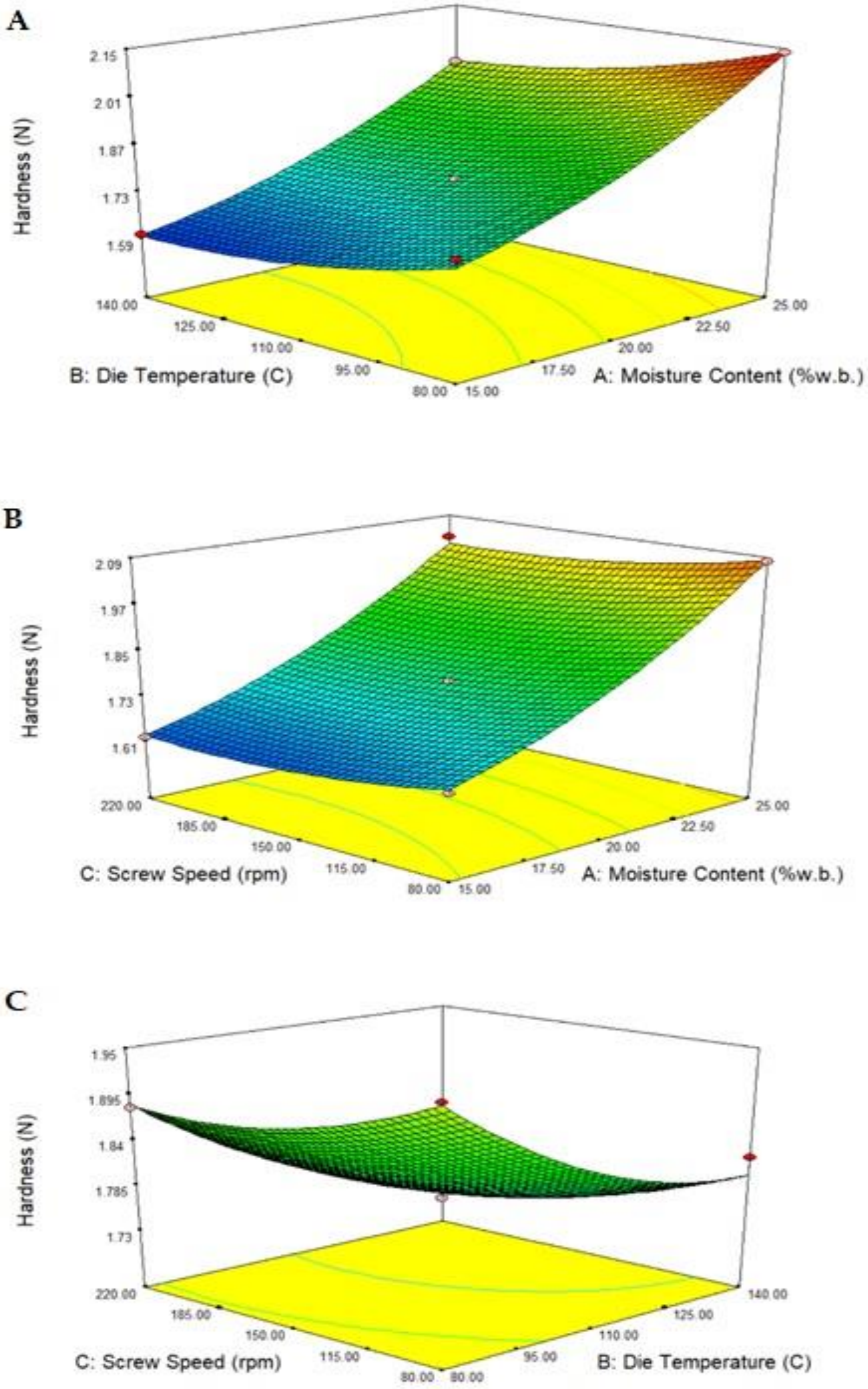


Figure 5.4: Response surface plots (3D) showing the effects on the Hardness (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

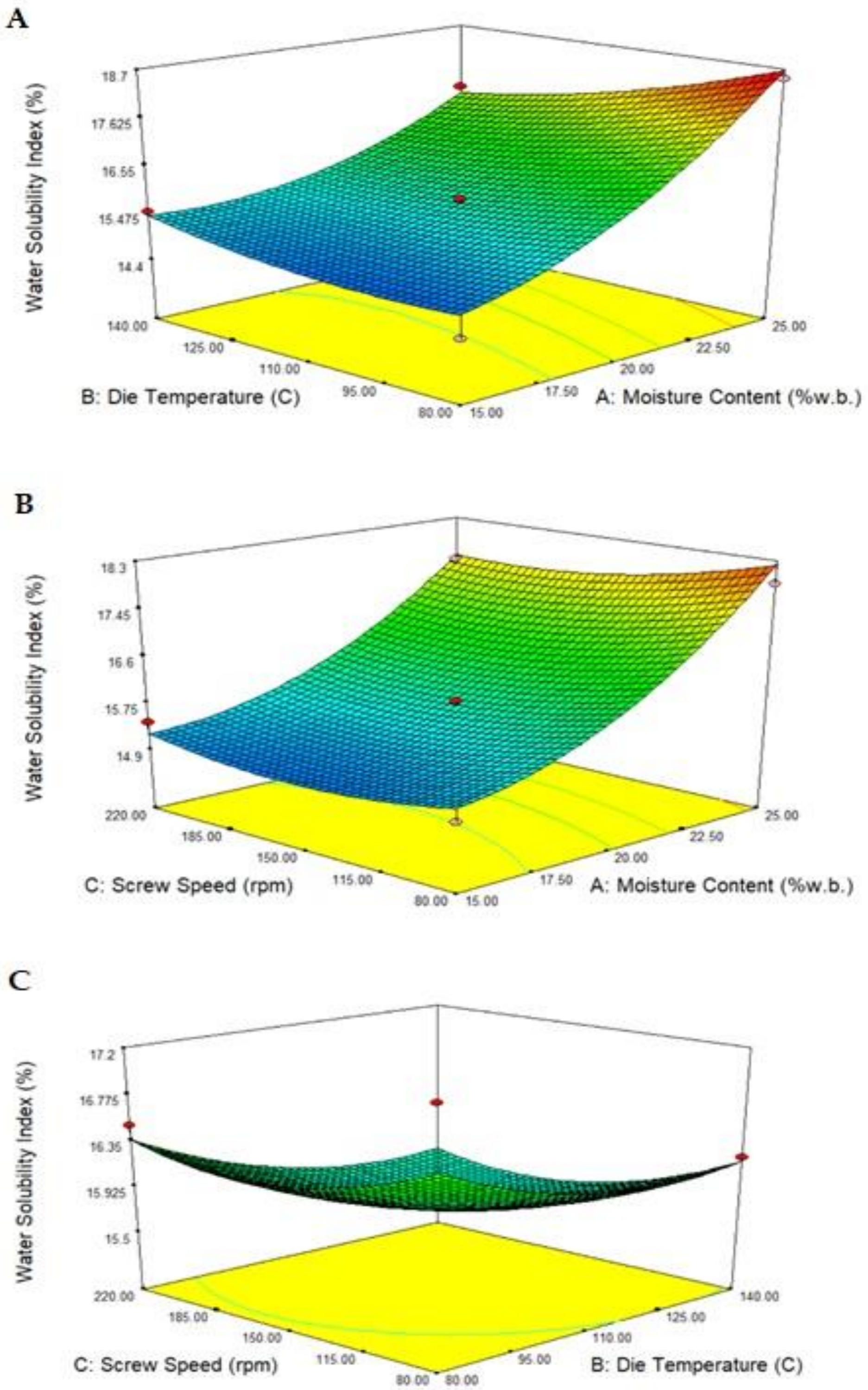


Figure 5.5: Response surface plots (3D) showing the effects on the Water Solubility Index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

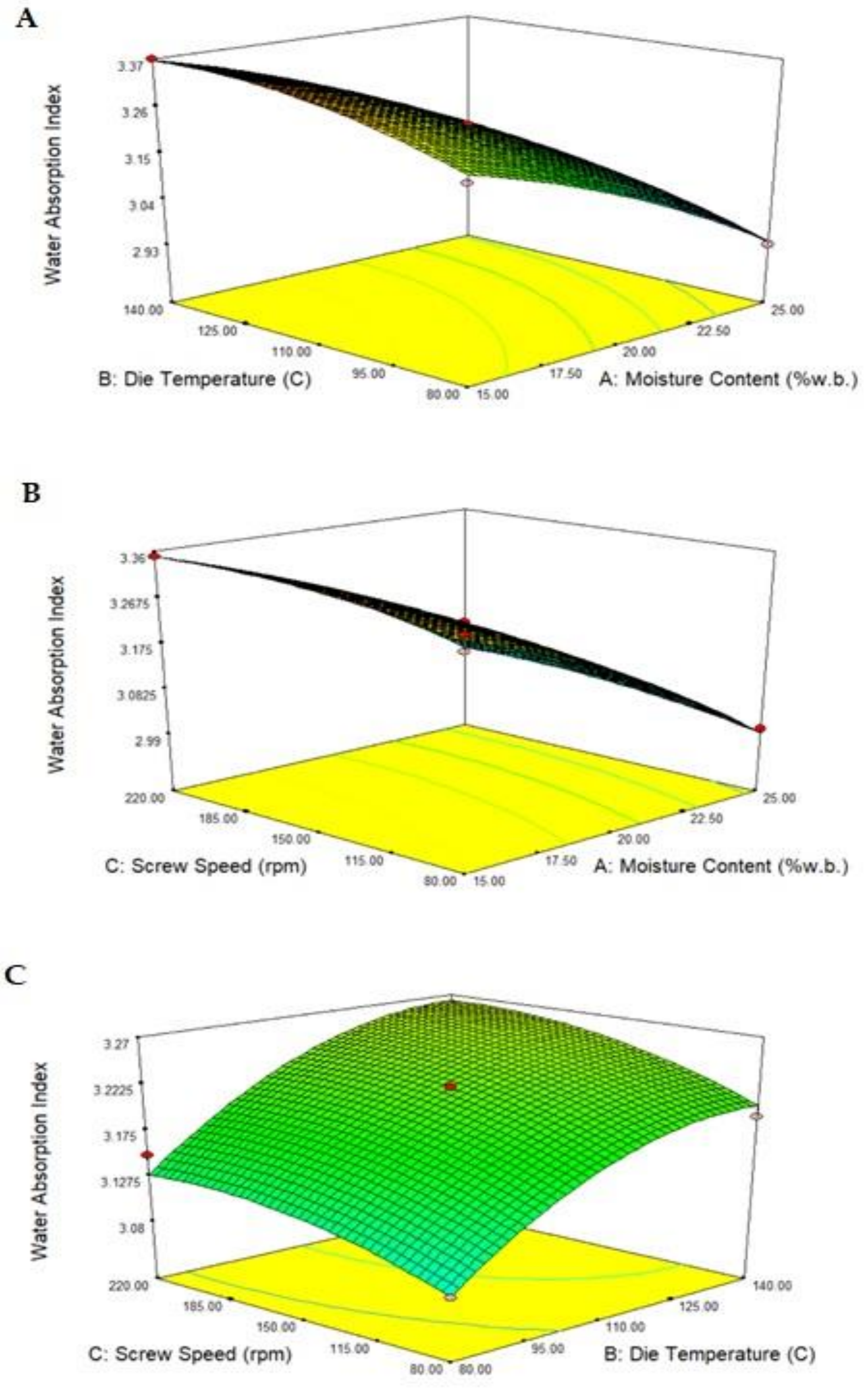


Figure 5.6: Response surface plots (3D) showing the effects on the Water Absorption Index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

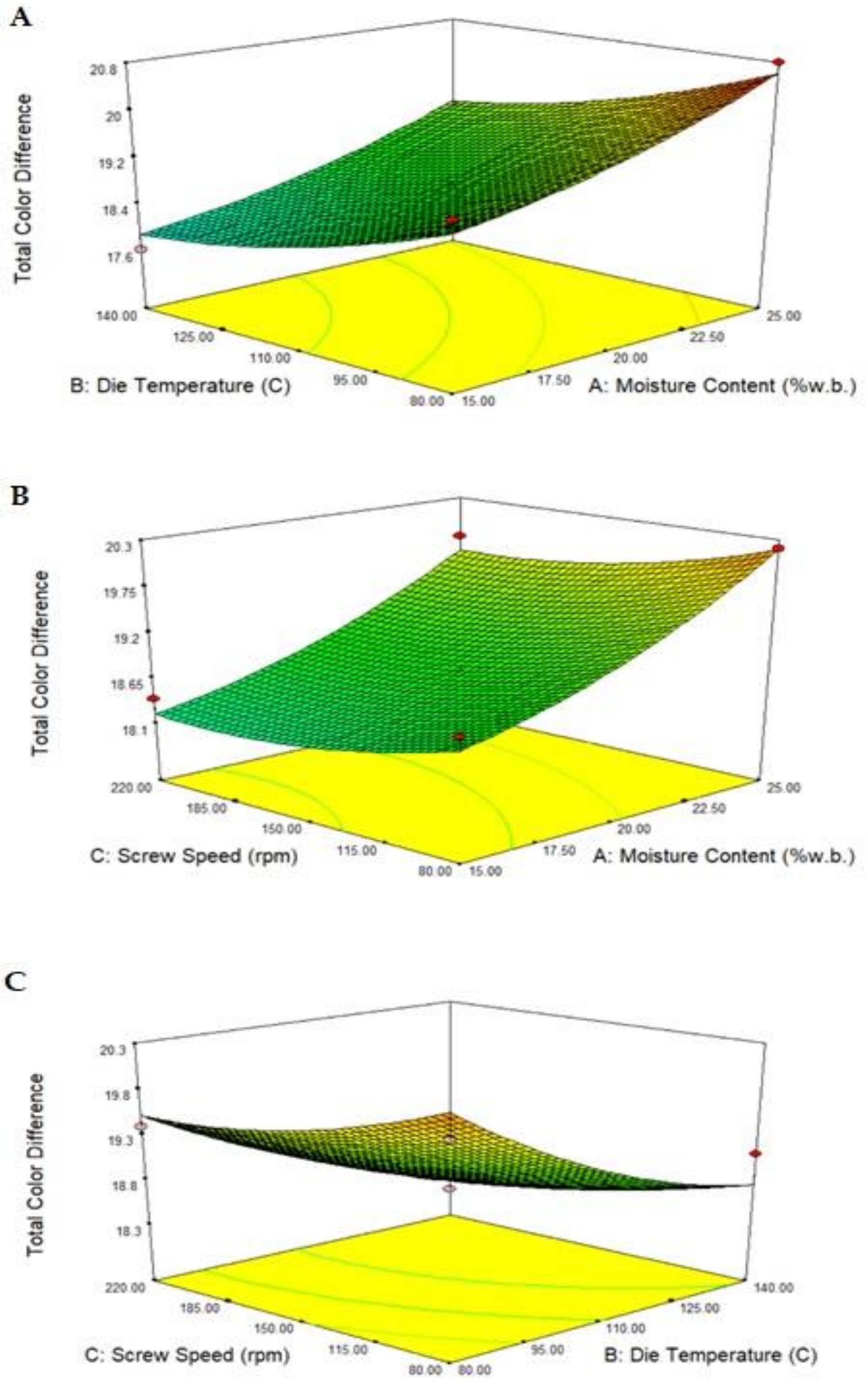


Figure 5.7: Response surface plots (3D) showing the effects on the Total Color Difference (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

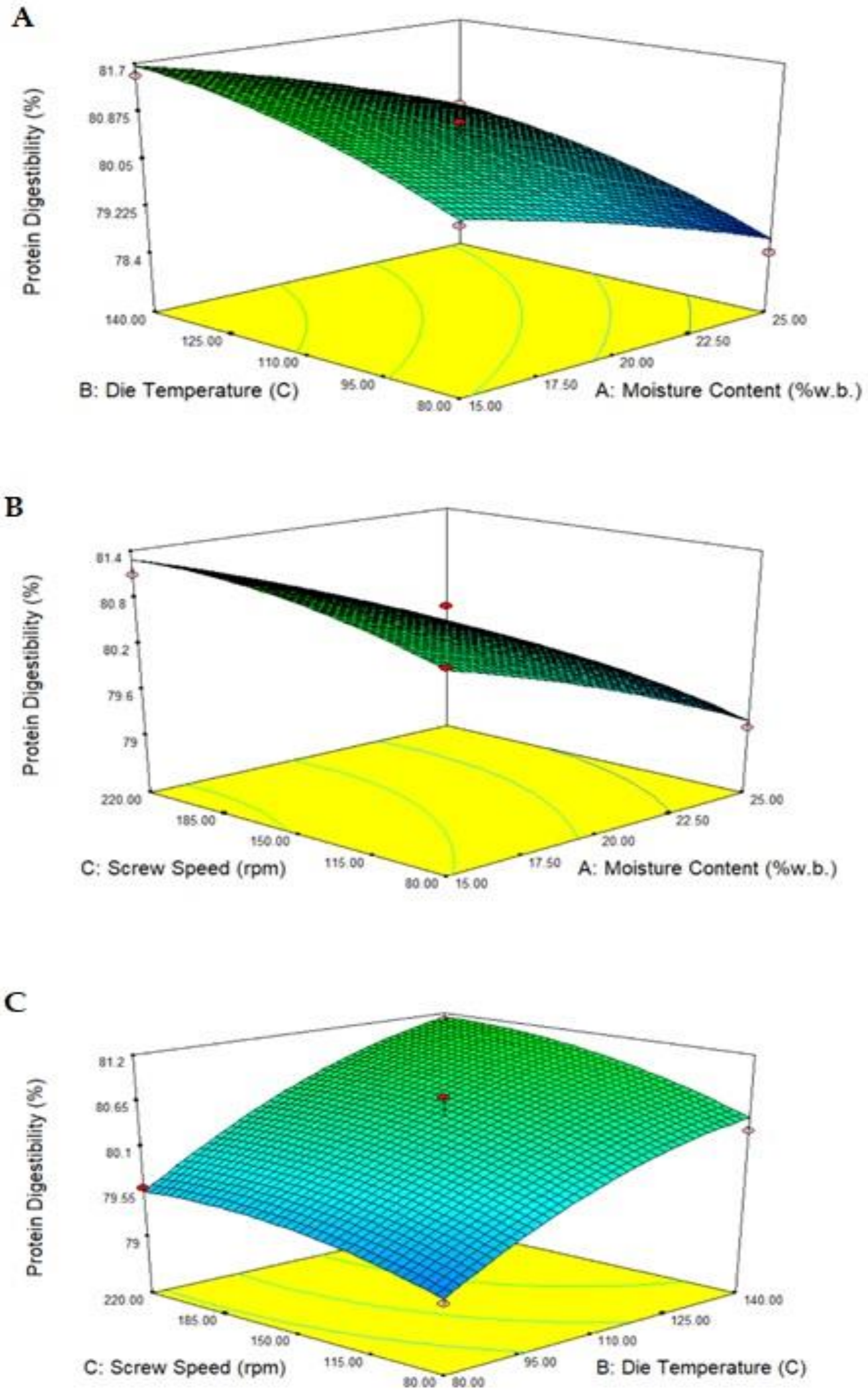


Figure 5.8: Response surface plots (3D) showing the effects on the Protein Digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

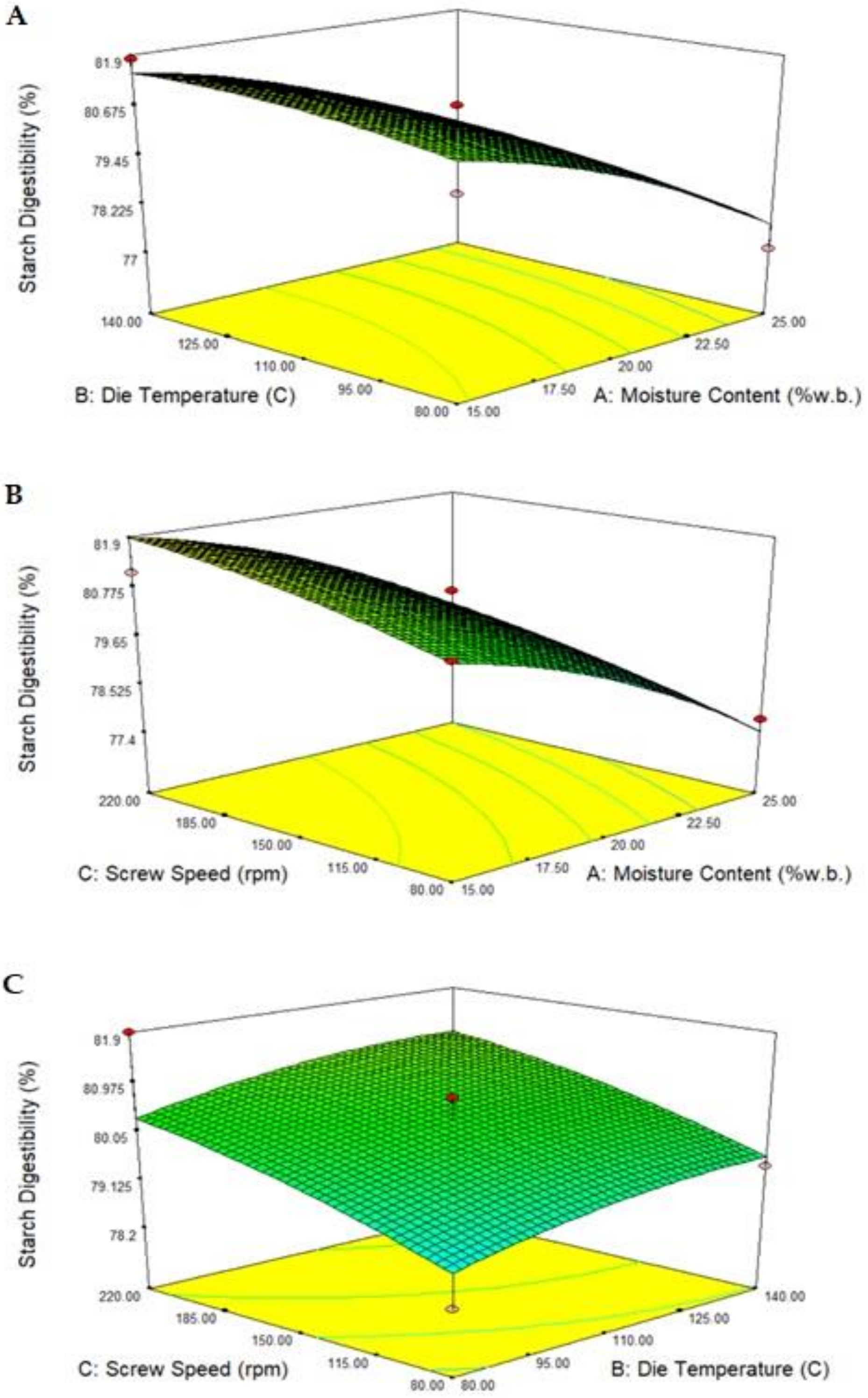


Figure 5.9: Response surface plots (3D) showing the effects on the Starch Digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

CHAPTER 6: EFFECT OF SINGLE-SCREW EXTRUSION PROCESS PARAMETERS ON THE PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF SPROUTED PROSO MILLET EXTRUDATES

Abstract

Soaking and germination pre-treatments of proso millet were carried out to reduce the phytic acid content and therefore increase protein and starch digestibility. Single-screw extrusion processing of sprouted proso millet flour was studied using a Box Behnken response surface design to understand the influence of feed moisture (15-25% w.b.), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of proso millet extrudates. The following responses of the extrudates were measured and the values ranged from: bulk density (101-137 kg/m^3), hardness (1.01-1.3N), water solubility index (14.8-18.7%), water absorption index (4.0-4.41), ΔE (13.0-17.1), expansion ratio (3.28-3.75), protein digestibility (78.2-86.6%) and starch digestibility (80.9-87.7%). Both feed moisture and extruder die temperature had statistically significant effects on all the extrudate characteristics. Extruder screw speed had minimal effect on the extrudate properties. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility.

Keywords: Proso Millet, Gluten-Free, Soaking, Germination, Phytic Acid, Digestibility

6.1. Introduction

Millets are small-seeded annual cereals grown for food, feed, forage, and fuel (D. D. Baltensperger, 2002). Millet ranks sixth among the world’s most important cereal grains, sustaining more than one-third of the world’s population (Bough, Colosi, & Cavers, 1986). Commonly cultivated millet species include proso millet (*Panicum miliaceum*), pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum setaceum*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*), and barnyard millet (*Echinochloa utilis*) (Colosi & Schaal, 1997). Proso millet is a summer annual grass. Grains are round, about 3 mm long and 2 mm wide, and enclosed in a smooth hull, which is typically white or creamy-white, yellow or red in color (Paridhi Gulati, Steven Weier, et al., 2016) White-seeded varieties are most often grown in the US followed by red-seeded varieties. Proso millet is among the preferred crops for planting after wheat in the Central Great Plains because it helps control weeds and conserve stored moisture. In 2019, the Central Great Plains states of Colorado, Nebraska, and South Dakota were the major producers of US (Romero et al., 2017). Other states producing significant quantities of proso millet include Kansas, Wyoming, Minnesota and North Dakota (Paridhi Gulati, Steven Weier, et al., 2016).

Millets are unique among the cereals because of their high calcium, iron, potassium, magnesium, phosphorous, zinc, dietary fiber, polyphenols, and protein content. Proso millet has the highest protein content among the various millets (Jones et al., 1970b). Millets are gluten-free, ideal for people who are gluten-intolerant, though millet flour cannot be used for raised bread. They contain a high amount of lecithin, which provides excellent support for nervous system health by helping to restore nerve cell function, regenerate myelin fiber, and intensify brain cell metabolism (Habiyaremye et al., 2017). Millets are also rich in micronutrients such as niacin, B-complex vitamins, Vitamin B6, and folic acid. Millets generally contain significant amounts of essential amino acids, particularly those containing sulfur (methionine and cysteine) (L. Zhang et al., 2014). Consumption of proso millet and other millets is associated with reduced risk of type-2 diabetes mellitus because whole grains like millet are a rich source of magnesium (Parameswaran & Sadasivam, 1994). Magnesium acts as a co-factor in several enzymatic reactions that regulate the secretion of glucose and insulin. Magnesium can also reduce the frequency of migraine headaches and heart attacks and is beneficial for people suffering from atherosclerosis and diabetic heart disease. Proso millet is used as bird feed predominantly but if it is converted to a value-added food product, farmers and food manufacturers would be benefitted. Because of its mild flavor, light color, gluten-free quality, and potential health benefits, proso millet has been receiving growing interest from the food industries in North America

Millet can be cooked and prepared in different ways. The grains can be boiled, steamed to make salad, or fully cooked like rice. Together with other ingredients, millet flour can be made into porridge. However, phytic acid poses an issue with cereals and pseudocereals. Phytic acid (myoinositol hexaphosphoric acid) reduces the availability of many minerals like iron, zinc, calcium and magnesium (Cheryan & Rackis, 1980). The formation of iron-phytate complexes of low solubility, in the small intestine is the basis for the interference of phytate with iron absorption. Although the iron content in cereals is usually high, the iron absorption from them is often poor due to the presence of high amounts of phytates. They are also found to inhibit the proteases and amylases of the intestinal tract. Soaking, sprouting and fermentation are some of the methods that can break down the phytic acid to increase the digestibility of the food as well as enhance mineral absorption (Egli et al., 2003).

Soaking and sprouting are an age-old technique however the benefits of these methods has not been completely exploited to increase the digestibility of food. Sprouted seeds are nutritionally superior to their respective seeds Germinated grains have higher levels of nutrients, lower levels of anti-nutrients such as phytic acid and flatulence-causing sugars, increased protein and starch digestibility, and increased bioavailability of some minerals (Carciochi et al., 2014). The inclusion of sprouted grains in the formulation of whole-grain-rich, fiber-rich, and gluten-free baked goods may provide a natural fortification and enrichment mechanism; it may improve nutritional quality because of the increased digestibility and bioavailability of some nutrients and the reduction of some anti-nutrients. Comprehensive reviews of the effects of germination on the nutrient composition of cereals and pseudocereals have been published (Carciochi et al., 2014) (Lombardi-Boccia et al., 1991) (Lorenz & D'Appolonia, 1980).

Sprouted grains have been consumed by people who have a strict diet schedule or by ones who have a rigorous exercise pattern. Not all people enjoy the flavour of a sprouted whole grain. Hence it is important to convert it to an interesting product before it reaches the consumer. Extrusion processing is a commonly used processing technology in the food industry with a wide number of applications. It is a processing system that utilizes a single screw or a set of screws to force food materials through a die opening. The food ingredients are subjected to high shear, temperature, and pressure for a short period of time (Gopirajah & Muthukumarappan, 2018). The role of the screw is to assist in imparting shear to the ingredient mixture and forcing the dough of the mixed ingredients out from the extruder through the die (Muthukumarappan & Karunanithy, 2012). The screws are also responsible for the build-up of pressure that occurs at the end of the extruder as well as added mixing of the ingredients. This helps to transform the ingredients from solid powders to a melt state inside of the extruder (Guy, 2001). The melted ingredients are then forced through a die at the end of the extruder into the atmosphere. The melt coming out of the extruder encounters a sudden drop in pressure, resulting in rapid expansion as well as a decrease in temperature, helping it to transform into a cooked product. The final quality and texture of the extruded product depends on various factors, including the ingredient mixture and its properties, extrusion processing conditions, and post-processing conditions (Harper & Clark, 1979). Because of the great flexibility of extrusion processing, it has found very diverse applications in the food industry. A single-screw extruder consists of only one screw housed in the barrel that often has a fluted or grooved design. Additionally, the screw in a single-screw extruder is usually designed with a decreasing pitch to create compression (Guy, 2001). The amount of decreasing pitch is referred to as the compression ratio. A twin-screw extruder has a pair of screws that are either intermeshing or non-intermeshing. The set of screws in the twin-screw extruder can be either co-rotating or counter-rotating. For any new material extrusion, a single screw extrusion is preferred because of the relatively low cost, straightforward design, ruggedness and favorable performance/cost ratio (Akdogan, 1999).

The effect of germination on the nutrition properties of grains has been studied by various authors (Donkor et al., 2012) (Hung et al., 2012) (Nelson et al., 2013). Similarly, the impact of extrusion variables on the proso millet extrudates have also been studied (P Gulati, S Weier, D Santra, J Subbiah, & DJ Rose, 2016). However, the method of producing highly digestible proso millet extrudates by removing phytic acid has not been researched. The objective of this research is to study the effect of germination on the changes in nutrients of proso millet and the impact of the single-screw extrusion variables such as moisture content of feed, temperature and screw speed of extruder on the physical and physico-chemical properties of extrudates made from the sprouted proso millet flour.

6.2 Materials and Methods

6.2.1 Raw materials

Proso millet seeds (white) were obtained from Foundation Seed Stock Agronomy, Horticulture and Plant Science department at South Dakota State University, SD. For the proximate composition analysis of proso millet seeds and flour, the following AACC methods were used: Moisture -Oven drying at 135°C (method no. 44–19), Ash - calcination at 550°C (method no. 08–01), Lipids - defatting in a Soxhlet apparatus with petroleum ether (method no. 30–25), Protein - micro Kjeldahl (N 6.25) (method no. 46–13), Carbohydrate – sugars and starch (method no. 80–04, 76-13) and total fiber - (method no. 32–07). Phytic acid content (hexaphosphate equivalents) were quantified using the method described by (Rathod & Annapure, 2017). All chemicals were purchased from Sigma–Aldrich, USA. All chemicals and reagents used were of analytical grade. All experiments were performed in triplicates.

6.2.2 Soaking and Germination

The proso millet (11 pounds) was soaked overnight (8 pm- 8 am) for 12 h in tap water (1:5 w/v) at room temperature. The soaked samples were then drained and washed. Excess water was drained, and the samples were wrapped in a moist cloth for 5 days (0 h – Day 0, 24 h - Day 1, 48 h - Day 2, 72 h – Day 3, 96 h – Day 4) at room temperature. All experiments were performed in triplicates.

6.2.3 Extrusion processing

The proso millet flour samples, each weighing around 200 g were randomly extruded using a 19.18 mm (0.755 in.) barrel inner diameter (i.d.), single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder® South Hackensack, NJ) as shown in Fig 6.1. It was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 200 rpm. The extruder had a barrel with length (L) to diameter (D) ratio of 20:1. A uniform 19.05 mm pitch screw having 381 mm (15.0 in.) screw length, a 19.05 mm (0.75 in.) constant outside (top of flight) diameter, a 3.81 mm (0.15 in.) initial screw feed depth, an 11.43 mm (0.45 in.) initial screw root diameter, and a screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiments. A die nozzle with 3 mm diameter was used. The extrudates were cooled to room temperature and sealed in polyethylene bags and stored at room temperature until measurements were taken.

6.2.4 Measurement of extrudate properties

6.2.4.1 Bulk Density

The bulk density (kg/m^3) was calculated as the ratio of the mass M (kg) to the volume V (m^3) of the measured and weighed extrudate sample. It was assumed that extrudates were of cylindrical shape. The bulk density of all 65 extrudates were measured and the measurements were done in triplicate.

$$Bulk\ Density = \frac{M}{V} \tag{6.1}$$

6.2.4.2 Expansion ratio (ER)

Expansion ratio was calculated by measuring the mean diameter of 10 randomly chosen locations on the extrudates with calipers for each extrusion process condition. Further it was divided by the die diameter of 3 mm.

$$ER = \frac{Mean\ diameter\ of\ the\ extrudate\ (mm)}{Die\ diameter\ (mm)} \tag{6.2}$$

6.2.4.3 Hardness

Hardness is defined as the maximum force per unit area of the proso millet extrudate that is necessary to breakdown the extrudate into two pieces. Hardness was tested by breaking the extrudate using a cylindrical probe (TA-4, Texture Technologies Corp, NY) attached to the crosshead of texture analyser. The sample was positioned in its natural position. A vertical force was applied at a speed of 50 mm/min. The force applied vs deformation was recorded and the maximum force recorded in the curve was considered as the hardness. The hardness of all 65 extrudates were measured and the measurements were done in triplicate.

6.2.4.4 Water Solubility Index and Water Absorption Index

The water absorption index and water solubility index of all extrudates were measured and the measurements were done in triplicate. The proso millet extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was mixed in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was mixed occasionally and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminium cup and dried at 135°C for 2h (AACC, 2000). The weight of the gel in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by:

$$Water\ Solubility\ Index(\%) = \frac{W_{ss}}{W_{ds}} * 100 \qquad (6.3)$$

$$Water\ Absorption\ Index = \frac{W_g}{W_{ds}} \qquad (6.4)$$

where, W_{ss} is the weight of dry solids of supernatant (g), W_{ds} is the weight of dry sample (g), and W_g is the weight of gel (g)

6.2.4.5 Color

The color of proso millet extrudate samples was measured using colorimeter (LabScan XE, HunterLab, Reston, VA) in triplicate in terms of Hunter Lab values

(L^*, a^*, b^*). The Hunter Lab value ‘L^*’ signifies lightness with 0 for dark and 100 for bright. The value ‘a^*’ measures the degree of green color in the range from -100 to 0 and red in the range 0 to 100. The ‘b^*’ value represents blue color in the range from -100 to 0 and yellow in the range from 0 to 100.The total color change (ΔE) is calculated as

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \qquad (6.5)$$

where $\Delta L = L^* - L_0$, $\Delta a = a^* - a_0$, and $\Delta b = b^* - b^*_0$; the subscript ‘‘0’’ represents initial color values before processing. All readings were taken in triplicate.

6.2.4.6 Starch and Protein Digestibility

The starch digestibility and protein digestibility of all 65 extrudates were measured and the measurements were done in triplicate. The proso millet extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The in-vitro starch digestibility was determined in the extrudate powder (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20 °C for 2 h according to the method of (Rathod & Annapure, 2017). At the end of the incubation period, 2 ml of 3,5-dini-trosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard.

A system consisting, 100 mg (0.1 g) dry and defatted samples were incubated at 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 °C for 3 h. Further it was neutralized with 7.5 ml 0.2N NaOH. Then 4 mg pancreatin in 7.5 ml phosphate buffer (pH 8) was added and incubated for 37 °C for 24 h, further 5 mg of TCA was added, centrifugated at 5000 rpm for 10 min. After that the supernatant dry residue at 40–50 °C was discarded and the micro Kjeldahl process for protein estimation was carried out. The percent in vitro protein digestibility was calculated using the following equation (Rathod & Annapure, 2017):

$$Protein\ digestibility\ (\%) = \frac{Total\ protein - Residual\ protein}{Total\ protein} * 100 \qquad (6.6)$$

6.2.5 Experimental Design and Statistical Analysis

Experiments were conducted using the Box Benhken design (BBD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA), consisting four factors namely moisture content (X_1), screw speed (X_2), temperature (X_3) and germination time (X_4). Three replicates were taken at the design center and the total number of observations were 75 [60 (not center points) and 15 (center points)]. Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN). The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA). The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

6.3 Results and Discussion

6.3.1 Effect of Soaking and Germination on the Sprouted Proso Millet extrudates

The nutritional properties of the sprouted proso millet are shown in Table 6.1. After soaking for 12 h, the white color hypocotyls appeared in the proso millet seeds on Day 1. The germination ratio was more than 90%. The protein content decreased significantly on Day 4 of germination and a simultaneous increase in the hypocotyl length was observed. The change in color of the sprouted flour indicates the breakdown of phytates. The optimised soaking time was 12 h and germination time was 96 h. In general, protein content of proso millet seeds ranges between 13.1% and 14.0% (James, 2009). The total protein content of proso millet is higher than that of grains such as rice, barley, corn, rye, sorghum and wheat (Navruz-Varli & Sanlier, 2016). The chief carbohydrate constituent of proso millet is starch, and it ranges from 62-64%. The total fiber was close to other grain products (7.0%-9.7%). Proso millet contains sugar by about 3%. It mostly contains maltose, D-galactose, and D-ribose in addition to low levels of fructose and glucose (Navruz-Varli & Sanlier, 2016).

The phytic acid concentration in proso millet seeds is comparable to whole grain rye flour (7.7 mg/g), whole grain wheat flour (8.7 mg/g), lentils (8.4 mg/g) and fava beans (8.0 mg/g). Phytic acid is not only present in the outer layers of proso millet, as in the case of rye and wheat but is also evenly distributed within the endosperm (Ahamed et al., 1998). Phytic acid binds minerals, such as Fe, Zn, Ca and Mg, and can make the mineral content of a cereal inadequate, especially for children (Khattak et al., 2007). Soaking and sprouting processes reduce the 'binding' effect of the phytic acid by activating the enzyme phytase (James, 2009). The amounts of phytic acid of the proso millet seeds obtained in this study are in concordance with the values for Cherry vanilla proso millet (Kowalski et al., 2016). A significant reduction in phytic acid content is observed with increase in germination time. Day 4 had more than 50% reduction in phytic acid. Extrusion further decreased the phytic acid content of pea flour (Alonso et al., 1998), bean flour (Anton et al., 2009) and other legumes (Lombardi-Boccia et al., 1991). Additionally, phytic acid content is expected to be reduced through either extrusion (Anuonye et al., 2010). The phytic acid content was significantly reduced by soaking and germination as shown in Table 6.1.

6.3.2 Effect of independent variables on the single-screw extrusion processing of sprouted proso millet

The results of the single screw extrusion process of sprouted proso millet along with the experimental design is presented in Appendix D.

6.3.2.1 Bulk Density

The bulk density of the extrudates varied between 101 and 137 kg/m^3. Low bulk density, which is a required characteristic of the extruded product, was achieved with low feed moisture content, average temperature and high screw speed combination. Fig 6.2 shows that feed moisture content had a significant effect on bulk density followed by screw speed, whereas temperature seemed to have a minor effect. Low product bulk density of 101 kg/m^3 was obtained at feed moisture of 15%, 115°C temperature, 220 rpm screw speed and Day 4 germinated samples.

6.3.2.2 Expansion ratio

The measured expansion ratio of proso millet extrudates ranged from 3.28 to 3.75. the expansion ratio was significantly affected by temperature and feed moisture content. High expansion ratio at low feed moisture content for expanded products is distinctive for cereals. However, Fig 6.3 shows that the temperature and screw speed also have a significant effect on the expansion ratio of the proso millet extrudates. Similar trends have been obtained for corn (Diaz et al., 2013), wheat (Pitts et al., 2014) and oats (Gopirajah & Muthukumarappan, 2018).

6.3.2.3 Hardness

Hardness, the maximum force to break the sample, ranged between 1.01 and 1.3 N. The coefficient of determination for hardness was high (R^2 = 0.97) and the moisture, temperature and germination period were found to have significant contributions to the hardness of the extrudates. At all feed moisture content levels, decrease in die temperature decreased the product hardness. Fig 6.4 shows the effect of moisture and screw speed on the product hardness. Previous studies have also reported similar trends for extrudates based on rice (Ding et al., 2006), wheat (Ding et al., 2006), oat bran and soy flour (Anuonye et al., 2010) and chickpea (Khattak et al., 2007). The hardness was high of 1.8 N at about 140°C, 150 rpm screw speed, 25% feed moisture content and Day 0 sprouted samples. Chemical changes occurring during extrusion cooking affect the development of textural and mechanical properties such as hardness in extrudates. The elastic swell effect and bubble growth effect equally contribute to the structural changes of starch during extrusion. Water performs as a plasticizer to the starch-based material decreasing its viscosity and the mechanical energy dissipation in the extruder. Hence the product becomes dense and bubble growth is compressed. The reduced starch conversion and compressed bubble growth result in a dense product and wetness of extrudate.

6.3.2.4 Water Solubility Index and Water Absorption Index

Water solubility index is related to the quantity of soluble solids, which is related to dextrinization. The coefficient of determination for water solubility index was highly significant as revealed by a high value of R^2 (0.98). The linear effects of feed moisture, screw speed and temperature had significant effect on water solubility index. in general, water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. fig 6.5 shows the significant effect of temperature and screw speed on water solubility index. the increase in water solubility index with increasing temperature was consistent with the results reported for oat extrudates (Gutkoski & El-Dash, 1999). The water solubility index decreased with the increase in moisture. Similar trends have been reported earlier for starch, maize grits, wheat and pea (Ding et al., 2006) (Singh et al., 2007). The poor correlation between water solubility index and most of the process and product responses is explained by the fact that water solubility index includes the opposing effects of starch dextrinization and the molecular level interactions between degraded components, which may not be favoured at the same condition (Lei et al., 2008). An increase in the amount of dextrinized starch during extrusion cooking results in an increase in water solubility index. however, molecular interactions between degraded starch, protein, and lipid components, which in turn lead to an increase in molecular weight, may decrease the solubility, thus water solubility index (Camire et al., 1990).

Water absorption index depends on the availability of hydrophilic groups and on the gel formation capacity of the macromolecules. It is a measure of damaged starch together with protein denaturation and new macromolecular complex formations. water absorption index of extrudates ranged between 4.00 and 4.41. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. The interaction effects except for die temperature-screw speed were significant as shown in Fig 6.6. Similar responses were observed in rice flour (Hagenimana et al., 2006), oats (Gutkoski & El-Dash, 1999), finger millet (Sawant et al., 2012) and cassava starch (Hashimoto et al., 2003). water absorption index had poor correlations with almost all process and product responses except product density. This is an expected result since it includes the effect of starch gelatinization, protein denaturation and molecular level cross-linking reactions which are not always favored at the same conditions (Doğan & Karwe, 2003).

6.3.2.5 Color

Color is an important quality parameter since it reflects the extent of chemical reactions and degree of cooking or degradation that take place during extrusion cooking. In this study, ΔE represents the total color difference compared to the color of unsoaked and unsprouted proso millet. Higher ΔE means darker products with more intense yellow and red color. The total color change in extruded products ranged between 13.0 and 17.1. Interaction effects of moisture content-screw speed and temperature-screw speed were found to have the highest contribution to total color change. As depicted in Fig 6.7, low feed moisture content and high temperature increased the total color change possibly due to increased extent of browning reactions under this condition. At low screw speeds a slight increase in color change observed due to longer residence times which might increase the extent of chemical reactions.

6.3.2.6 Starch Digestibility (SD) and Protein Digestibility (PD)

Enhancement of starch digestibility during soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility (Linko et al., 1983). The degree of starch gelatinisation of germinated samples Day 4 is higher than in Day 0 and it is thus more readily hydrolysed. The rupture of starch granules of sprouted proso millet, making the substrate more accessible, facilitated the amylolysis. On the other hand, reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility.

The results show that both protein digestibility and starch digestibility of the proso millet extrudates were increased significantly by soaking, germination and extrusion. Fig 6.8 and Fig 6.9 illustrate the effect of the independent variables on the protein and starch digestibility respectively. The linear effects of all the variables and interaction effect of moisture and screw speed had a significant effect on the protein and starch digestibility. Germination produced a more significant improvement of protein digestibility and starch digestibility in proso millet. The increases of digestibility produced by the different treatments were higher in protein than in starch. Improvement of protein digestibility after processing could be attributable to the reduction or elimination of different anti-nutrients. Phytic acid, as well as condensed tannins and polyphenols, are known to interact with protein to form complexes. This interaction increases the degree of cross-linking, decreasing the solubility of proteins and making protein complexes less susceptible to proteolytic attack than the same protein alone (Alonso et al., 1998). Extrusion produced a higher increase in protein digestibility than the other processing methods. This can be related to the higher efficiency of this thermal treatment in reduction of anti-nutrient activities in addition to soaking and germination.

6.3.3 Box-Behnken design and analysis

This response surface methodology is an empirical modeling technique used to evaluate the relationship between the experimental and the predicted results (Muthukumarappan & Swamy, 2018). In the present study, the Box Behnken Design was used to obtain a proper model for the optimization of the extrusion process variables. Experiments were carried out according to the experimental design in order to explore the optimum conditions and study the influence of process variables on the physical and physico-chemical properties of the extrudates. The predicted values were attained by the model fitting technique in Design Expert software version 8.0.7.1 and the values correlated well with the experimental values. Fitting of the data to various models such as the linear, interactive, quadratic and cubic models was performed to acquire the regression equations. Sequential model sum of squares and model summary statistics tests were carried out to decide the adequacy of models in the present study and the results are presented in Table 6.2. From Table 6.2, it is observed that the quadratic model is highly significant for the extrusion of sprouted proso millet. The model summary statistics demonstrated that the quadratic model had maximum R- Squared, Adjusted R-Squared and Predicted R-Squared values. The cubic model was not suggested for the extrusion process since the BBD matrix had enough data to understand the outcome of the system. Hence, quadratic model was chosen for further analysis of data.

6.3.4 Fitting of polynomial equations and statistical analysis

Based on the Box-Behnken experimental design model, the empirical relationship between the input variables and experimental results obtained were expressed by a second-order polynomial equation with interaction terms (Swamy et al., 2014). The equations generated in coded factors are presented in Table 6.3.

The investigation and optimization of a fitted response surface might generate poor or disingenuous results. Therefore, it was essential to ensure the fitness of the model. Regression analysis and Pareto analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. The results pointed out that the equation sufficiently presented the actual relationship between the input parameters and responses. ANOVA is a statistical technique that subdivides the total variation in a dataset into component parts allied with sources of variation for testing hypotheses on the variables of the model (Swamy & Muthukumarappan, 2017).

ANOVA followed by Fisher’s statistical test (F-test) was conducted to analyse the significance of each independent variable. The F-value is the ratio of the regression mean square and the real error mean. It indicates the influence of each controlled factor on the tested model. The ANOVA results in Table 6.4 shows the F-value for the responses, implying that the model is highly significant. The large Fisher value signifies that the variation in the responses can be explicated by the regression equation (Asokapandian et al., 2016). The associated p value estimates if F value is large enough to show statistical significance. The p values that are lower than 0.0001 indicate that the model and the associated terms are statistically significant.

Coefficient of determination (R^2) and adjusted-R^2 were computed to verify the sufficiency and fitness of the model. The R^2 signifies the proportion of the total variation in the response expected by the model, thus indicating the ratio of the regression sum of squares (SSR) and total sum of squares (SST) (Sangamithra et al., 2015). The values of R^2 imply that 95% of experimental data was well-suited. The high R^2 coefficient gives a satisfactory adjustment of the quadratic model to the experimental data. The purpose of the adjusted-R^2 is to analyse the model adequacy and fitness. The adjusted-R^2 value adjusts the R^2 value for the sample size and for the number of terms in the model (Albarracín et al., 2015). The high value of adjusted-R^2 supports a high correlation between the experimental and the predicted values. The high coefficient of determination and very small p-value (<0.0001) demonstrate that the quadratic polynomial model is significant and adequate to characterize the actual relationship between the response and input.

The coefficient of variation (CV) shows the scattering of the experimental points from the predicted values of the second order polynomial model (Swamy & Muthukumarappan, 2017). A high coefficient of variation points out that there is extreme variation in the mean value and does not adequately develop a sufficient model. The very low coefficient of variation value undoubtedly represents a high degree of precision and reliability of experiments conducted.

6.3.5 Selection of optimum conditions

Optimum conditions for the extrusion process were deduced to get desired extrudate properties. Second order polynomial models attained in the study were used for each response to acquire specified optimum conditions. To optimize the independent variables, the Derringer's desirability function method was utilized. After satisfying the necessities for each response in the design, the Derringer's desirability function seeks for a combination of factor levels that together optimize a set of responses. By the conversion of each into a dimensionless desirability scale which defines a partial desirability function, combining the discrete desirability values to attain the global desirability function and then maximizing the global function and recognizing the optimal factor settings, the optimization process is accomplished. The desirability function scale operates between 0 and 1 with 0 signifying a completely undesirable response and 1 representing a fully desired response. The individual desirability for each response is achieved by stating the goals, i.e., minimize, maximize or target the response, and boundaries essential for each response. A weight factor (defines the shape of the desirability function for each response) is then allocated. The weights range between 0.1 and 10 with the larger ones relating to more significant responses. A weight factor of 1 was selected for the individual desirability in this research. The "importance" of the goals can be arranged sequentially according to increasing importance. It can vary from 1 (least importance) to 5 (most important). To optimize the process the following parameters (1) Moisture (15-25%) (2) Temperature (90-140°C) (3) Screw speed (80-220 rpm) and (4) Germination period (Day 0- Day 4) respectively, were set for maximum desirability. By applying the methodology of the desired function, the optimized process variables were obtained. It indicated that a moisture 15%, temperature 110°C, screw speed 120 rpm and Day 4 germinated flour input will produce an extrudate with bulk density107.7 kg/m^3, hardness 1.02 N, water solubility index 18.2, water absorption index 4.3, ΔE=16.4, expansion ratio=3.7, protein digestibility=86.2% and starch digestibility=87.6% respectively with overall desirability value of 1.

6.3.6 Verification of optimized conditions and predictive model

The fitness of the model equations for predicting optimum response values was investigated under the conditions: moisture content 15%, temperature 110°C, screw speed 120 rpm and Day 4 germinated flour. These conditions were optimum by the RSM optimization approach. To verify the validity of the optimized conditions, experiments were performed to evaluate the experimental results vs predicted values of the output using the model equation. The experiments were carried out thrice and the average values were presented in Table 6.5. The results expected from the optimum solution was with bulk density107.7 kg/m^3, hardness 1.02 N, water solubility index 18.2, water absorption index 4.3, ΔE=16.4, expansion ratio=3.7, protein digestibility=86.2% and starch digestibility=87.6% and the experimental value was bulk density=107.8±0.02 kg/m^3, hardness=1.02±0.06 N, water solubility index=18.2±0.03, water absorption index=4.3±0.09, ΔE=16.4±0.07, expansion ratio=3.7±0.08, protein digestibility=86.2±0.05% and starch digestibility=87.6±0.05%. The mean values obtained through the experiments were compared with the predicted values. The values obtained through confirmation experiments are within 95% of predicted values. This shows that the developed quadratic models are well-suited. It is also evident that the optimal values are valid within the specified range of process variables.

6.4 Conclusions

As proso millet offers an excellent nutritional quality, this study was needed to increase to reveal its nutritional benefits and to investigate its effects on digestibility. In this study, soaking, germination and extrusion procedures were used to reduce anti-nutrient levels and improve the protein and starch digestibility. The influence of feed moisture (15-25% w.b.), temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of proso millet extrudates were studied. Both feed moisture and extruder temperature had significant effects on the extrudate characteristics. Changing the level of moisture content and temperature had significant effect ($p<0.0001$) on all physical properties. The total color change in extruded products ranged between 13.0 and 17.1. water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. Hardness ranged between 1.01 and 1.3 N and the coefficient of determination for hardness was high ($R^2 = 0.97$). Extruder screw speed had minimal effect on the extrudate properties. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility. By applying the methodology of the desired function, the optimized process variables were moisture 15%, temperature 110°C, screw speed 120 rpm and Day 4 germinated flour bulk density 107.7 kg/m^3, hardness 1.02 N, water solubility index 18.2, water absorption index 4.3, $\Delta E=16.4$, expansion ratio=3.7, protein digestibility=86.2% and starch digestibility=87.6% respectively with overall desirability value of 1. Overall, the proso millet studied in the range extrusion conditions showed considerable expansion characteristics as compared high protein cereal grains, suggesting that proso millet may be combined with starch-based flours to make puffed snacks.

Table 6.1: Impact of soaking and germination on the nutritional properties of proso millet

	Raw proso millet	Day 0	Day 1	Day 2	Day 3	Day 4
Total Protein [a]	13.1±0.5	13.1±0.15	13.2±0.03	13.3±0.05	13.4±0.01	13.1±0.07
Carbohydrates[a]	63.2±0.6	63.2±0.16	63.3±0.03	63.4±0.07	63.4±0.09	63.6±0.02
Moisture [a]	11.1±0.2	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid [b]	1.10±0.07	0.69±0.05	0.55±0.05	0.46±0.02	0.33±0.04	0.21±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 6.2: Results of model adequacy tested in the Box-Behnken design

Source	**Sum of squares**	**DF**	**Mean square**	**F value**	**Prob > F**	**Remarks**
Sequential model sum of squares for ***Bulk Density***						
Quadratic	158.3	3	52.7	30.2	< 0.0001	Suggested
Sequential model sum of squares for ***Hardness***						
Quadratic	0.03	3	0.01	58.3	< 0.0001	Suggested
Sequential model sum of squares for ***Water Solubility Index***						
Quadratic	10.6	3	3.5	196.03	< 0.0001	Suggested
Sequential model sum of squares for ***Water Absorption Index***						
Quadratic	0.05	3	0.02	1537.84	< 0.0001	Suggested
Sequential model sum of squares for ***Total Color Difference ΔE***						
Quadratic	21.5	3	7.05	123.2	< 0.0001	Suggested
Sequential model sum of squares for ***Expansion Ratio***						
Quadratic	0.1	3	0.04	318.5	< 0.0001	Suggested
Sequential model sum of squares for ***Protein Digestibility***						
Quadratic	6.5	3	2.17	131.2	< 0.0001	Suggested
Sequential model sum of squares for ***Starch Digestibility***						
Quadratic	20.9	3	6.97	232.2	< 0.0001	Suggested

Source	**Std. Dev.**	**R-squared**	**Adjusted R-squared**	**Predicted R-squared**	**PRESS**	
Model summary statistics for ***Bulk Density***						
Quadratic	1.32	0.9817	0.9739	0.9512	274.42	Suggested
Model summary statistics for ***Hardness***						
Quadratic	0.013	0.9731	0.9617	0.9273	0.027	Suggested
Model summary statistics for ***Water Solubility Index***						
Quadratic	0.13	0.9859	0.9800	0.9618	2.91	Suggested
Model summary statistics for ***Water Absorption Index***						
Quadratic	0.003	0.9992	0.9988	0.9977	0.001	Suggested
Model summary statistics for ***Total Color Difference ΔE***						
Quadratic	0.24	0.9575	0.9394	0.8841	9.19	Suggested
Model summary statistics for ***Expansion Ratio***						
Quadratic	0.012	0.9924	0.9892	0.9801	0.023	Suggested
Model summary statistics for ***Protein Digestibility***						
Quadratic	0.13	0.9961	0.9945	0.9916	2.12	Suggested
Model summary statistics for ***Starch Digestibility***						
Quadratic	0.17	0.9922	0.9890	0.9796	4.66	Suggested

+ case(s) with leverage of 1.0000: PRESS statistic not defined

Table 6.3: Second order polynomial equations representing the relationship between independent and dependent variables

Bulk density	$117.6 + 9.55A -4.97B - 2.93C +2.78D_1 +1.31 D_2 +0.19D_3 -1.4D_4 + AB +0.7AC +0.33AD_1 +0.2AD_2 -0.55AD_3 -0.05AD_4 -0.65BC -0.4 BD_1 -0.28BD_2 +0.35BD_3 -0.15BD_4 +0.18CD_1 -0.075CD_2 -0.2CD_3 -0.2CD_4 -0.73A^2 +2.62B^2 +0.43 C^2$
Hardness	$1.10 +0.069A -0.036B -0.037C +0.024D_1 +0.013D_2 +0.0008D_3 -0.0121D_4 -0.024AB +0.004AC +0.002AD_1 -0.001AD_2 -0.004BC -0.006BD_1 -0.002BD_2 +0.002BD_3 +0.002BD_4 -0.002CD_1 -0.002CD_2 -0.0007CD_3 -0.0007CD_4 +0.002A^2 +0.022 B^2 -0.029C^2$
Water Solubility Index	$17.70 -1.12 A +0.43B +0.30C -0.25D_1 -0.12 D_2 +0.011D_3 +0.12D_4 +0.12AB -0.13AC -0.015AD_1 +1.000E-002AD_2 +1.000E-002AD_3 +1.000E-002AD_4 +0.015BC +0.073BD_1 +0.035BD_2 -0.015BD_3 -0.028BD_4 +0.018CD_1 +5.000E-003CD_2 +5.000E-003CD_3 -7.500E-003CD_4 -0.59A^2 -0.16B^2 -0.32C^2$
Water Absorption Index	$4.24 -0.13A +0.044B +0.032C -0.020D_1 -9.765E-003D_2 -3.529E-004D_3 +9.647E-003D_4 -2.500E-003AB +1.000E-003AC +2.250E-003AD_1 +2.250E-003AD_2 -1.500E-003AD_3 -1.500E -003AD_4 +9.500E -003BC +2.500E-004BD_1 +2.500E -004BD_2 +2.500E-004BD_3 +2.500E -004BD_4 -1.000E -003CD_1 -1.000E -003CD_2 +2.500E-004CD_3 +2.500E-004CD_4 -0.019A^2 -9.000E-003B^2 -0.045C^2$
Total Color Difference ΔE	$16.20 +0.20A -1.03B +0.21C -0.13D_1 -0.073D_2 +3.529E-003D_3 +0.056D_4 +0.47AB -0.040AC +0.030AD_1 +0.017AD_2 -7.500E-003AD_3 -0.020AD_4 +0.37BC -0.27 BD_1 -0.14BD_2 +2.500E-$

	003BD_3 +0.14BD_4 -7.500E-003CD_1 -7.500E-003CD_2 +5.000E-003CD_3 +5.000E-003CD_4 -0.048A^2 -0.89B^2 -0.39C^2
Expansion Ratio	3.63 -0.14A +0.046B +0.042C -0.027D_1 -0.014D_2 -1.176E-004D_3 +0.013D_4 +0.011AB +7.000E-003AC -2.250E-003AD_1 -1.000E-003AD_2 +2.500E-004AD_3 +1.500E-003AD_4 +4.000E-003BC +1.500E-003BD_1 +2.750E-003BD_2 +1.500E-003BD_3 -2.250E-003BD_4 +3.750E-003CD_1 +1.250E-003CD_2 +1.250E-003CD_3 -1.250E-003CD_4 -0.059A^2 -5.000E-003B^2 -0.052C^2
Protein Digestibility	84.26 -2.16A +0.34B +0.36C -1.39D_1 +0.15D_2 +0.27D_3 +0.41D_4 +1.000E-002AB +0.035AC -0.73AD_1 +0.17AD_2 +0.17AD_3 +0.19AD_4 +0.055BC +0.23BD_1 -0.055BD_2 -0.055BD_3 -0.043BD_4 +0.16CD_1 -5.000E-003CD_2 -0.030CD_3 -0.042CD_4 -0.39A^2 +0.015B^2 -0.38C^2
Starch Digestibility	86.46 -1.44A +0.48B +0.34C -2.23D_1 +0.36 D_2 +0.49D_3 +0.62D_4 +0.11AB +0.045AC -0.13AD_1 +0.020AD_2 +0.020AD_3 +0.032AD_4 +0.040BC +0.19BD_1 -0.045BD_2 -0.020BD_3 -0.045BD_4 +0.18CD_1 -5.000E-003CD_2 -0.030CD_3 -0.068CD_4 -0.79A^2 -0.15B^2 -0.52C^2

A – Moisture Content (%w.b.); B – Screw Speed (rpm); C – Die Temperature; D_1, D_2, D_3, D_4 – Germination time (Days)

Table 6.4: Pareto analysis of variance (ANOVA) analysis and significant statistical parameters of the model

Source	**Sum of squares**	**DF**	**Mean square**	**F-value**	**p-value**
Bulk density					
Model	5521.09	25	220.84	126.56	< 0.0001
A-moisture content	3648.10	1	3648.10	2090.64	< 0.0001
B-screw speed	990.02	1	990.02	567.36	< 0.0001
C-die temperature	342.23	1	342.23	196.12	< 0.0001
D – germ period	334.05	4	83.51	47.86	< 0.0001
B^2	145.07	1	145.07	83.13	< 0.0001
C.V. %	1.11				
Hardness					
Model	0.37	25	0.015	85.32	< 0.0001
A-moisture content	0.19	1	0.19	1101.25	< 0.0001
C-die temperature	0.053	1	0.053	310.4	< 0.0001
D – germ period	0.026	4	0.026	314.67	< 0.0001
B^2	0.009	1	0.083	58.01	< 0.0001
C^2	0.018	1	0.083	104.92	< 0.0001
C.V. %	1.16				

Water Solubility Index					
Model	75.2	25	3.01	165.40	< 0.0001
A-moisture content	50.4	1	50.4	2771.39	< 0.0001
B-screw speed	7.31	1	7.31	401.97	< 0.0001
C-die temperature	3.48	1	3.48	191.41	< 0.0001
D – germ period	2.55	4	0.64	35.02	< 0.0001
A^2	7.20	1	7.20	396.17	< 0.0001
B^2	0.51	1	0.51	27.81	< 0.0001
C^2	2.16	1	2.16	118.54	< 0.0001
C.V. %	0.78				
Water Absorption Index					
Model	0.87	25	0.035	2845.94	< 0.0001
A-moisture content	0.67	1	0.67	55339.84	< 0.0001
B-screw speed	0.076	1	0.076	6220.16	< 0.0001
C-die temperature	0.042	1	0.042	3418.87	< 0.0001
D – germ period	0.017	4	0.004	345.30	< 0.0001
BC	0.001	1	0.001	148.33	< 0.0001
A^2	0.007	1	0.007	592.12	< 0.0001
B^2	0.001	1	0.001	140.14	< 0.0001
C^2	0.044	1	0.044	3581.73	< 0.0001
C.V. %	0.083				

Total Color Difference					
ΔE					
Model	75.95	25	3.04	53.11	< 0.0001
A-moisture content	1.52	1	1.52	26.59	< 0.0001
B-screw speed	42.23	1	42.23	738.22	< 0.0001
C-die temperature	1.72	1	1.72	30.11	< 0.0001
BC	2.66	1	2.66	46.58	< 0.0001
B^2	16.77	1	16.77	293.15	< 0.0001
C^2	3.24	1	3.24	56.7	< 0.0001
C.V. %	1.54				
Expansion Ratio					
Model	1.13	25	0.045	309.48	< 0.0001
A-moisture content	0.8	1	0.8	5464.95	< 0.0001
B-screw speed	0.085	1	0.085	577.55	< 0.0001
C-die temperature	0.072	1	0.072	493.0	< 0.0001
D – germ period	0.032	4	0.007	54.01	< 0.0001
A^2	0.073	1	0.073	500.06	< 0.0001
C^2	0.057	1	0.057	388.44	< 0.0001
C.V. %	0.34				

Protein Digestibility					
Model	251.28	25	10.05	607.14	< 0.0001
A-moisture content	186.19	1	186.19	11247.0	< 0.0001
B-screw speed	4.69	1	4.69	283.44	< 0.0001
C-die temperature	5.04	1	5.04	304.5	< 0.0001
D – germ period	42.6	4	10.65	643.25	< 0.0001
AD	5.33	1	1.33	80.55	< 0.0001
BD	0.55	1	0.14	8.25	< 0.0001
A^2	3.12	1	3.12	188.5	< 0.0001
C^2	3.04	1	3.04	183.63	< 0.0001
C.V. %	0.15				
Starch Digestibility					
Model	226.5	25	9.06	301.75	< 0.0001
A-moisture content	83.52	1	83.52	2781.81	< 0.0001
B-screw speed	9.31	1	9.31	310.16	< 0.0001
C-die temperature	4.69	4	4.69	156.28	< 0.0001
D – germ period	106.79	1	26.70	889.18	< 0.0001
A^2	13.14	1	13.14	437.62	< 0.0001
C^2	5.80		5.80	193.27	< 0.0001
C.V. %	0.20				

Table 6.5: Predicted and experimental values of the responses at optimum conditions

Moisture Content (%w.b.) = 15; Die Temperature (°C) = 110; Screw Speed (rpm) = 120; Germination = Day 4

	Optimized values[a] (predicted values)	Experimental values[b]
Bulk density(kg/m^3)	107.8	107.8±0.02
Hardness (N)	1.02	1.02±0.06
Water Solubility Index (%)	18.2	18.2±0.03
Water absorption Index	4.3	4.3±0.09
Total Color Difference ΔE	16.4	16.4±0.07
Expansion Ratio	3.7	3.7±0.08
Protein Digestibility (%)	86.2	86.2±0.05
Starch Digestibility (%)	87.6	87.6±0.05

a Predicted using response surface quadratic model

b Mean ± standard deviation of triplicate determinations from experiments

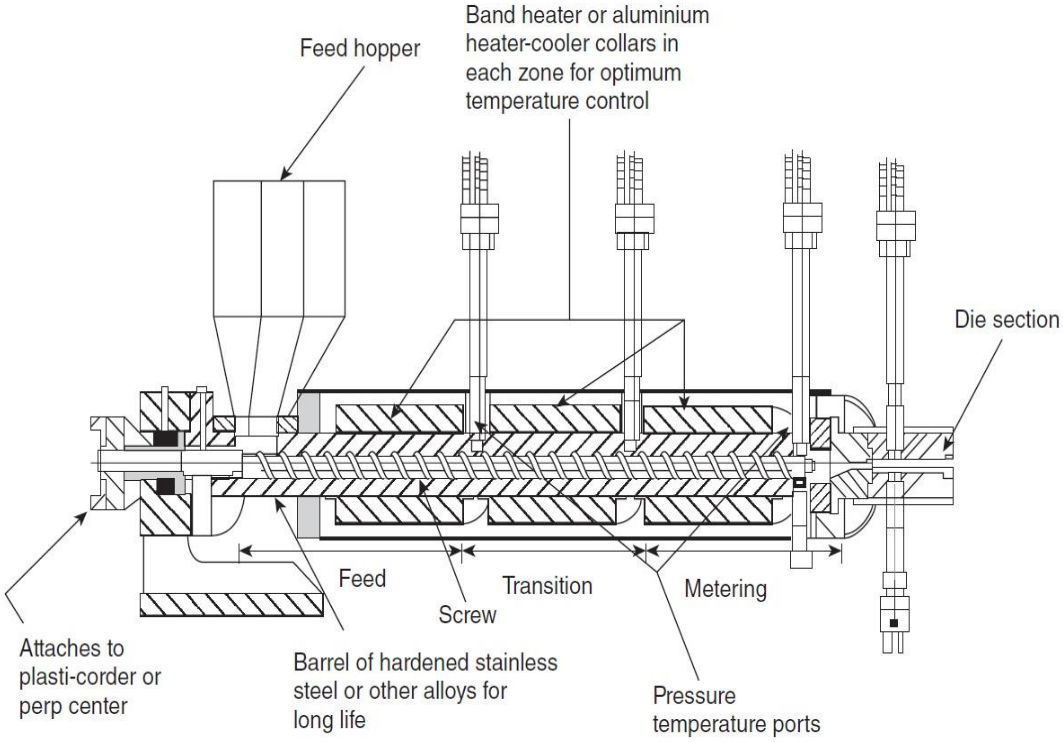


Figure 6.1: Single screw laboratory extruder

(Brabender Intelli-Torque Plasti-Corder® South Hackensack, NJ)

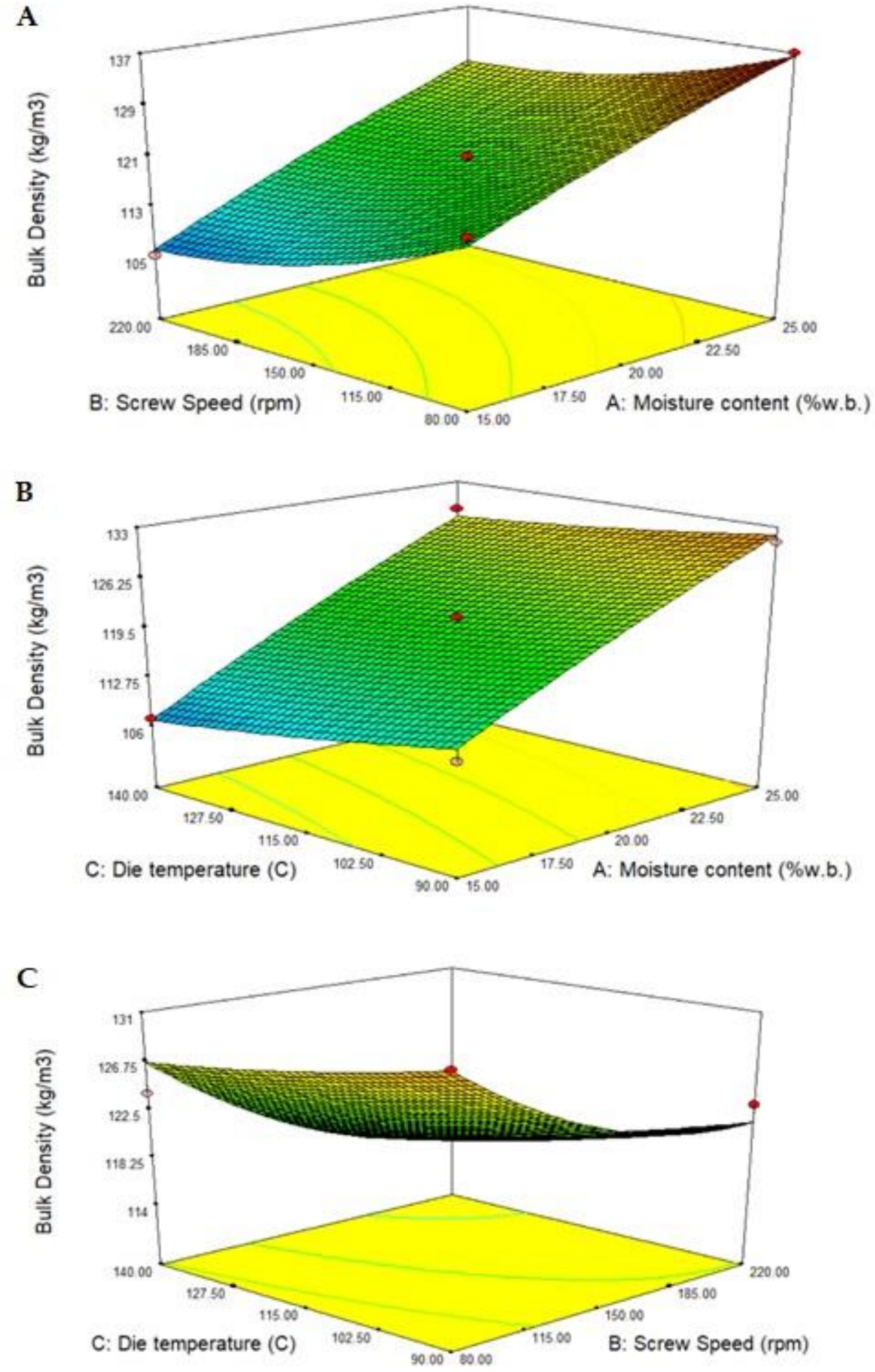


Figure 6.2: Response surface plots (3D) showing the effects on the Bulk Density (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

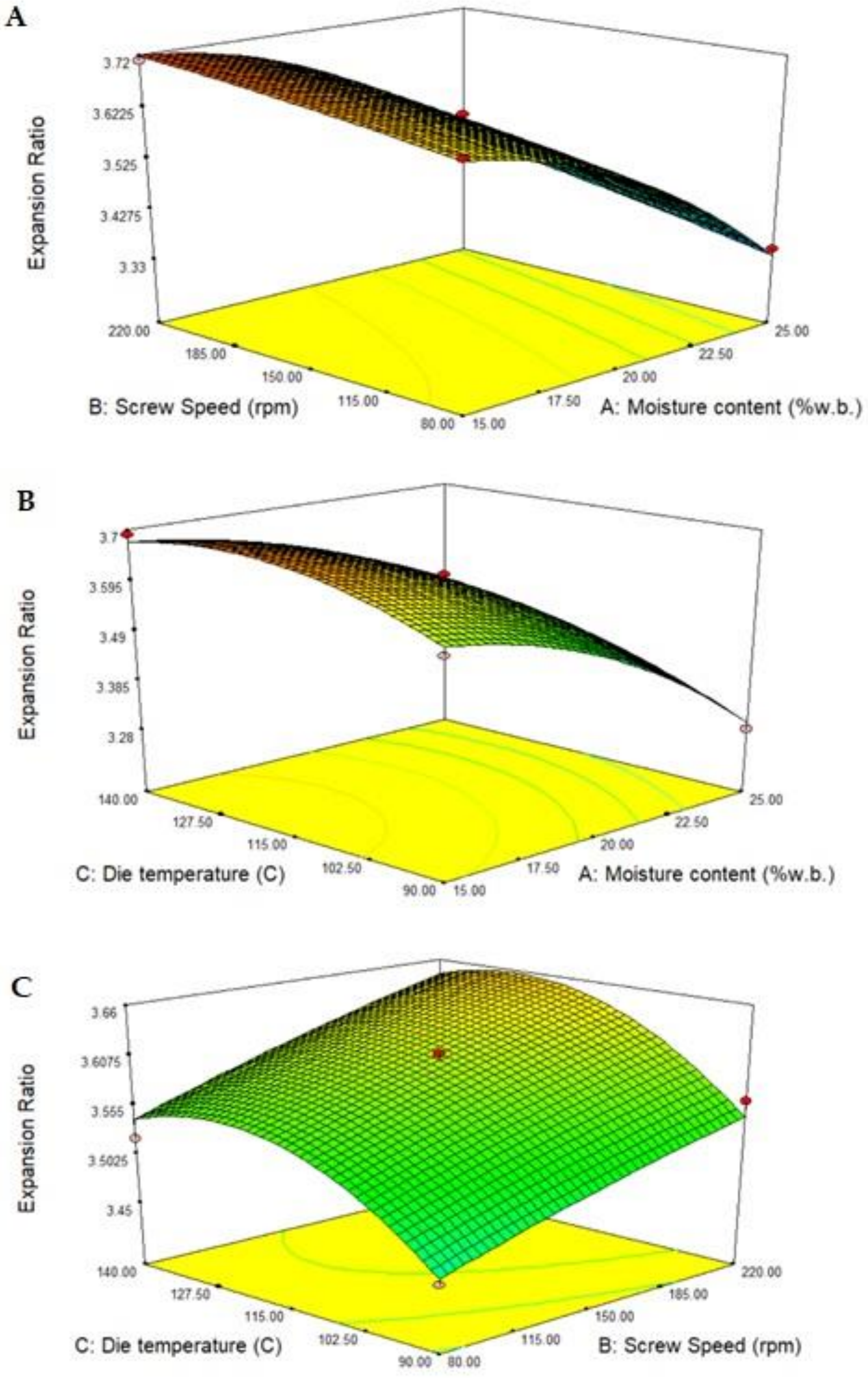


Figure 6.3: Response surface plots (3D) showing the effects on the Expansion Ratio (A) Moisture Content and Screw speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

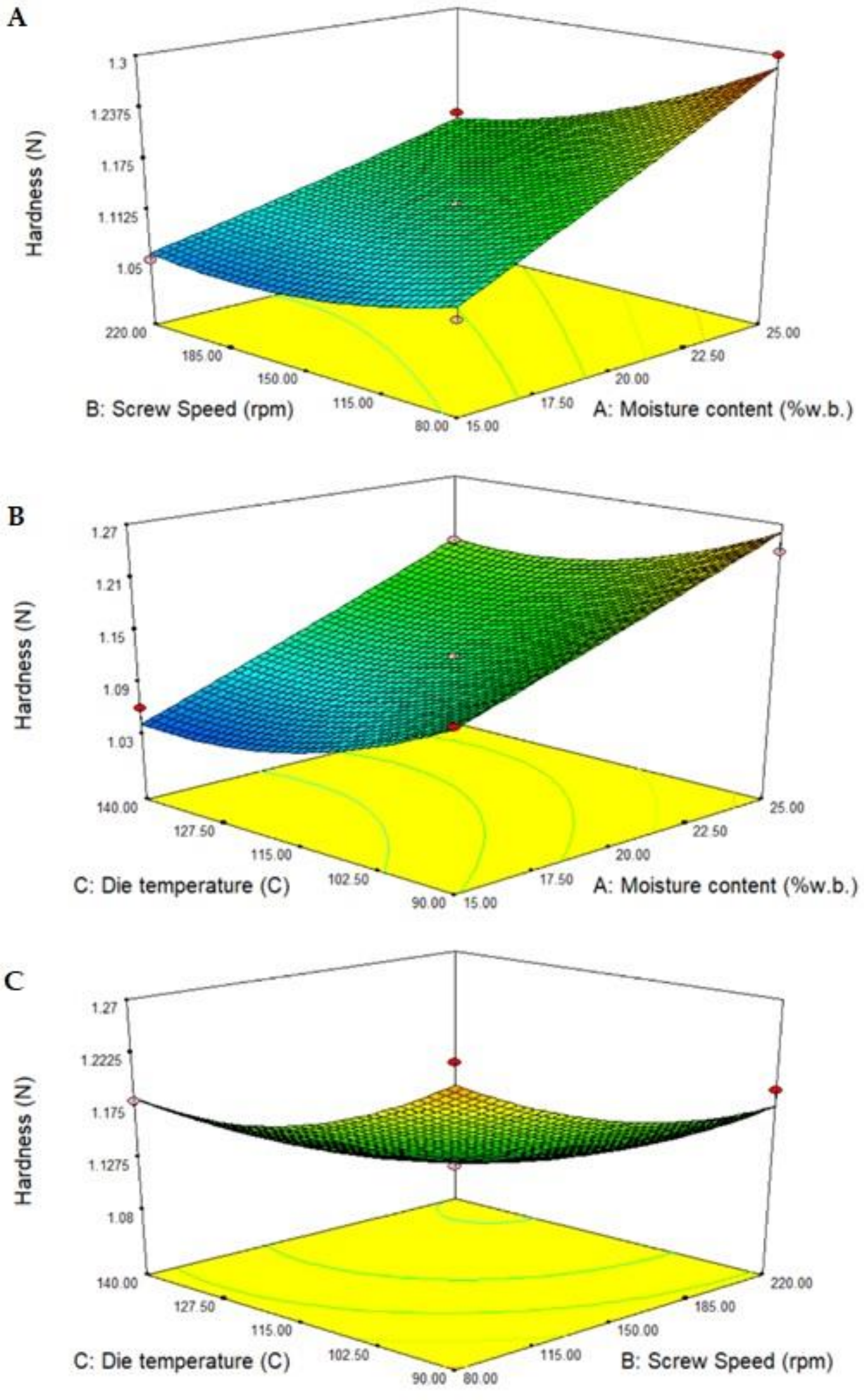


Figure 6.4: Response surface plots (3D) showing the effects on the Hardness (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

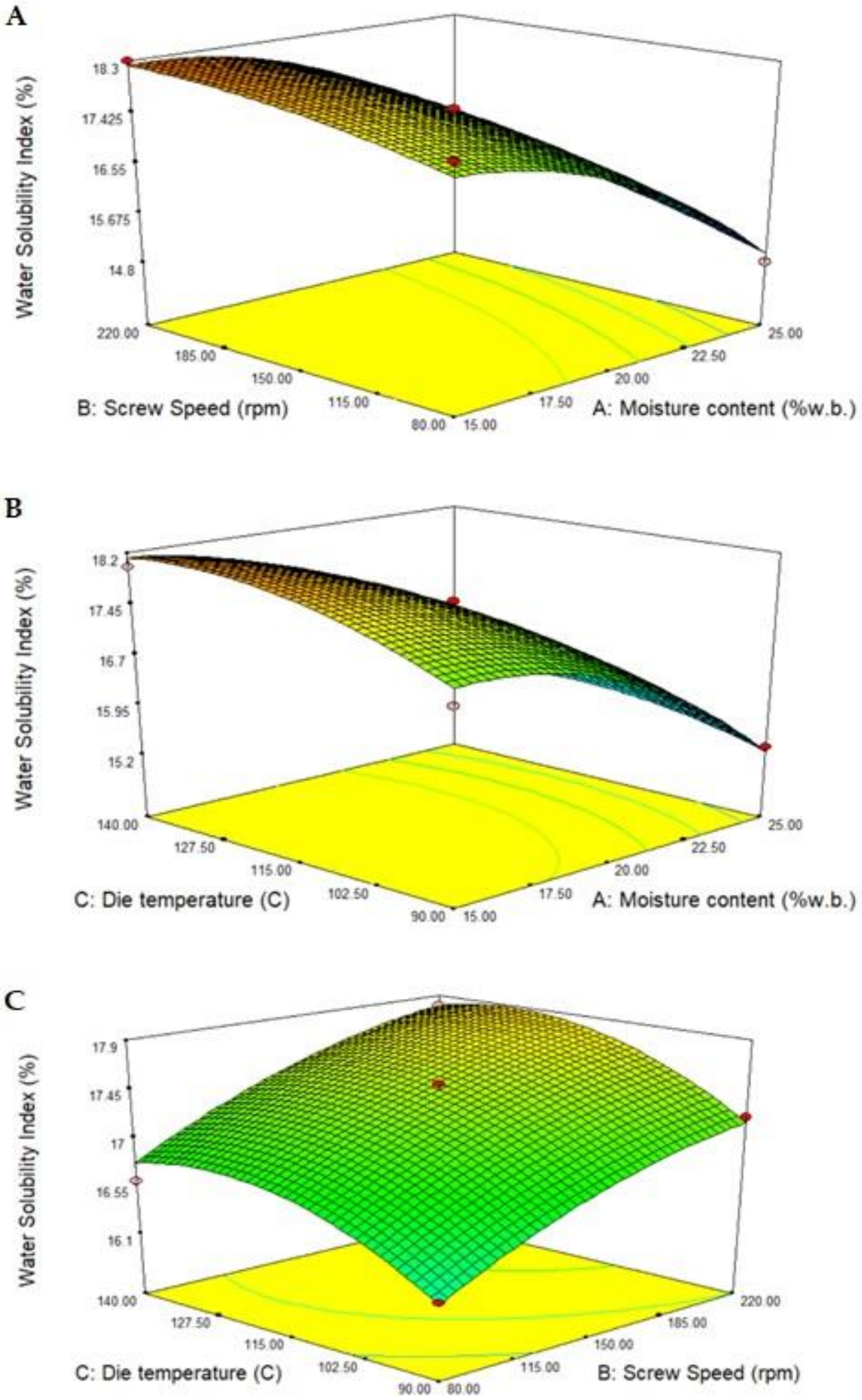


Figure 6.5: Response surface plots (3D) showing the effects on the Water Solubility Index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

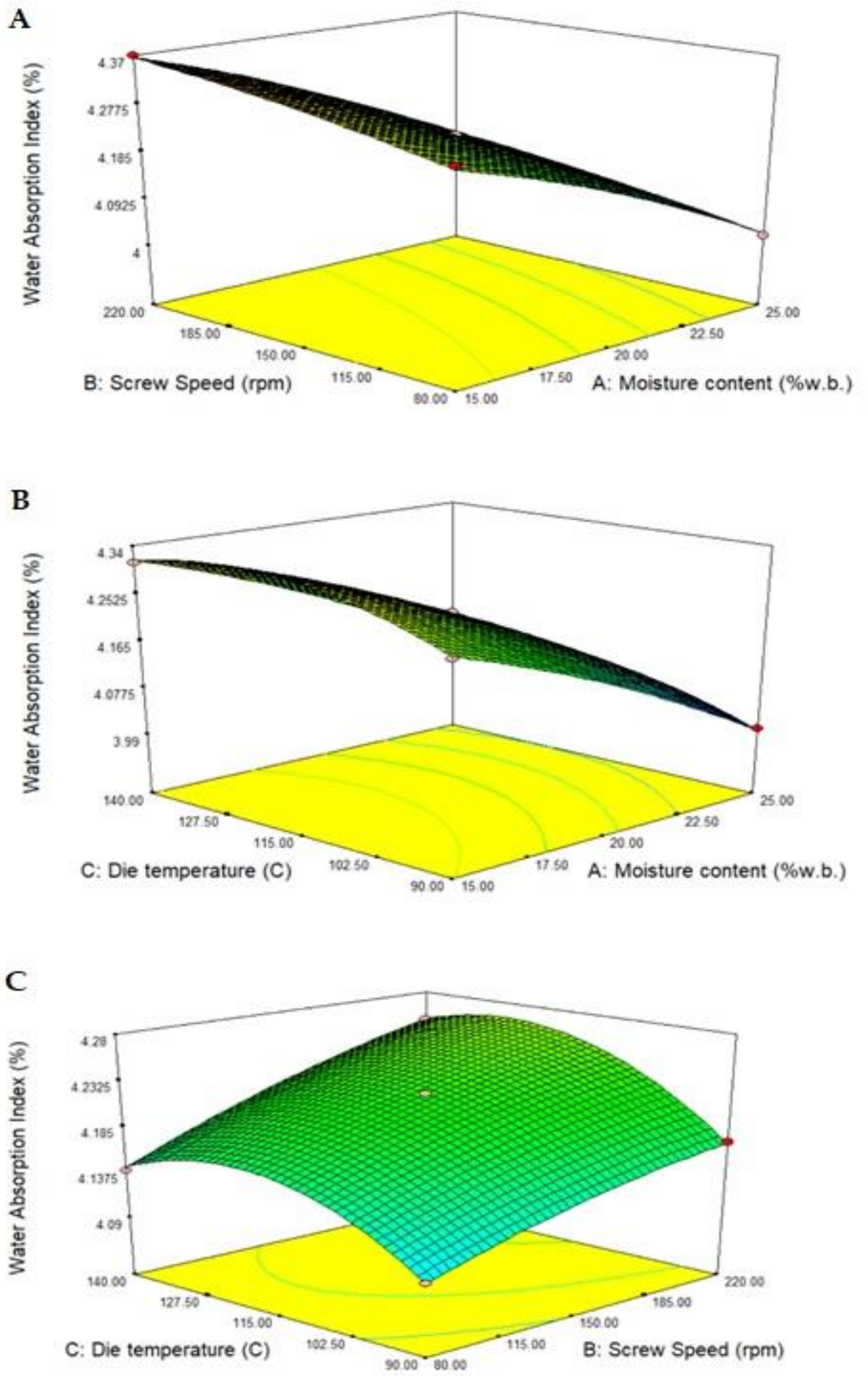


Figure 6.6: Response surface plots (3D) showing the effects on the Water Absorption Index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

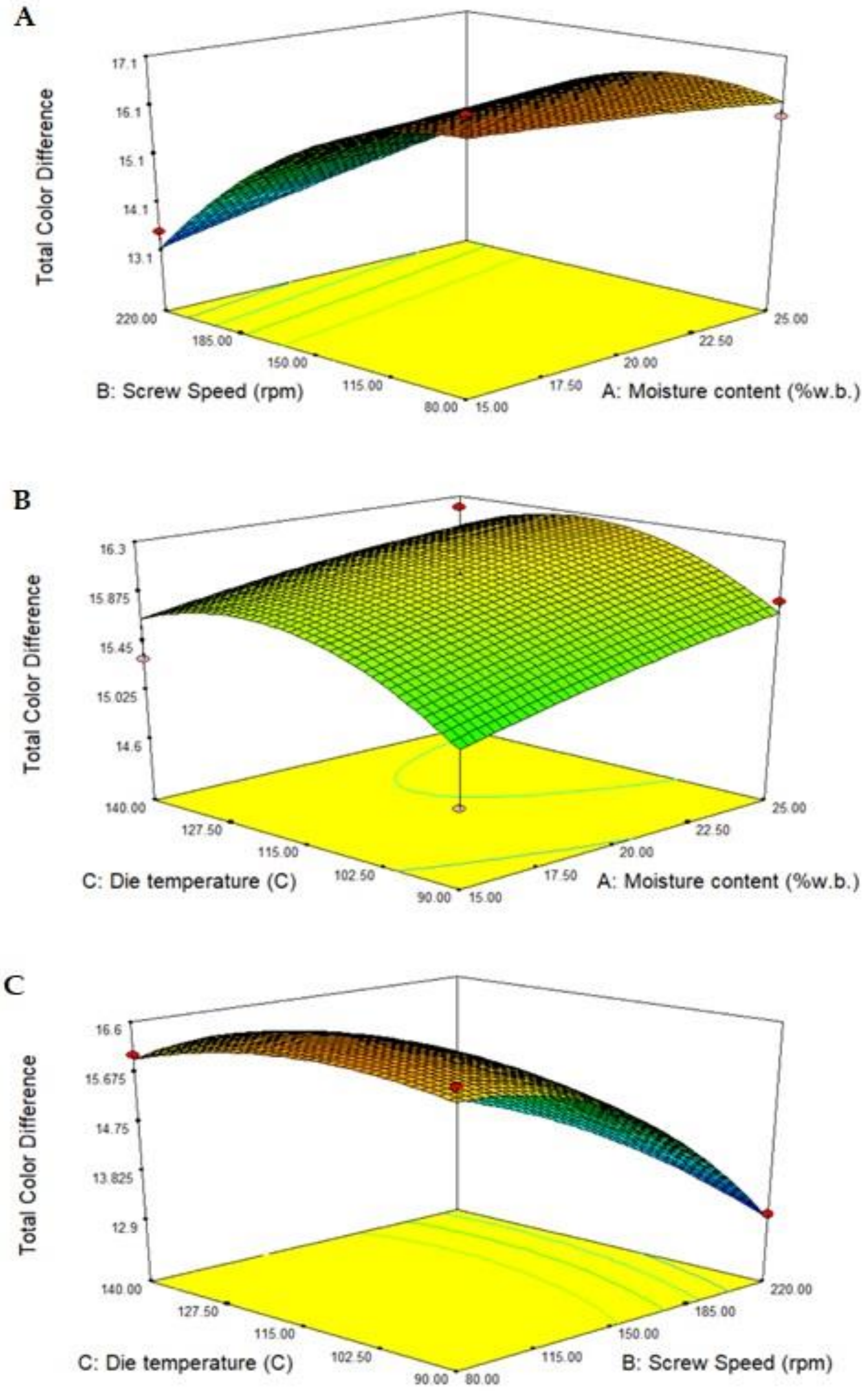


Figure 6.7: Response surface plots (3D) showing the effects on the Total Color Difference (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

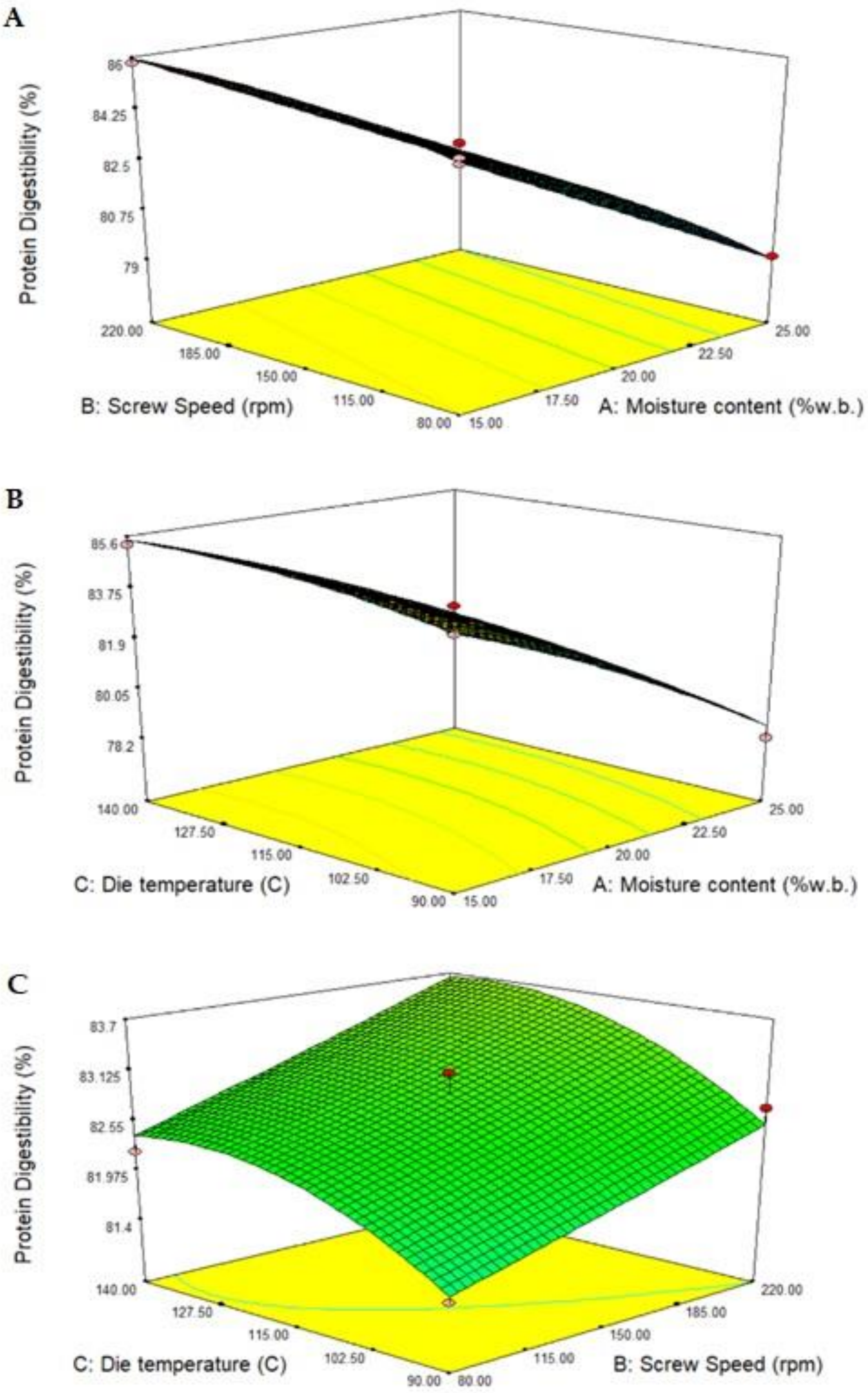


Figure 6.8: Response surface plots (3D) showing the effects on the Protein Digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

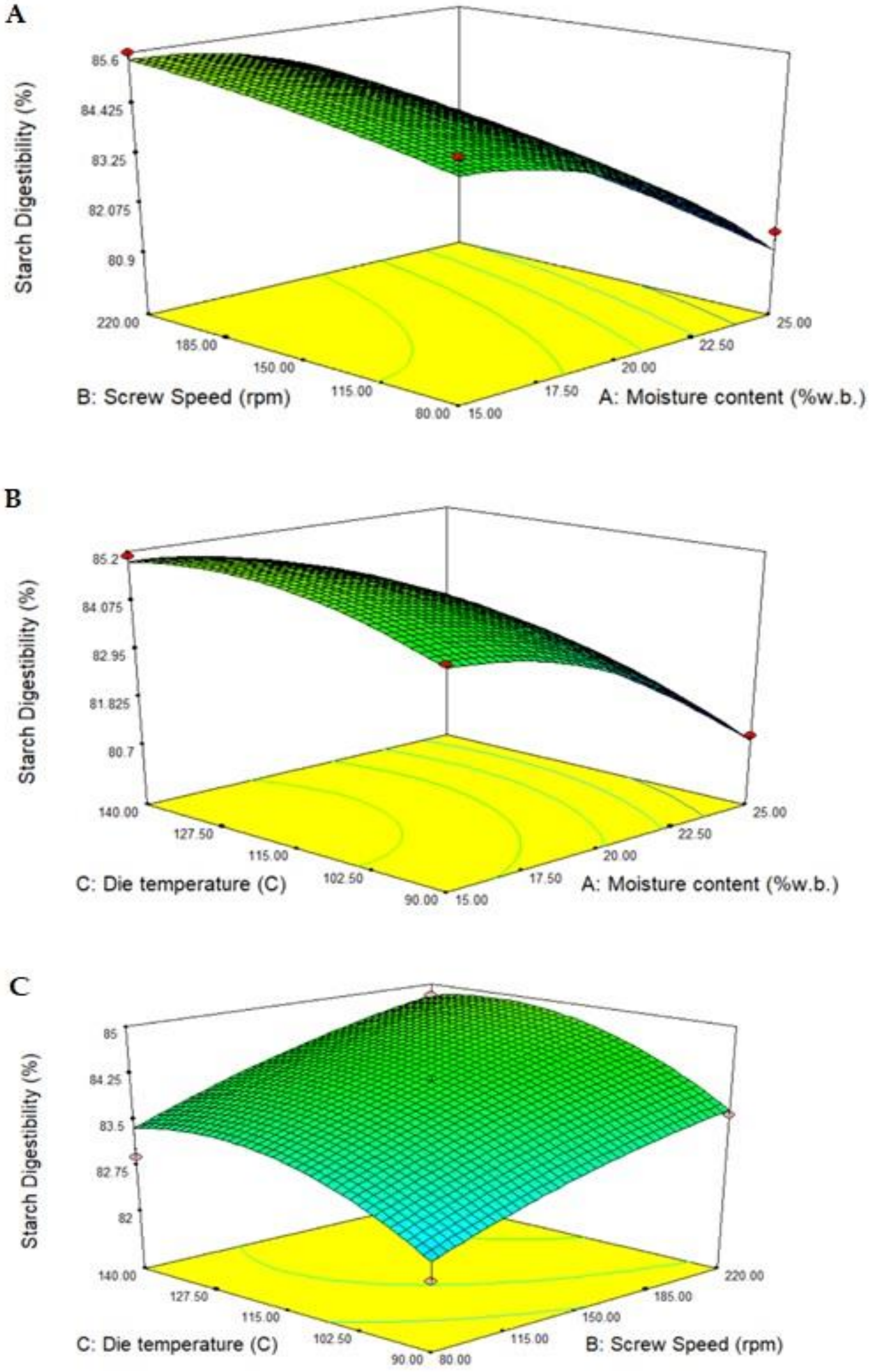


Figure 6.9: Response surface plots (3D) showing the effects on the Starch Digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

CHAPTER 7: EFFECT OF SINGLE-SCREW EXTRUSION PROCESS PARAMETERS ON THE PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF SPROUTED PROSO MILLET-CORN MEAL EXTRUDATES

Abstract

With the aim of creating a puffed extrudate using sprouted proso millet, a blend of sprouted proso millet and corn meal was prepared. Single-screw extrusion processing of sprouted proso millet-corn meal blend was studied using a response surface design to understand the influence of feed moisture (15-25% w.b.), die temperature (80-140°C), screw speed (90-220 rpm), corn meal ratio (0-30%) and proso millet germination time (Days 0-4) on the physical and physico-chemical properties of sprouted proso millet-corn meal blend extrudates. The following responses of the extrudates were measured and the values ranged from: bulk density (100-143 kg/m^3), hardness (0.84-1.59 N), water solubility index (12.5-20.1%), water absorption index (2.55-3.90), total color difference ΔE (13.07-20.37), expansion ratio (2.74-3.96), protein digestibility (78.5-85.9%) and starch digestibility (77.7-85.8%). The addition of corn meal (30%) significantly increased the expansion ratio from 3.29-3.96. Increasing the corn meal ratio to 30% increased the total color difference between the extrudates to 13.07 – 16.17 from 20.9 – 21.3 (only sprouted quinoa flour). The disadvantages of adding corn meal includes reduction of starch and protein digestibility. The addition of corn meal (30%) reduced the starch digestibility from 85.8 % to 77.7% and protein digestibility from 85.9% to 78.5%, respectively?

Keywords: Proso millet, Soaking, Germination, Corn meal, Gluten-free, Digestibility

7.1. Introduction

Millets are unique among the cereals because of their high calcium, iron, potassium, magnesium, phosphorous, zinc, dietary fiber, polyphenols, and protein content. Proso millet has the highest protein content among the various millets (Jones, Beckwith, Khoo, & Inglett, 1970). Millets are gluten-free, ideal for people who are gluten-intolerant, though millet flour cannot be used for raised bread. Commonly cultivated millet species include proso millet (*Panicum miliaceum*), pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), kodo millet (*Paspalum setaceum*), foxtail millet (*Setaria italica*), little millet (*Panicum sumatrense*), and barnyard millet (*Echinochloa utilis*) (Colosi & Schaal, 1997). Proso millet is a summer annual grass. Grains enclosed in a smooth hull, which is typically white or creamy-white, yellow or red in color (Paridhi Gulati, Steven Weier, Dipak Santra, Jeyamkondan Subbiah, & Devin Rose, 2016) White-seeded varieties are most often grown in the US followed by red-seeded varieties. Proso millet is among the preferred crops for planting after wheat in the Central Great Plains because it helps control weeds and conserve stored moisture. In 2019, the Central Great Plains states of Colorado, Nebraska, and South Dakota were the major producers of US (Romero, Santra, Rose, & Zhang, 2017). Other states producing significant quantities of proso millet include Kansas, Wyoming, Minnesota and North Dakota (Paridhi Gulati et al., 2016).

Millets generally contain significant amounts of essential amino acids, particularly those containing sulfur (methionine and cysteine) (Zhang, Liu, & Niu, 2014). They contain a high amount of lecithin, which provides excellent support for nervous system health by helping to restore nerve cell function, regenerate myelin fiber, and intensify brain cell metabolism (Habiyaremye et al., 2017). Millets are also rich in micronutrients such as niacin, B-complex vitamins, Vitamin B6, and folic acid. Magnesium acts as a co-factor in several enzymatic reactions that regulate the secretion of glucose and insulin. Magnesium can also reduce the frequency of migraine headaches and heart attacks and is beneficial for people suffering from atherosclerosis and diabetic heart disease. Proso millet is used as bird feed predominantly but if it is converted to a value-added food product, farmers and food manufacturers would be benefitted. Because of its mild flavor, light color, gluten-free quality, and potential health benefits, proso millet has been receiving interest from the food industries in North America.

Phytic acid (myoinositol hexaphosphoric acid) reduces the availability of many minerals like iron, zinc, calcium and magnesium (Cheryan & Rackis, 1980). The formation of iron-phytate complexes of low solubility, in the small intestine is the basis for the interference of phytate with iron absorption. Although the iron content in cereals is usually high, the iron absorption from them is often poor due to the presence of high amounts of phytates. They are also found to inhibit the proteases and amylases of the intestinal tract. Sprouted seeds are nutritionally superior to their respective seeds Germinated grains have higher levels of nutrients, lower levels of anti-nutrients such as phytic acid and flatulence-causing sugars, increased protein and starch digestibility, and increased bioavailability of some minerals (Carciochi et al., 2014). The inclusion of sprouted grains in the formulation of whole-grain-rich, fiber-rich, and gluten-free baked goods may provide a natural fortification and enrichment mechanism; it may improve nutritional quality because of the increased digestibility and bioavailability of some nutrients and the reduction of some anti-nutrients. Comprehensive reviews of the effects of germination on the nutrient composition of cereals and pseudocereals have been published (Carciochi et al., 2014; Lombardi-Boccia et al., 1991; Lorenz & D'Appolonia, 1980).

Corn originated approximately 7000 years ago in Mexico. Currently, the United States, Brazil, Mexico, Argentina, India, France, Indonesia, South Africa, and Italy produce 79% of the world's corn production. In general, there are several uses of corn in food as well as feed industry such as the production of flour, corn meal, grits, sweeteners, starches, alcoholic beverages, cooking oil, tortilla, snacks, breakfast foods and other products. There are two basic categories of industrial processing employed for transforming corn into products for human consumption. They are known as dry and wet milling. In the wet milling process, corn is separated into relatively pure chemical compound classes of starch, protein, oil, and fiber. Industrial dry milling includes particle size reduction of clean whole maize with or without screening separation, retaining all or some of the original maize germ and fiber. Corn meal is coarse flour ground from dried corn. It the most common primary ingredient used in corn-based extruded snacks. Because of its composition, ratio of vitreous to floury endosperm, and particle size, under optimal extruding conditions corn meal makes for a light, highly expanded, crunchy and soft product. Corn has been blended with various cereals and legumes such as soybean (Yu et al., 2012), teff and soy (Forsido et al., 2011), wheat (Ryu & Ng, 2001) and pea (Sobota & Rzedzicki, 2009) to create puffed snacks.

With the rise in global population, food researchers’ focus is to directly and efficiently transform raw agricultural products consisting of starch, plant protein, and fat into foods of high acceptability (Albarracín et al., 2015). Extruders can continuously combine, cook and texturize food components quickly and efficiently, thus making it ideally suited for the development of puffed snack foods and breakfast cereals. The food material is sheared, mixed and compressed during extrusion cooking and then texturized and shaped in the die (Alvarez-Jubete et al., 2010). The thermomechanical action during extrusion brings about gelatinization of starch, denaturation of protein and inactivation of enzymes, microbes and many anti-nutritional factors; all this occurs in a shear environment, resulting in a plasticized continuous mass (Ding et al., 2006). The rate and extent of heating, mixing, shearing, and compressing of the materials inside the barrel, and subsequently the die, is strongly related to the properties of the raw materials and process conditions used.

Single screw extrusion processing is a commonly used processing technology in the food industry with a wide number of applications. It is a processing system that utilizes a single screw or a twin screw to force food materials through a die opening. The food ingredients are subjected to high shear, temperature, and pressure for a short period of time (Gopirajah & Muthukumarappan, 2018). The melted ingredients are then forced through a die at the end of the extruder into the atmosphere. The melt coming out of the extruder encounters a sudden drop in pressure, resulting in rapid expansion as well as a decrease in temperature, helping it to transform into a cooked and expanded product. The final quality and texture of the extruded product depends on various factors, including the ingredient mixture and its properties, extrusion processing conditions, and post-processing conditions (Harper & Clark, 1979).

A single-screw extruder consists of only one screw housed in the barrel that often has a fluted or grooved design. Additionally, the screw in a single-screw extruder is usually designed with a decreasing pitch to create compression (Guy, 2001). The amount of decreasing pitch is referred to as the compression ratio. The role of the screw is to assist in imparting shear to the ingredient mixture and forcing the dough of the mixed ingredients out from the extruder through the die (Muthukumarappan & Karunanithy, 2012). The screws are also responsible for the build-up of pressure that occurs at the end of the extruder as well as added mixing of the ingredients. This helps to transform the ingredients from solid powders to a melt state inside of the extruder For any new material extrusion, a single screw extrusion is preferred because of the relatively low cost, straightforward design, ruggedness and favorable performance/cost ratio (Akdogan, 1999; Guy, 2001) Because of the great flexibility of extrusion processing, it has found very diverse applications in the food industry.

Proso millet has high protein and is more suited to produce noodles or pasta. Wheat and corn meal flours are the most blended materials for food extrusion to produce a puffed cereal. Other materials like rice flour, soy, potato, rye, barley, oats, sorghum, cassava, tapioca, buckwheat, pea flour and other related materials can also be used. The objective of this research is to understand the effect of germination on the changes in nutrients of proso millet and the impact of the single-screw extrusion variables such as moisture content of feed, temperature, screw speed of extruder and germination time on the physical and physico-chemical properties of extrudates made from the sprouted proso millet flour-corn meal blend.

7.2 Materials and Methods

7.2.1 Raw materials

Proso millet seeds (white) were obtained from Foundation Seed Stock in Agronomy, Horticulture and Plant Science department at South Dakota State University, SD. Corn meal was obtained from a national grain processor (Bob's Red Mill Natural Foods, Milwaukie, OR). For the proximate composition analysis of proso millet and corn meal, the following AACC methods were used: Moisture -Oven drying at 135°C (method no. 44–19), Ash - calcination at 550°C (method no. 08–01), Lipids - defatting in a Soxhlet apparatus with petroleum ether (method no. 30–25), Protein - micro Kjeldahl (N 6.25) (method no. 46–13), Carbohydrate – sugars and starch (method no. 80–04, 76-13) and total fiber - (method no. 32–07). Saponin content and phytic acid content (hexaphosphate equivalents) were quantified using the method described by (Rathod & Annapure, 2017). All chemicals were purchased from

Sigma–Aldrich, USA. All chemicals and reagents used were of analytical grade. All experiments were performed in triplicates.

7.2.2 Soaking and Germination of Proso millet

The proso millet (11 pounds) was soaked overnight (8 pm- 8 am) for 12 h in tap water (1:5 w/v) at room temperature (~25°C). The soaked samples were then drained and washed. Excess water was drained, and the samples were wrapped in a moist cloth for 5 days (0 h – Day 0, 24 h - Day 1, 48 h - Day 2, 72 h – Day 3, 96 h – Day 4) at room temperature. All experiments were performed in triplicates.

7.2.3 Extrusion processing

The sprouted proso millet-corn meal blend, each weighing around 200 g were randomly extruded using a 19.18 mm (0.755 in.) barrel inner diameter (i.d.), single-screw laboratory extruder (Brabender Intelli-Torque Plasti-Corder® South Hackensack, NJ) as shown in Fig 7.1. It was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 200 rpm. The extruder had a barrel with length (L) to diameter (D) ratio of 20:1. A uniform 19.05 mm pitch screw having 381 mm (15.0 in.) screw length, a 19.05 mm (0.75 in.) constant outside (top of flight) diameter, a 3.81 mm (0.15 in.) initial screw feed depth, an 11.43 mm (0.45 in.) initial screw root diameter, and a screw compression ratio (feed channel depth to metering channel depth) of 1.5:1 was used in the experiments. A die nozzle with 3 mm diameter was used. The extrudates were cooled to room temperature and sealed in polyethylene bags and stored at room temperature until measurements were taken.

7.2.4 Measurement of extrudate properties

7.2.4.1 Bulk Density

The bulk density (kg/m^3) was calculated as the ratio of the mass M (kg) to the volume V (m^3) of the measured and weighed extrudate sample. It was assumed that extrudates were of cylindrical shape. The bulk density of all extrudates were measured and the measurements were done in triplicate.

$$Bulk\ Density = \frac{M}{V} \tag{7.1}$$

7.2.4.2 Expansion ratio (ER)

Expansion ratio was calculated by measuring the mean diameter of 10 randomly chosen locations on the extrudates with calipers for each extrusion process condition. Further it was divided by the die diameter of 3 mm.

$$Expansion\ Ratio = \frac{Mean\ diameter\ of\ the\ extrudate\ (mm)}{Die\ diameter\ (mm)} \tag{7.2}$$

7.2.4.3 Hardness

Hardness is defined as the maximum force per unit area of the sprouted proso millet-corn meal extrudate that is necessary to breakdown the extrudate into two pieces. Hardness was tested by breaking the extrudate using a cylindrical probe (TA-4, Texture Technologies Corp, NY) attached to the crosshead of texture analyser. The sample was positioned in its natural position. A vertical force was applied at a speed of 50 mm/min. The force applied vs deformation was recorded and the maximum force recorded in the curve was considered as the hardness. The hardness of all extrudates were measured and the measurements were done in triplicate.

7.2.4.4 Water Solubility Index and Water Absorption Index

The water absorption index and water solubility index of all extrudates were measured and the measurements were done in triplicate. The proso millet-corn meal extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was mixed in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was mixed occasionally and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminium cup and dried at 135°C for 2h (AACC, 2000). The weight of the gel in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by:

$$Water\ Solubility\ Index\ (\%) = \frac{W_{ss}}{W_{ds}} * 100 \tag{7.3}$$

$$Water\ Absorption\ Index = \frac{W_g}{W_{ds}} \tag{7.4}$$

where, W_{ss} is the weight of dry solids of supernatant (g), W_{ds} is the weight of dry sample (g), and W_g is the weight of gel (g)

7.2.4.5 Color

The color of proso millet-corn meal extrudate samples was measured using colorimeter (LabScan XE, HunterLab, Reston, VA) in triplicate in terms of Hunter Lab values (L^{*}, a^{*}, b^{*}). The Hunter Lab value 'L^{*}' signifies lightness with 0 for dark and 100 for bright. The value 'a^{*}' measures the degree of green color in the range from -100 to 0 and red in the range 0 to 100. The 'b^{*}' value represents blue color in the range from -100 to 0 and yellow in the range from 0 to 100.The total color change (ΔE) is calculated as

$$Total\ Color\ Difference\ \Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \tag{7.5}$$

where $\Delta L = L^* - L_0$, $\Delta a = a^* - a_0$, and $\Delta b = b^* - b^*_0$; the subscript ‘‘0’’ represents initial color values before processing. All readings were taken in triplicate.

7.2.4.6 Starch and Protein Digestibility

The starch digestibility and protein digestibility of all extrudates were measured and the measurements were done in triplicate. The proso millet-corn meal extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The in-vitro starch digestibility was determined in the extrudate powder (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20 °C for 2 h according to the method of (Rathod & Annapure, 2017). At the end of the incubation period, 2 ml of 3,5-dini-trosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard.

A system consisting, 100 mg (0.1 g) dry and defatted samples were incubated at 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 °C for 3 h. Further it was neutralized with 7.5 ml 0.2N NaOH. Then 4 mg pancreatin in 7.5 ml phosphate buffer (pH 8) was added and incubated for 37 °C for 24 h, further 5 mg of TCA was added, centrifugated at 5000 rpm for 10 min. After that the supernatant dry residue at 40–50 °C was discarded and the micro Kjeldahl process for protein estimation was carried out. The percent in vitro protein digestibility was calculated using the following equation (Rathod & Annapure, 2017):

$$Protein\ digestibility\ (\%) = \frac{Total\ protein - Residual\ protein}{Total\ protein} * 100 \qquad (7.6)$$

7.2.5 Experimental Design and Statistical Analysis

Experiments were conducted using the Box Benhken design (BBD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA), consisting four factors namely moisture content (X_1), temperature (X_2), screw speed (X_3), corn meal ratio (X_4) and germination time (X_5). Three replicates were taken at the design center and the total number of observations were 135. Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN). The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA). The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

7.3 Results and Discussion

7.3.1 Effect of Soaking and Germination on the proso millet flour

The nutritional properties of the sprouted proso millet flour are presented in Table 7.1 and sprouted proso millet-corn meal blend are shown in Table 7.2. The addition of corn meal increased the protein content of the blend. In general, protein content of proso millet seeds ranges between 13.1% and 14.0% (James, 2009). The total protein content of proso millet is higher than that of grains such as rice, barley, corn, rye, sorghum and wheat (Navruz-Varli & Sanlier, 2016). The chief carbohydrate constituent of proso millet and corn meal is It ranges from 62-64% in proso millet and 68-74% in corn meal. The total fiber was close to other grain products (7.0%-9.7%). Proso millet contains sugar by about 3%. It mostly contains maltose, D-galactose, and

D-ribose in addition to low levels of fructose and glucose (Navruz-Varli & Sanlier, 2016). Corn meal is nutritious, providing fiber, which aids in digestion, plus folate, thiamin, phosphorus, vitamin C, and magnesium.

Phytic acid binds minerals, such as Fe, Zn, Ca and Mg, and can make the mineral content of a cereal inadequate, especially for children (Khattak et al., 2007). Soaking and sprouting processes reduce the 'binding' effect of the phytic acid by activating the enzyme phytase (James, 2009). The amounts of phytic acid of the proso millet seeds obtained in this study are in concordance with the values for Cherry vanilla (Kowalski et al., 2016). The phytic acid concentration in proso millet seeds is comparable to whole grain rye flour (7.7 mg/g), whole grain wheat flour (8.7 mg/g), lentils (8.4 mg/g) and fava beans (8.0 mg/g). Phytic acid is not only present in the outer layers of proso millet, as in the case of rye and wheat but is also evenly distributed within the endosperm (Ahamed et al., 1998). A significant reduction in phytic acid content is observed with increase in germination time. Day 4 germinated proso millet samples had more than 50% reduction in phytic acid. Extrusion further decreased the phytic acid content of pea flour (Alonso et al., 1998), bean flour (Anton et al., 2009) and other legumes (Lombardi-Boccia et al., 1991).

7.3.2 Effect of independent variables on the single extrusion process of sprouted proso millet – corn meal blend

The results of the single extrusion process of sprouted proso millet – corn meal blend along with the experimental design is presented in Appendix E.

7.3.2.1 Bulk Density

The bulk density of the extrudates varied between 100 and 143 kg/m^3. Low bulk density of 100 kg/m^3, which is a required characteristic of the extruded product, was achieved with low feed moisture content (15%), average temperature (115°C) and high screw speed combination (220 rpm). The addition of corn meal (15-30%) significantly reduced the bulk density creating a less dense and higher expansion ratio. Fig 7.2 shows that feed moisture content had a significant effect on bulk density followed by screw speed, whereas temperature seemed to have a minor effect. Similar results were reported in oat-corn meal extrudates (Liu et al., 2000) and soy-corn meal extrudates (Yu et al., 2012).

7.3.2.2 Expansion ratio

The measured expansion ratio of proso millet-corn meal extrudates ranged from 2.74 to 3.96. The expansion ratio was significantly affected by the higher starch content of corn meal. As the corn meal content was increased from 30%, the expansion ratio increased from 3.29-3.97. In addition, temperature and feed moisture content played a significant role in increasing the expansion ratio. Fig 7.3 shows that the temperature and corn meal ratio have a significant effect on the expansion ratio of the extrudates. High expansion ratio at low feed moisture content for expanded products is distinctive for cereals. Similar trends have been obtained for corn (Diaz et al., 2013), wheat (Pitts et al., 2014) and oats (Gopirajah & Muthukumarappan, 2018).

7.3.2.3 Hardness

Hardness, the maximum force to break the sample, ranged between 0.84 and 1.59 N. The coefficient of determination for hardness was high ($R^2 = 0.9$) and the corn meal ratio, moisture, temperature and germination period were found to have significant contributions to the hardness of the extrudates. At 30% corn meal ratio, the hardness of the extrudate was 0.84 N whereas the control sample (only proso millet and no corn meal) had a hardness of 1.59 N. Chemical changes occurring during extrusion cooking affect the development of textural and mechanical properties such as hardness in extrudates. The elastic swell effect and bubble growth effect equally contribute to the structural changes of starch during extrusion. Water performs as a plasticizer to the starch-based material decreasing its viscosity and the mechanical energy dissipation in the extruder. Hence the product becomes dense and bubble growth is compressed. The reduced starch conversion and compressed bubble growth result in a dense product and wetness of extrudate. At all feed moisture content levels, decrease in die temperature decreased the product hardness. Screw speed did not have a significant effect on the extrudate hardness. Fig 7.4 shows the effect of moisture and screw speed on the product hardness. Previous studies have also reported similar trends for extrudates based on rice (Ding et al., 2006), wheat (Ding et al., 2006), oat bran and soy flour (Anuonye et al., 2010) and chickpea (Khattak et al., 2007). The hardness was high of 1.8 N at about 140°C, 150 rpm screw speed, 25% feed moisture content, 0% corn meal ratio and Day 0 sprouted samples.

7.3.2.4 Water Solubility Index and Water Absorption Index

Water solubility index is related to the quantity of soluble solids, which is related to dextrinization. The coefficient of determination for water solubility index was highly significant as revealed by a high value of R^2 (0.9). Higher content of corn meal in the blend increased the water solubility of the extrudates. The linear effects of feed moisture, screw speed and temperature had significant effect on water solubility index. In general, water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy favours starch dextrinization. The poor correlation between water solubility index and most of the process and product responses is explained by the fact that water solubility index includes the opposing effects of starch dextrinization and the molecular level interactions between degraded components, which may not be favoured at the same condition (Lei et al., 2008). An increase in the amount of dextrinized starch during extrusion cooking results in an increase in water solubility index. However, molecular interactions between degraded starch, protein, and lipid components, which in turn lead to an increase in molecular weight, may decrease the solubility, thus water solubility index (Camire et al., 1990). Fig 7.5 shows the significant effect of corn meal ratio, temperature and screw speed on water solubility index. The increase in water solubility index with increasing temperature was consistent with the results reported for oat extrudates (Gutkoski & El-Dash, 1999). The water solubility index decreased with the increase in moisture. Similar trends have been reported earlier for starch, maize grits, wheat and pea (Ding et al., 2006; Singh et al., 2007)

Water absorption index depends on the availability of hydrophilic groups and on the gel formation capacity of the macromolecules. It is a measure of damaged starch together with protein denaturation and new macromolecular complex formations. Water absorption index of extrudates ranged between 2.55 and 3.90. The increased corn meal ratio in the blend significantly increased the water absorption ratio. This is an expected result since it includes the effect of starch gelatinization, protein denaturation and molecular level cross-linking reactions which are always favoured at the same conditions (Doğan & Karwe, 2003) The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. The interaction effects except for temperature-screw speed were significant as shown in Fig 7.6. Similar responses were observed in rice flour (Hagenimana et al., 2006), oats (Gutkoski & El-Dash, 1999), finger millet (Sawant et al., 2012) and cassava starch (Hashimoto et al., 2003). Water absorption index had poor correlations with almost all process and product responses.

7.3.2.5 Color

Color is an important quality parameter since it reflects the extent of chemical reactions and degree of cooking or degradation that take place during extrusion cooking. Higher color difference ΔE means darker products with more intense yellow and red color. The total color change in extruded products ranged between 13.07 and 20.37. Germination increased the color of the proso millet flour by breaking down the reducing sugars. The addition of corn meal masked the effects of germination on color. Hence the total color difference was low (13.07) when 30% corn meal was added. Linear effects of temperature and moisture content were found to have the highest contribution to total color change. As depicted in Fig 7.7, low feed moisture content and high temperature increased the total color change possibly due to increased extent of browning reactions under this condition. Although, the screw speed was not a significant parameter, at low screw speeds a slight increase in color change observed due to longer residence times which might increase the extent of chemical reactions.

7.3.2.6 Starch Digestibility (SD) and Protein Digestibility (PD)

The results show that both protein digestibility and starch digestibility of corn meal-free extrudates were increased significantly by soaking, germination and extrusion. Fig 7.8 and Fig 7.9 illustrate the effect of the independent variables on the protein and starch digestibility respectively. The addition of 30% corn meal decreased the starch digestibility to 77.7% and protein digestibility to 78.5%. This is attributed to the reason that the complex starch and protein were not broken down into simpler substances. However, extrusion was the reason which partially increased the digestibility by breaking down the starch and protein. This can be related to the higher efficiency of this thermal treatment in reduction of anti-nutrient activities in addition to soaking and germination. The linear effects of temperature and interaction effect of moisture and screw speed had a significant effect on the protein and starch digestibility. Enhancement of starch digestibility during soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility (Linko et al., 1983). The rupture of starch granules of sprouted proso millet, making the substrate more accessible, facilitated the amylolysis. On the other hand, reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility.

7.3.3 Box-Behnken design and analysis

This response surface methodology is an empirical modeling technique used to evaluate the relationship between the experimental and the predicted results (Muthukumarappan & Swamy, 2018). In the present study, the Box Behnken Design was used to obtain a proper model for the optimization of the extrusion process variables. Experiments were carried out according to the experimental design in order to explore the optimum conditions and study the influence of process variables on the physical and physico-chemical properties of the extrudates. The predicted values were attained by the model fitting technique in Design Expert software version 8.0.7.1 and the values correlated well with the experimental values. Fitting of the data to various models such as the linear, interactive, quadratic and cubic models was performed to acquire the regression equations. Sequential model sum of squares and model summary statistics tests were carried out to decide the adequacy of models in the present study and the results are presented in Table 7.3. From Table 7.3, it is observed that the quadratic model is highly significant for the extrusion of the blend. The model summary statistics demonstrated that the quadratic model had maximum R- Squared, Adjusted R-Squared and Predicted R-Squared values. The cubic model was not suggested for the extrusion process since the BBD matrix had enough data to understand the outcome of the system. Hence, quadratic model was chosen for further analysis of data.

7.3.4 Fitting of polynomial equations and statistical analysis

Based on the Box-Behnken experimental design model, the empirical relationship between the input variables and experimental results obtained were expressed by a second-order polynomial equation with interaction terms (Swamy et al., 2014). The equations generated in coded factors are presented in Table 7.4.

The investigation and optimization of a fitted response surface might generate poor or disingenuous results. Therefore, it was essential to ensure the fitness of the model. Regression analysis and Pareto analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. The results pointed out that the equation sufficiently presented the actual relationship between the input parameters and responses. ANOVA is a statistical technique that subdivides the total variation in a dataset into component parts allied with sources of variation for testing hypotheses on the variables of the model (Swamy & Muthukumarappan, 2017).

ANOVA followed by Fisher’s statistical test (F-test) was conducted to analyse the significance of each independent variable. The F-value is the ratio of the regression mean square and the real error mean. It indicates the influence of each controlled factor on the tested model. The ANOVA results in Table 7.5 shows the F-value for the responses, implying that the model is highly significant. The large Fisher value signifies that the variation in the responses can be explicated by the regression equation (Asokapandian et al., 2016). The associated p value estimates if F value is large enough to show statistical significance. The p values that are lower than 0.0001 indicate that the model and the associated terms are statistically significant.

Coefficient of determination (R^2) and adjusted-R^2 were computed to verify the sufficiency and fitness of the model. The R^2 signifies the proportion of the total variation in the response expected by the model, thus indicating the ratio of the regression sum of squares (SSR) and total sum of squares (SST) (Sangamithra et al., 2015). The values of R^2 imply that 95% of experimental data was well-suited. The high R^2 coefficient gives a satisfactory adjustment of the quadratic model to the experimental data. The purpose of the adjusted-R^2 is to analyse the model adequacy and fitness. The adjusted-R^2 value adjusts the R^2 value for the sample size and for the number of terms in the model (Albarracín et al., 2015). The high value of adjusted-R^2 supports a high correlation between the experimental and the predicted values. The high coefficient of determination and very small p-value (<0.0001) demonstrate that the quadratic polynomial model is significant and adequate to characterize the actual relationship between the response and input.

The coefficient of variation (CV) shows the scattering of the experimental points from the predicted values of the second order polynomial model (Swamy & Muthukumarappan, 2017). A high coefficient of variation points out that there is extreme variation in the mean value and does not adequately develop a sufficient model. The very low coefficient of variation value undoubtedly represents a high degree of precision and reliability of experiments conducted.

7.3.5 Selection of optimum conditions

Optimum conditions for the extrusion process were deduced to get desired extrudate properties. Second order polynomial models attained in the study were used for each response to acquire specified optimum conditions. To optimize the independent variables, the Derringer's desirability function method was utilized. After satisfying the necessities for each response in the design, the Derringer's desirability function seeks for a combination of factor levels that together optimize a set of responses. By the conversion of each into a dimensionless desirability scale which defines a partial desirability function, combining the discrete desirability values to attain the global desirability function and then maximizing the global function and recognizing the optimal factor settings, the optimization process is accomplished. The desirability function scale operates between 0 and 1 with 0 signifying a completely undesirable response and 1 representing a fully desired response. The individual desirability for each response is achieved by stating the goals, i.e., minimize, maximize or target the response, and boundaries essential for each response. A weight factor (defines the shape of the desirability function for each response) is then allocated. The weights range between 0.1 and 10 with the larger ones relating to more significant responses. A weight factor of 1 was selected for the individual desirability in this research. The "importance" of the goals can be arranged sequentially according to increasing importance. It can vary from 1 (least importance) to 5 (most important). To optimize the process the following parameters (1) Moisture (15-25% w.b.) (2) Temperature (90-140°C) (3) Screw speed (80-220 rpm) (4) Corn meal ratio (0-30%) and (5) Germination period (Day 0- Day 4) respectively, were set for maximum desirability. By applying the methodology of the desired function, the optimized process variables were obtained. It indicated that a moisture 15%, temperature 115°C, screw speed 185 rpm and Day 4 germinated flour input will produce an extrudate with bulk density 107.5kg/m^3, hardness 1.10 N, water solubility index 18.45, water absorption index 3.6, total color difference ΔE=16.59, expansion ratio=3.78, protein digestibility=84.8% and starch digestibility=85.8% respectively with overall desirability value of 0.827.

7.3.6 Verification of optimized conditions and predictive model

The fitness of the model equations for predicting optimum response values was investigated under the conditions: moisture 15%, temperature 140°C, screw speed 219 rpm, corn meal ratio 6% and Day 4 germinated proso millet flour. These conditions were optimum by the RSM optimization approach. To verify the validity of the optimized conditions, experiments were performed to evaluate the experimental results vs predicted values of the output using the model equation. The experiments were carried out thrice and the average values were presented in Table 7.6. The results expected from the optimum solution was with bulk density 107.5kg/m^3, hardness 1.10 N, water solubility index 18.45, water absorption index 3.6, total color difference ΔE=16.59, expansion ratio=3.78, protein digestibility=84.8% and starch digestibility=85.8% and the experimental value was bulk density=107.5±0.07 kg/m^3, hardness=1.10±0.09N, water solubility index=18.45±0.06, water absorption index=3.6±0.07, total color difference ΔE=16.59±0.12, expansion ratio=3.78±0.05, protein digestibility=84.8±0.04 and starch digestibility=85.8±0.09. The mean values obtained through the experiments were compared with the predicted values. The values obtained through confirmation experiments are within 95% of predicted values. This shows that the developed quadratic models are well-suited. It is also evident that the optimal values are valid within the specified range of process variables.

7.4 Conclusions

In this study, the effect of corn meal addition to the sprouted proso millet flour was studied. The influence of feed moisture (15-25% w.b.), temperature (80-140°C), screw speed (90-220 rpm), corn meal ratio (0-30%) and germination time (Days 0-4) on the physical and physico-chemical properties of sprouted proso millet-corn meal extrudates were studied. The levels of corn meal ratio significantly improved the color and expansion ratio of the extrudates. The total color change in extruded products ranged between 13.07 and 20.37. Both feed moisture and extruder temperature had significant effects on the extrudate characteristics. Changing the level of moisture content and temperature had significant effect ($p<0.001$) on all physical properties. The regression analysis showed that the linear effect of moisture, screw speed and temperature were highly significant on water absorption index. Hardness ranged between 0.84 and 1.59 N and the coefficient of determination for hardness was high ($R^2 = 0.9$). Extruder screw speed had minimal effect on the extrudate properties. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility. The addition of corn meal however significantly decreased the starch and protein digestibility of the extrudates. By applying the methodology of the desired function, the optimized process variables were moisture 15%, temperature 115°C, screw speed 185 rpm and Day 4 germinated flour with bulk density 107.5kg/m^3, hardness 1.10 N, water solubility index 18.45, water absorption index 3.6, total color difference $\Delta E=16.59$, expansion ratio=3.78, protein digestibility=84.8% and starch digestibility=85.8% respectively with overall desirability value of 0.827. The results therefore clearly showed that highly expanded products can be produced by blending corn meal with proso millet. However, the starch and protein digestibility of the extrudates is significantly reduced.

Table 7.1: Impact of soaking and germination on the nutritional properties of proso millet flour

	Raw proso millet	Day 0	Day 1	Day 2	Day 3	Day 4
Total Protein[a]	13.1±0.5	13.1±0.15	13.2±0.03	13.3±0.05	13.4±0.01	13.1±0.07
Carbohydrates[a]	63.2±0.6	63.2±0.16	63.3±0.03	63.4±0.07	63.4±0.09	63.6±0.02
Moisture[a]	11.1±0.2	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid[b]	1.10±0.07	0.69±0.05	0.55±0.05	0.46±0.02	0.33±0.04	0.21±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 7.2: Nutritional properties of sprouted proso millet-corn meal blend

	Raw proso millet + corn meal	Day 0 + corn meal	Day 1 + corn meal	Day 2 + corn meal	Day 3 + corn meal	Day 4 + corn meal
Total Protein[a]	14.4±0.5	14.9±0.09	15.2±0.03	15.3±0.05	15.4±0.01	15.1±0.07
Carbohydrates[a]	71.2±0.7	71.2±0.16	72.3±0.03	73.4±0.07	74.4±0.09	74.9±0.02
Moisture[a]	11.4±0.4	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.4±0.02
Phytic acid[b]	1.01±0.07	0.61±0.05	0.45±0.05	0.36±0.02	0.23±0.04	0.11±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 7.3: Results of model adequacy tested in the Box-Behnken design

Source	Sum of squares	DF	Mean square	F value	Prob > F	Remarks
*Sequential model sum of squares for **Bulk Density***						
Quadratic	541.33	4	135.33	30.10	< 0.0001	Suggested
*Sequential model sum of squares for **Hardness***						
Quadratic	0.29	4	0.074	22.42	< 0.0001	Suggested
*Sequential model sum of squares for **Water Solubility Index***						
Quadratic	10.6	3	3.5	196.03	< 0.0001	Suggested
*Sequential model sum of squares for **Water Absorption Index***						
Quadratic	1.93	4	0.48	13.46	< 0.0001	Suggested
*Sequential model sum of squares for **Total Color Difference ΔE***						
Quadratic	21.5	3	7.05	123.2	0.0004	Suggested
*Sequential model sum of squares for **Expansion Ratio***						
Quadratic	0.29	4	0.074	22.42	< 0.0001	Suggested
*Sequential model sum of squares for **Protein Digestibility***						
Quadratic	6.5	3	2.17	131.2	< 0.0001	Suggested
*Sequential model sum of squares for **Starch Digestibility***						
Quadratic	26.64	4	6.66	14.87	< 0.0001	Suggested

Source	**Std. Dev.**	**R-square**	**Adjusted R-square**	**Predicted R-square**	**PRESS**	
Model summary statistics for ***Bulk Density***						
Quadratic	2.12	0.9613	0.9481	0.9254	866.14	Suggested
Model summary statistics for ***Hardness***						
Quadratic	0.013	0.9731	0.9617	0.9273	0.027	Suggested
Model summary statistics for ***Water Solubility Index***						
Quadratic	0.70	0.8925	0.8560	0.7929	93.73	Suggested
Model summary statistics for ***Water Absorption Index***						
Quadratic	0.19	0.8713	0.8275	0.7520	6.92	Suggested
Model summary statistics for ***Total Color Difference ΔE***						
Quadratic	0.003	0.9992	0.9988	0.9977	0.001	Suggested
Model summary statistics for ***Expansion Ratio***						
Quadratic	0.057	0.9629	0.9502	0.9284	0.63	Suggested
Model summary statistics for ***Protein Digestibility***						
Quadratic	0.93	0.8918	0.8550	0.7916	165.95	Suggested
Model summary statistics for ***Starch Digestibility***						
Quadratic	0.012	0.9924	0.9892	0.9801	0.023	Suggested

+ case(s) with leverage of 1.0000: PRESS statistic not defined

Table 7.4: Second order polynomial equations representing the relationship between independent and dependent variables

Bulk Density (kg/m^3)	12.00 +8.97A -4.17B -2.57C -7.90D +2.28E1 +0.95E2 -0.052E3 -1.05E4 -2.25AB +1.55AC +1.20AD -0.050AE1 -0.22AE2 +0.033AE3 +0.033AE4 -2.00BC +1.25BD -0.45CD -0.10CE1 -0.017CE2 +0.067CE3 +0.067CE4 -0.52DE1 -0.100DE2 +0.23DE3 +0.23DE4 -0.30A^2 +3.00B^2 -0.75C^2 +2.75D^2
Hardness (N)	1.21 +0.18A -0.079B -0.045C -0.12D +0.030E1 +0.016E2 -0.027E3 -5.037E-003E4 +5.000E-004AB -0.075AC -9.500E-003AD -0.014AE1 -0.015AE[2] +0.060AE3 -0.014AE4 -0.020BC -0.017BD +0.016BE1 +0.015BE2 -0.060BE3 +0.015BE4 -0.042CD +5.000E-004CE1 +5.000E-004CE2 -3.333E-004CE3 -3.333E-004CE4 -9.167E-003DE1 -8.333E-004DE2 +1.667E-003DE3 +4.167E-003DE4 +0.034A^2 +1.000E-003B^2 +0.033C^2 -0.040D^2
Water Solubility Index (%)	11.40 -1.89A +0.51B +0.23C +0.21D -0.22E1 -0.093E2 +7.407E-003E3 +0.10E4 -0.12AB -0.15AC -0.46AD -6.667E-003AE1 +1.667E-003AE2 +1.667E-003AE3 +1.667E-003AE4 -0.45BD +6.667E-003BE1 -1.667E-003BE2 -1.667E-003BE3 -1.667E-003BE4 +0.14CD +1.000E-002CE1 +1.667E-003CE2 +1.667E-003CE3 -6.667E-003CE4 +0.053DE1 -5.000E-003DE2 -5.000E-003DE3 -0.013DE4 +2.08A^2 -0.19B^2 -0.15C^2 +6.667E-003D^2
Water Absorption Index	3.44 -0.40A +0.045B +0.043C +0.43D -0.029E1 -8.296E-003E2 +2.444E-003E3 +0.012E4 -0.11AB -0.10AC +0.20AD -2.667E-003AE1 +6.667E-004AE2 +6.667E-004AE3 +6.667E-004AE4 -0.034 BC -0.039BD +2.333E-003BE1 +6.667E-004 BE2 -

	1.000E-003BE3 -1.000E-003BE4 +0.063CD +1.667E-004CE1 -6.667E-004CE2 +1.667E-004CE3 +1.667E-004CE4 +1.500E-003DE1 -1.000E-003DE2 -1.667E-004DE3 -1.667E-004DE4 -0.23A^2 -0.14B^2 -0.11C^2 -0.20D^2
Total Color Difference ΔE	7.03 -0.41A +0.12B +0.11C -1.40D -0.046 * E1 +5.926E-003E2 +9.630E-003E3 +0.013E4 +0.62AB +0.41AC +1.35AD -0.12AE1 -0.045AE2 +5.000E-003AE3 +0.055AE4 -0.010 BC +0.17BD +0.060BE1 +1.000E-002BE2 -6.667E-003BE3 -0.023BE4 +1.27CD -0.053CE1 -0.012CE2 +5.000E-003CE3 +0.022CE4 -0.22DE1 -0.070DE2 +0.013DE3 +0.097DE4 +0.048A^2 -0.019B^2 +0.47C^2 -0.20D^2
Expansion Ratio	4.4 -0.28A +0.063B +0.059C +0.22D -0.031E1 -8.815E-003E2 +2.296E-003E3 +0.013E4 -0.022AB -0.015AC +0.047AD +3.333E-003AE2 +8.333E-004AE3 +0.051BC -0.026BD -6.667E-004BE1 -6.667E-004BE2 +1.667E-004BE3 +1.667E-004BE4 -7.500E-003CD -3.167E-003CE1 +1.667E-004 * CE2 +1.000E-003CE3 +1.000E-003CE4 +4.167E-003DE1 -2.500E-003DE2 -1.667E-003DE3 -8.333E-004DE4 -0.052A^2 -0.095 B^2 -0.025C^2 -0.067D^2
Protein Digestibility (%)	9.58 -0.74A +0.70B +0.38C -2.81D -0.48E1 -0.036 E2 +0.045E3 +0.19E4 +0.51AB +0.57AC -0.27AD +0.64AE1 -0.14AE2 -0.21AE3 -0.15AE4 -0.040BC +0.12BD -0.34BE1 +0.048BE2 +0.082BE3 +0.11BE4 +0.13CD +0.17CE1 -0.020CE2 -0.095CE3 -0.028CE4 +0.36DE1 -0.13DE2 -0.095DE3 -0.070DE4 +1.54A^2 +0.37B^2 +0.18C^2 +1.83D^2

Starch Digestibility (%)	$11.20 -0.94A +0.78B +1.05C -2.44D -0.57E1 -6.667E-003E2 +0.10E3 +0.20E4 -0.075AB +0.55AC +0.15AD +0.37AE1 -0.092AE2 -0.092AE3 -0.092AE4 -0.020BC -0.51BD +0.040BE1 -0.018BE2 -0.027BE3 -0.027BE4 +0.35CD +5.000E-003CE1 +0.013CE2 +0.013CE3 +0.013CE4 +0.20DE1 -0.057DE2 -0.048DE3 -0.048DE4 +0.45A^2 -0.11B^2 -0.32C^2 +0.65D^2$

A – Moisture Content (%w.b.); B – Screw Speed (rpm); C – Die Temperature; D – Corn meal ratio; E_1, E_2, E_3, E_4 – Germination time (Days)

Table 7.5: Pareto analysis of variance (ANOVA) analysis and significant statistical parameters of the model

Source	Sum of squares	DF	Mean square	F-value	p-value
Bulk Density					
Model	5521.09	34	0.30	77.95	< 0.0001
A-moisture content	3648.10	1	0.035	9.16	< 0.0001
B-screw speed	990.02	1	395.27	87.92	< 0.0001
C- die temperature	342.23	1	0.083	21.87	< 0.0001
D – corn meal ratio	334.05	1	3744.60	832.91	< 0.0001
E – germ period	145.07	4	79.20	17.62	< 0.0001
AB	101.25	1	101.25	22.52	< 0.0001
BC	80.00	1	80.00	10.69	< 0.0001
B^2	240.00	1	240.00	6.41	< 0.0001
D^2	201.67	1	201.67	44.86	< 0.0001
C.V. %	1.71				
Hardness					
Model	0.015	34	0.10	25.28	< 0.0001
A- moisture content	0.19	1	1.85	2.16	< 0.0001
C- die temperature	0.053	1	0.30	0.54	< 0.0001
D – corn meal ratio	0.026	1	0.035	0.30	< 0.0001
E – germ period	0.083	4	395.27	2.77	< 0.0001
A^2	0.083	1	0.083	25.28	< 0.0001
C.V. %	4.10				

Water Solubility Index					
Model	50.4	34	6.11	2771.39	< 0.0001
A- moisture content	7.31	1	119.37	401.97	< 0.0001
B-screw speed	3.48	1	25.28	191.41	< 0.0001
C- die temperature	0.64	1	2.16	35.02	< 0.0001
D – corn meal ratio	7.20	1	0.54	396.17	< 0.0001
E – germ period	0.51	4	0.30	27.81	< 0.0001
A^2	2.77	1	2.77	60.77	< 0.0001
C.V. %	1.51				
Water Absorption Index					
Model	6.54	34	0.26	6220.16	< 0.0001
A- moisture content	4.73	1	0.076	3418.87	< 0.0001
B-screw speed	0.87	1	0.042	345.30	< 0.0001
C- die temperature	0.13	1	0.017	148.33	< 0.0001
D – corn meal ratio	187.06	1	0.001	546.76	< 0.0001
E – germ period	119.37	4	0.007	2616.91	< 0.0001
AB	0.26	1	0.076	110.85	< 0.0001
AC	0.063	1	0.042	27.23	< 0.0001
A^2	0.27	1	0.27	118.79	< 0.0001
B^2	0.068	1	0.068	29.58	< 0.0001
C^2	0.062	1	0.062	26.73	< 0.0001
C.V. %	1.75				

Total Color Difference ΔE					
Model	261.57	34	42.23	42.23	< 0.0001
A- moisture content	187.06	1	1.72	1.72	< 0.0001
D – corn meal ratio	0.87	1	2.66	2.66	< 0.0001
A^2	6.32	1	16.77	16.77	< 0.0001
C.V. %	3.09				
Expansion Ratio					
Model	0.045	34	0.34	0.085	< 0.0001
A- moisture content	0.8	1	6.67	0.072	< 0.0001
B-screw speed	0.085	1	0.78	0.032	< 0.0001
C- die temperature	0.072	1	0.46	0.073	< 0.0001
D- corn meal ratio	0.068	4	0.063	27.23	< 0.0001
B^2	0.19	1	0.19	35.88	< 0.0001
C^2	0.11	1	0.11	20.32	< 0.0001
C.V. %	2.24				
Protein Digestibility					
Model	138.02	34	5.52	84.63	< 0.0001
A- moisture content	18.91	1	18.91	289.81	< 0.0001
B-screw speed	5.55	1	5.55	85.08	< 0.0001
C- die temperature	3.60	1	3.60	55.18	< 0.0001
D – corn meal ratio	103.76	1	25.94	397.62	< 0.0001
E- germ period	0.78	4	0.78	147.77	< 0.0001
AB	1.57	1	1.57	24.04	< 0.0001
B^2	1.83	1	1.83	28.08	< 0.0001
C.V. %	0.30				

Starch Digestibility					
Model	142.81	34	5.71	251.28	< 0.0001
A- moisture content	9.03	1	9.03	186.19	< 0.0001
B-screw speed	10.05	1	6.64	4.69	< 0.0001
C- die temperature	186.19	1	6.97	5.04	< 0.0001
D – corn meal ratio	4.69	1	28.98	42.6	< 0.0001
E – germ period	5.04	4	1.57	251.28	< 0.0001
AB	10.65	1	0.48	6.98	< 0.0001
B^2	1.33	1	1.61	23.34	< 0.0001
C^2	10.05		1.72	24.95	< 0.0001
C.V. %	0.31				

Table 7.6: Predicted and experimental values of the responses at optimum conditions

Moisture Content (%) = 15; Temperature (°C) = 140; Screw Speed (rpm) = 219; Corn meal ratio = 6%; Germination = Day 4

	Optimized values[a] (predicted values)	Experimental values[b]
Bulk density(kg/m^3)	107.5	107.5±0.07
Hardness (N)	1.10	1.10±0.09
Water Solubility Index (%)	18.45	18.45±0.06
Water absorption Index	3.6	3.6±0.07
Total Color Difference ΔE	16.59	16.59±0.12
Expansion Ratio	3.78	3.78±0.05
Protein Digestibility (%)	84.8	84.8±0.04
Starch Digestibility (%)	85.8	85.8±0.09

a Predicted using response surface quadratic model

b Mean ± standard deviation of triplicate determinations from experiments

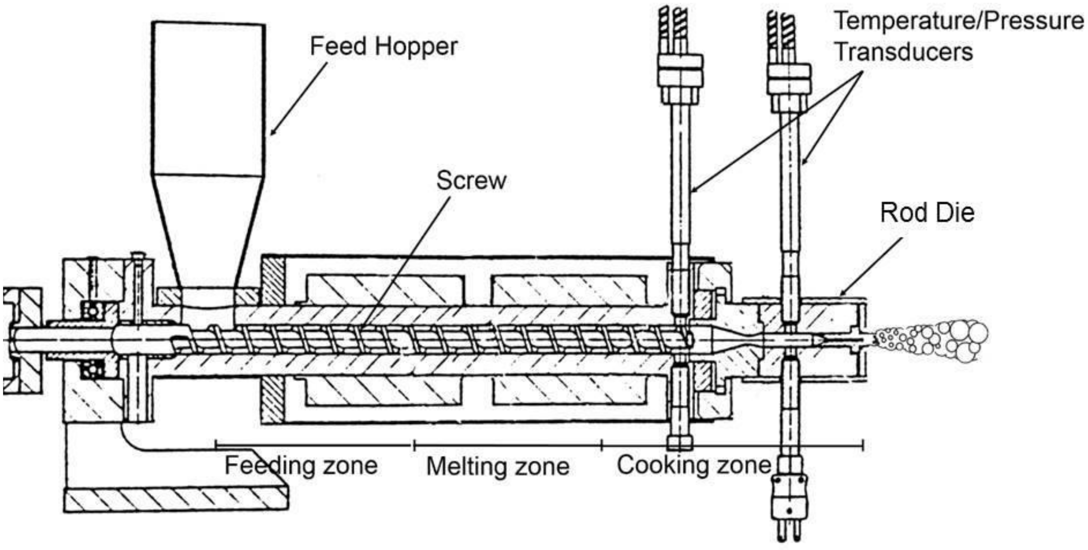


Figure 7.1: Single-screw extruder barrel zones

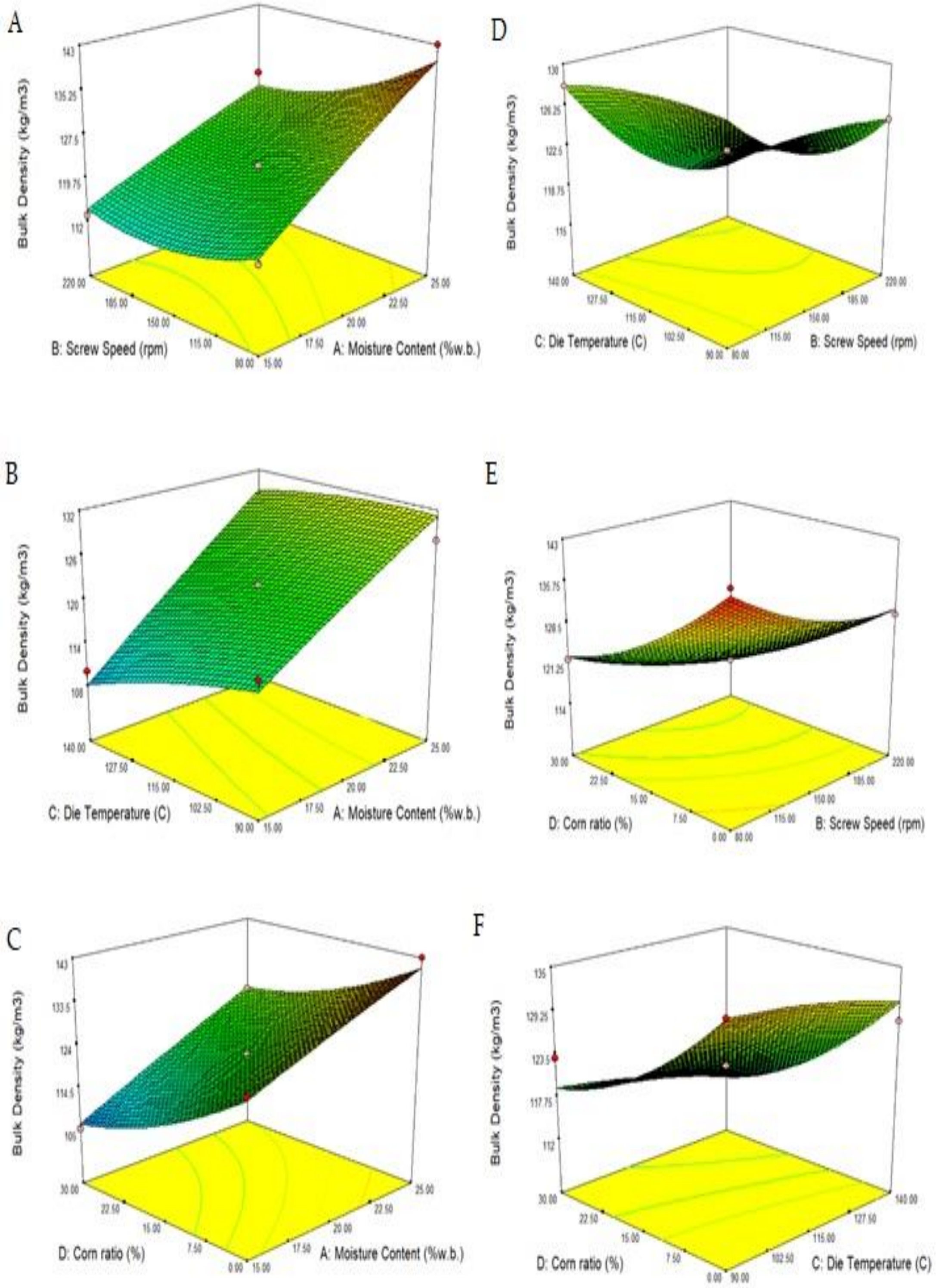


Figure 7.2 Response surface plots (3D) showing the effects on the bulk density (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

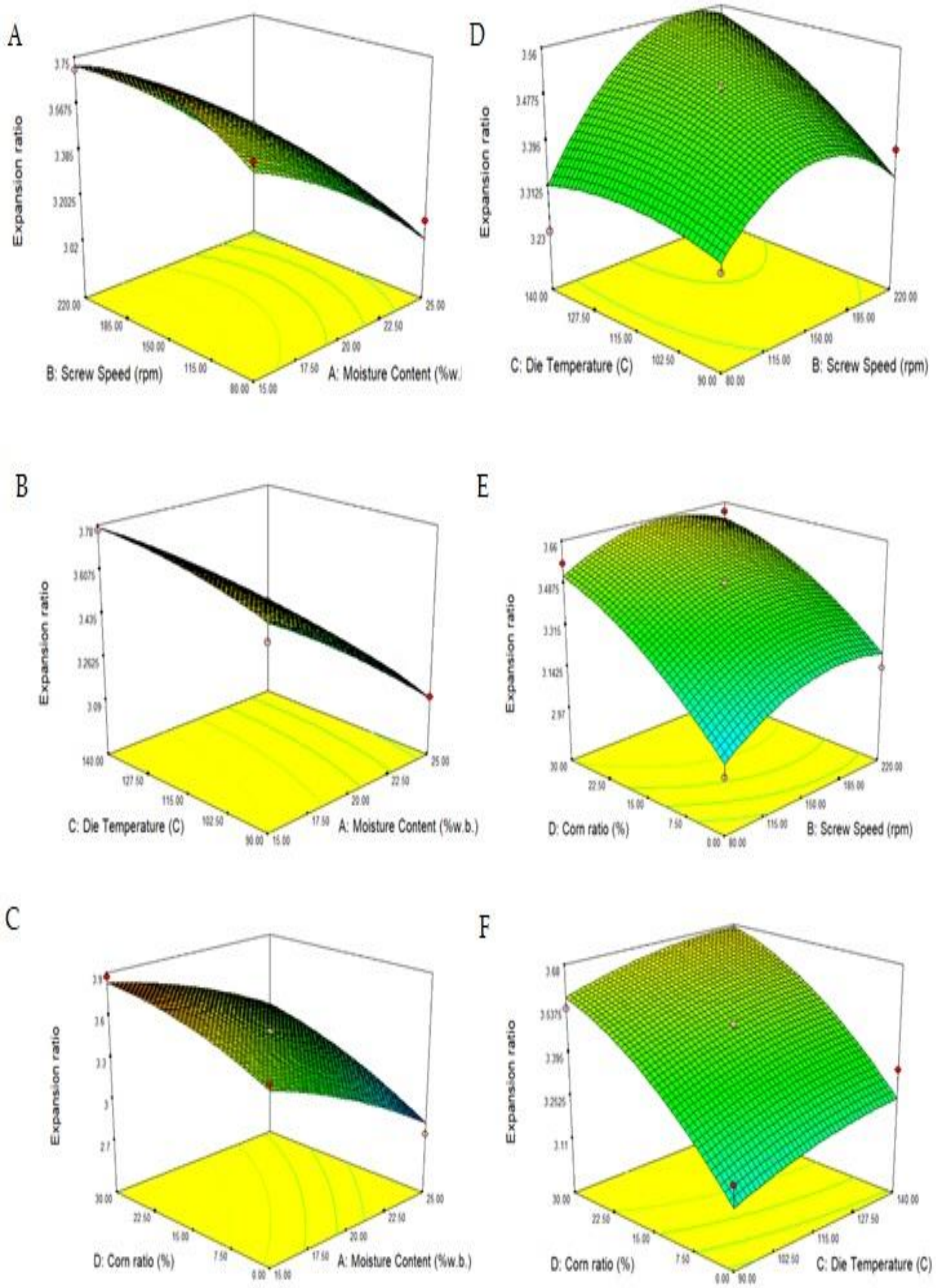


Figure 7.3: Response surface plots (3D) showing the effects on the expansion ratio (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

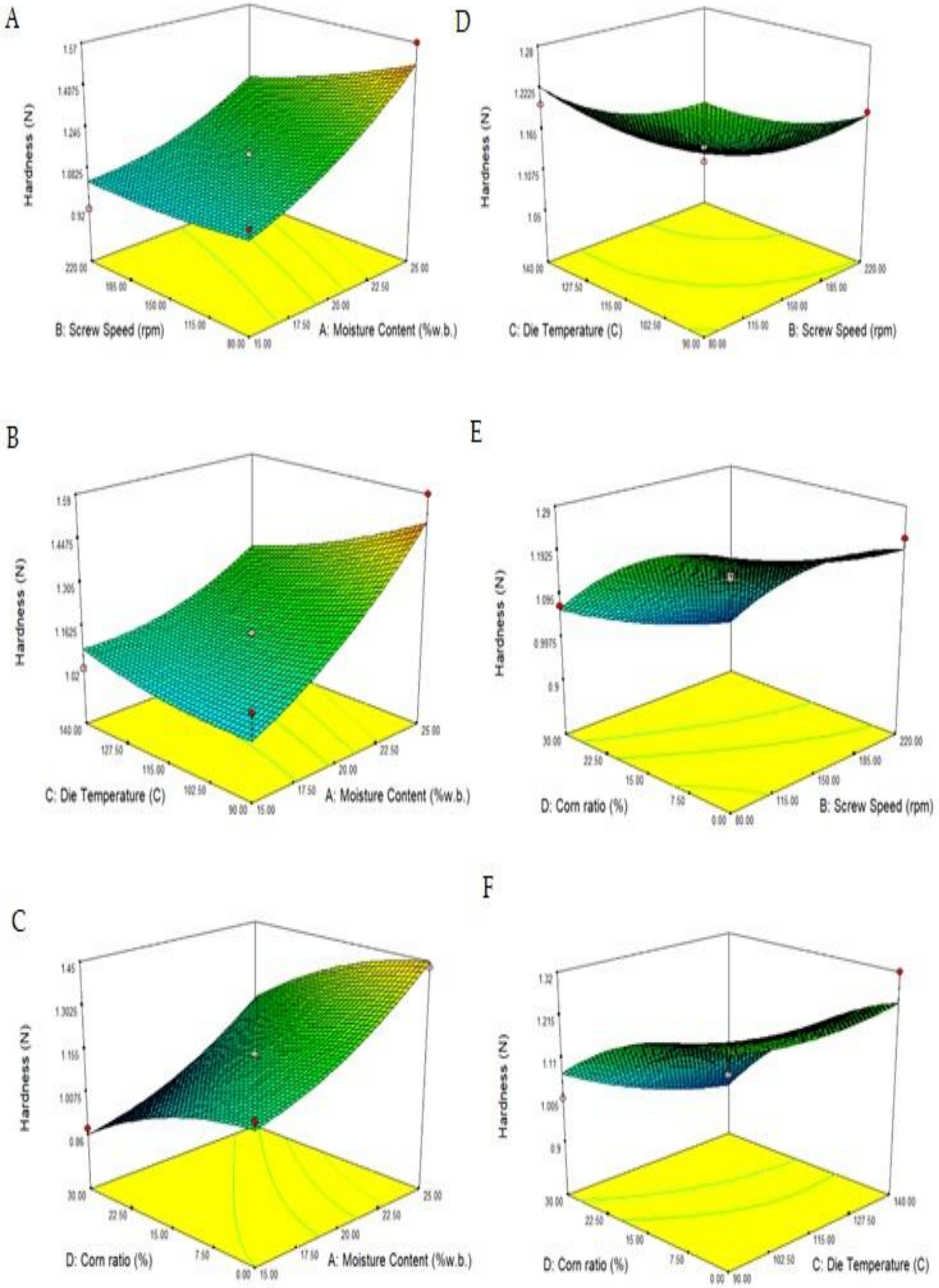


Figure 7.4: Response surface plots (3D) showing the effects on the hardness (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

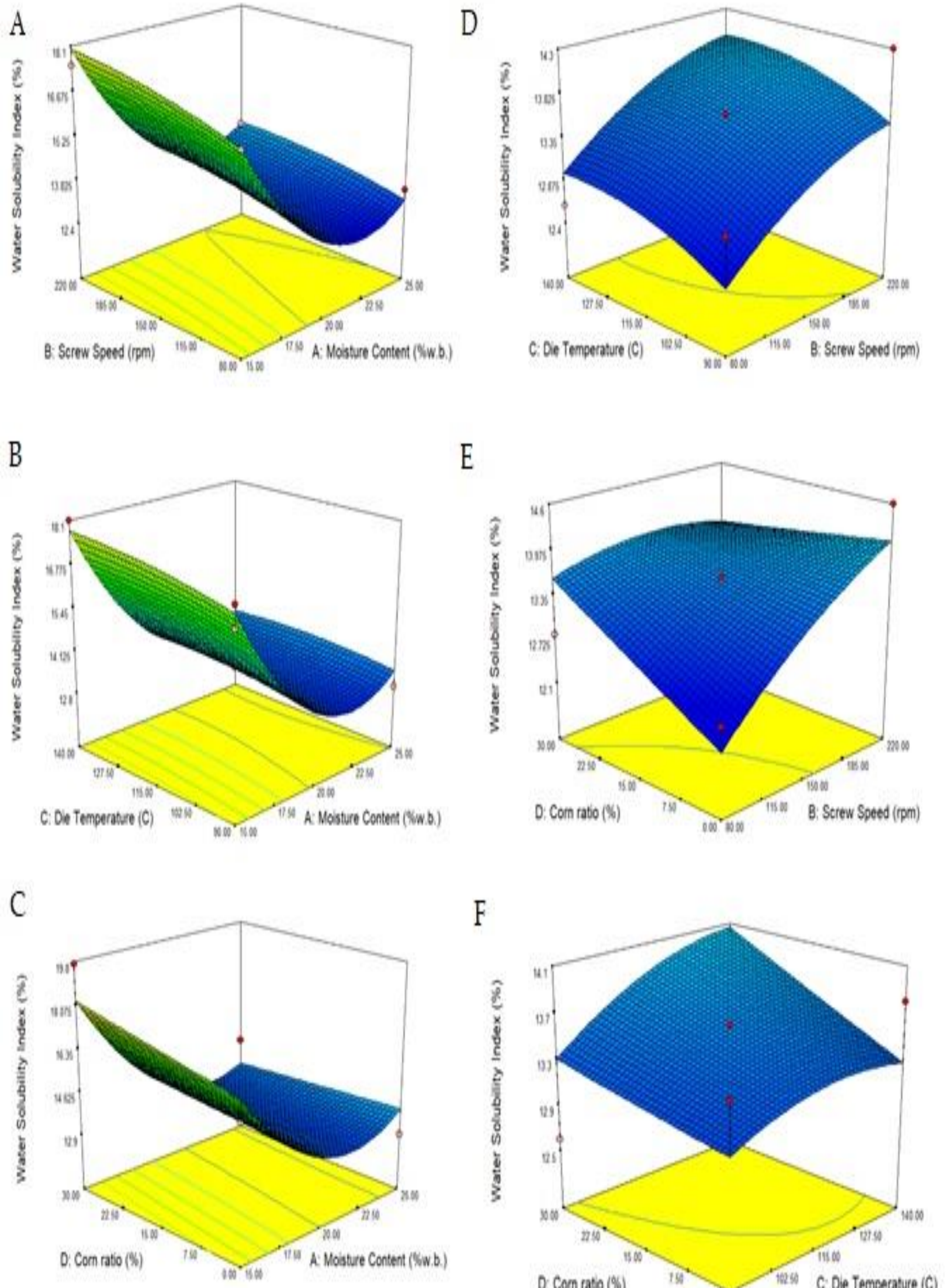


Figure 7.5: Response surface plots (3D) showing the effects on the water solubility index (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

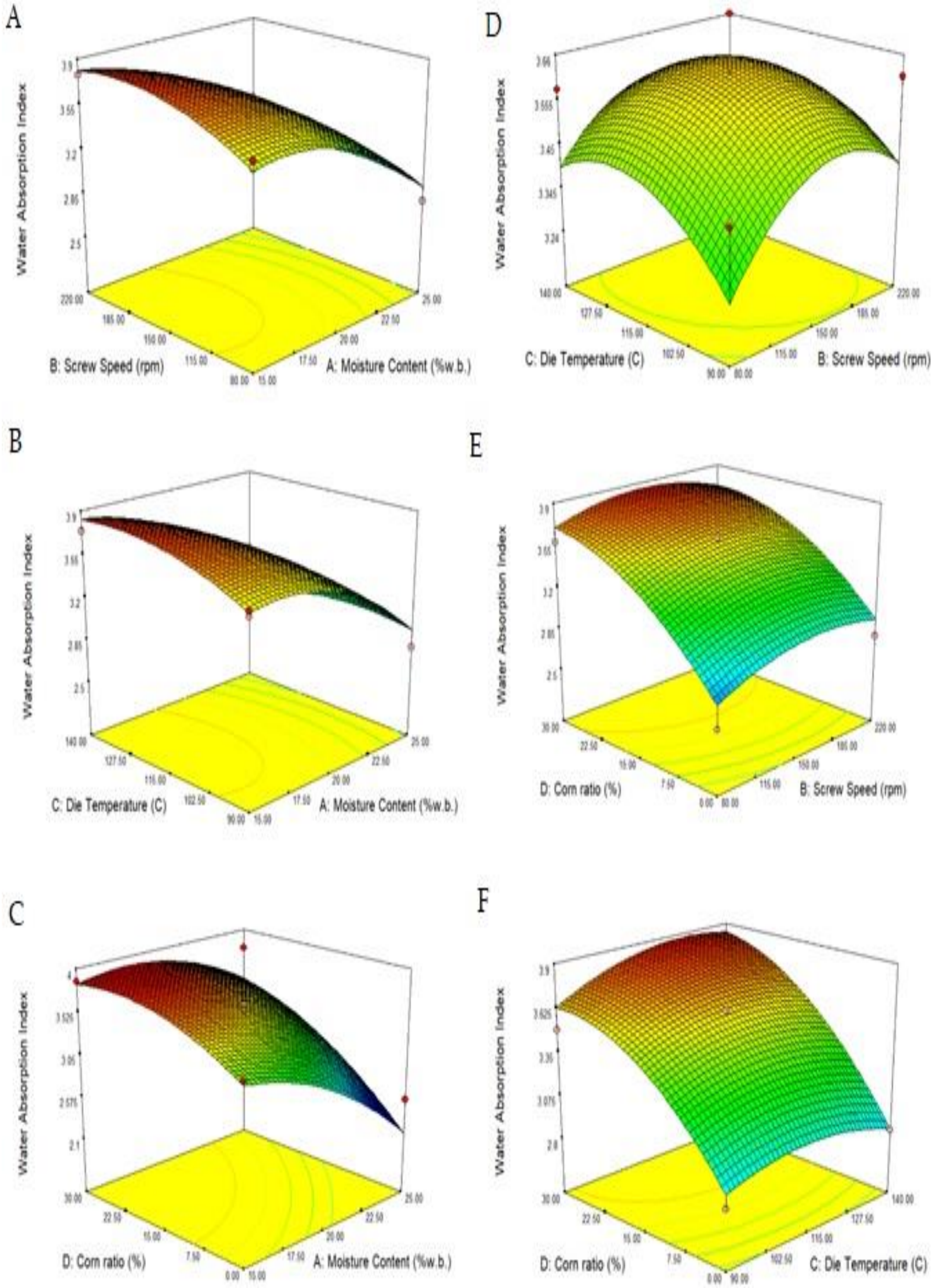


Figure 7.6: Response surface plots (3D) showing the effects on the water absorption index (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

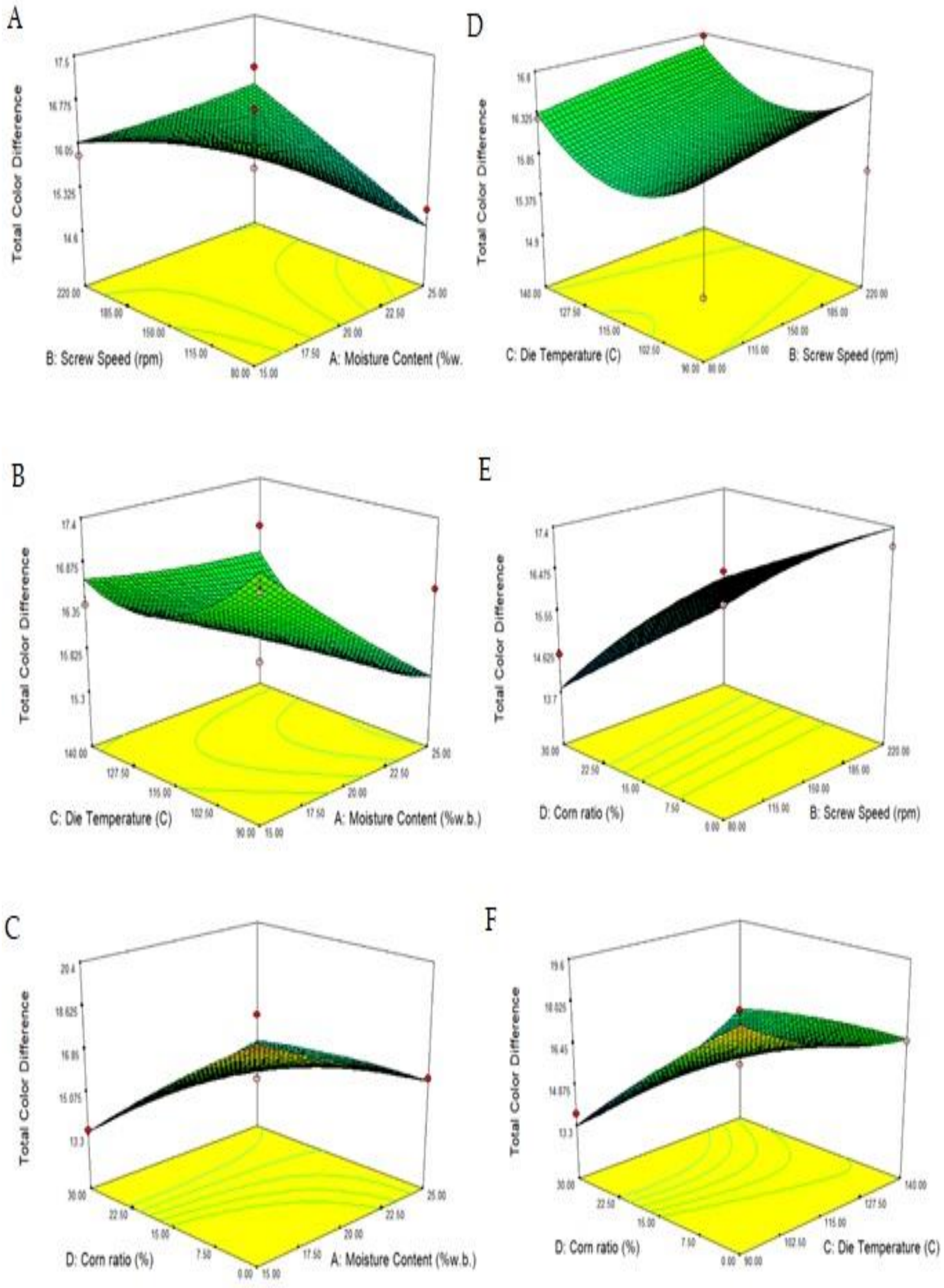


Figure 7.7: Response surface plots (3D) showing the effects on the total color difference (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

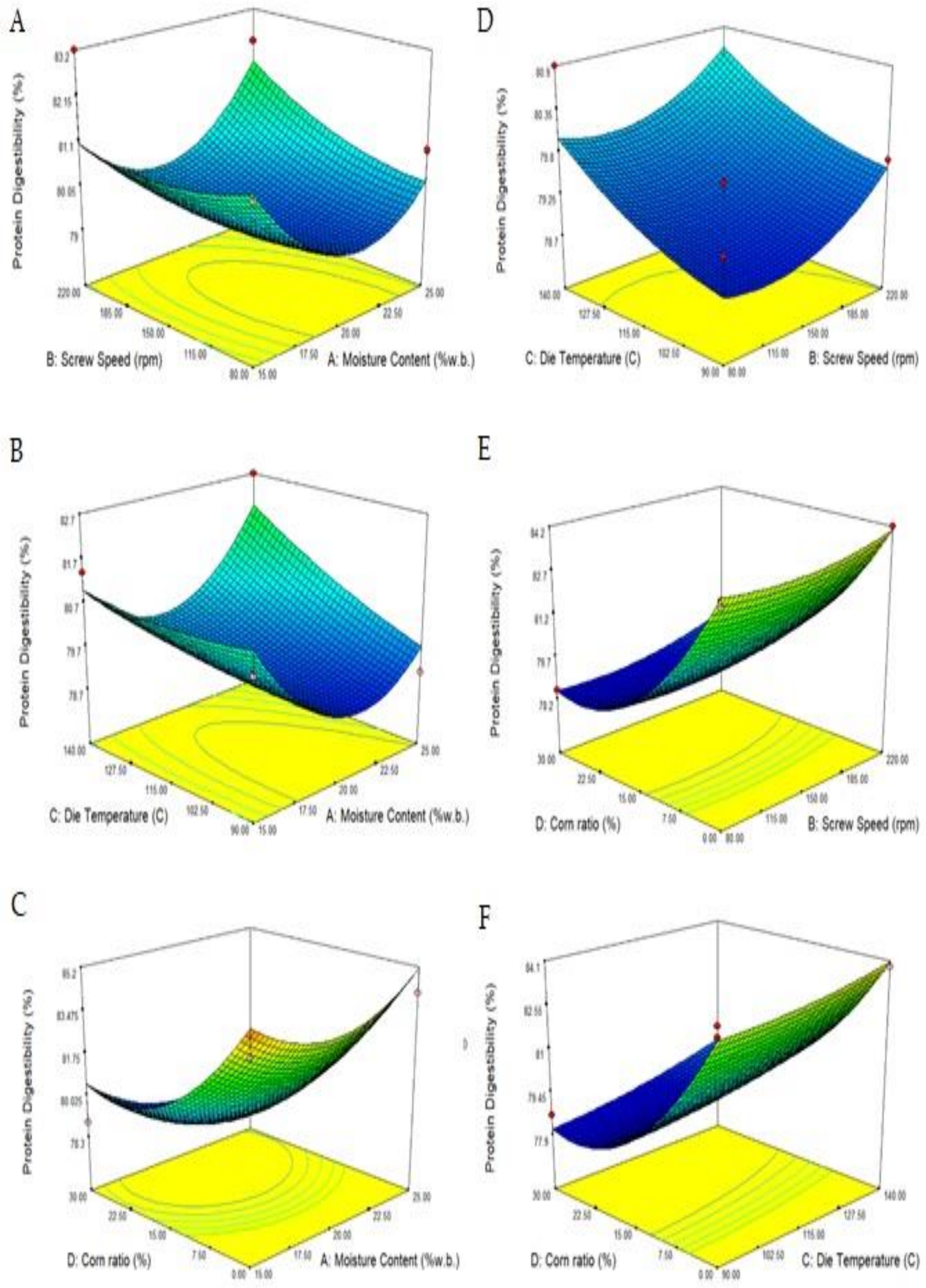


Figure 7.8: Response surface plots (3D) showing the effects on the protein digestibility (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

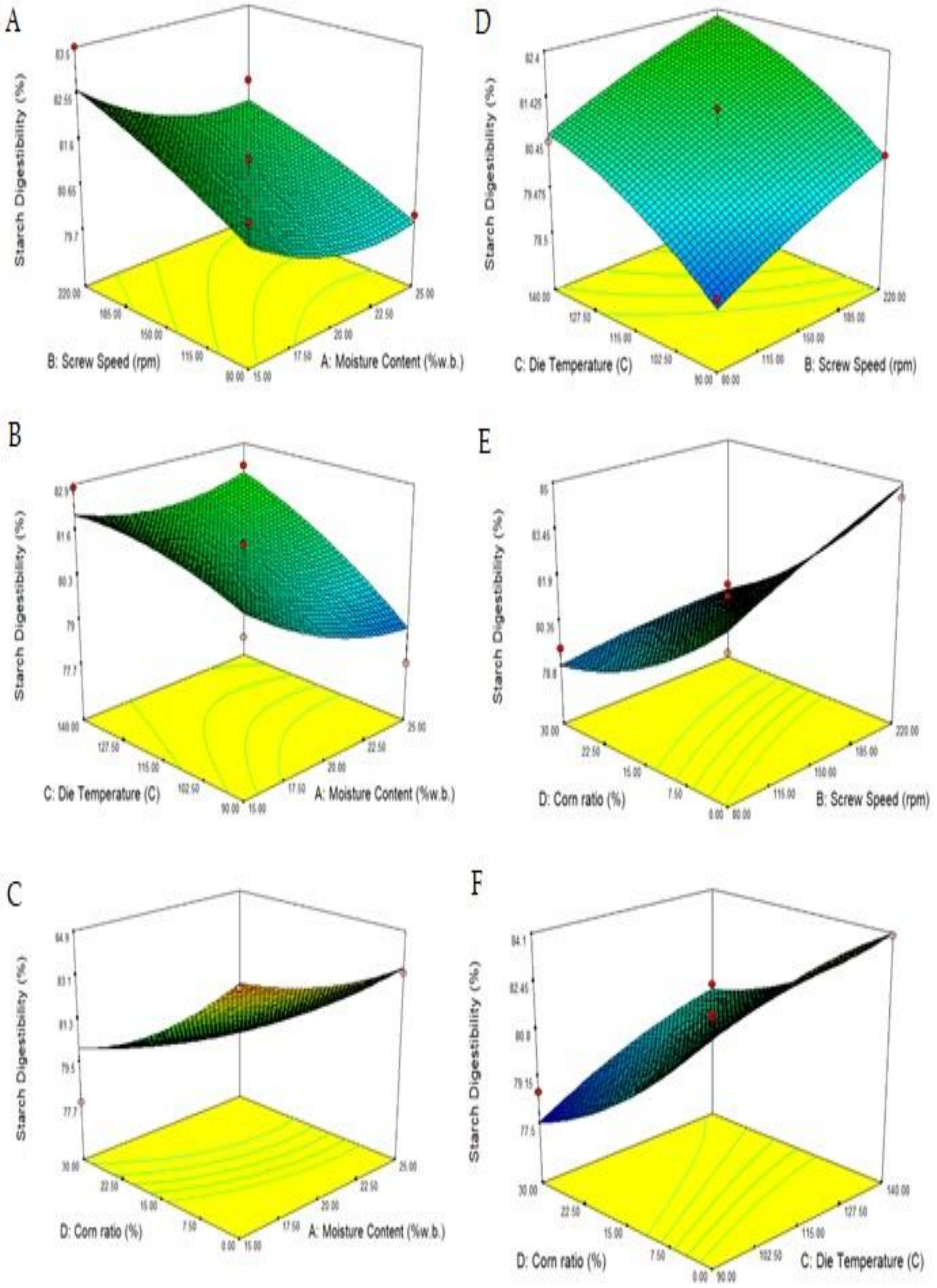


Figure 7.9: Response surface plots (3D) showing the effects on the starch digestibility (A) moisture and screw speed (B) die temperature and moisture (C) corn meal ratio and moisture (D) temperature and screw speed (E) corn meal ratio and screw speed (F) corn meal ratio and die temperature

CHAPTER 8: EFFECT OF TWIN-SCREW EXTRUSION PROCESS PARAMETERS ON THE PHYSICAL AND PHYSICO-CHEMICAL PROPERTIES OF SPROUTED PROSO-MILLET EXTRUDATES

Abstract

Twin-screw extrusion processing of sprouted proso millet flour was studied using a response surface design to understand the influence of feed moisture (15-25% w.b.), die temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on the physical and physico-chemical properties of proso millet extrudates. Soaking and germination pre-treatments of proso millet were carried out to reduce the phytic acid content and therefore increase protein and starch digestibility. The following responses of the extrudates were measured and the values ranged from: bulk density (132-175 kg/m^3), hardness (1.56-2.14 N), water solubility index (14.4-18.5%), water absorption index (2.93-3.41), ΔE (16.7-20.8), expansion ratio (2.28-2.83), protein digestibility (78.4-84.1%) and starch digestibility (77.1-83.4%). All independent parameters had statistically significant effects on all the extrudate characteristics. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility.

Keywords: Proso millet, saponin, gluten-free, soaking, germination, digestibility

8.1. Introduction

Coeliac disease, also known as gluten-sensitive enteropathy, is one of the common food intolerances on a global scale (Green & Jabri, 2003). Tiny hair-like projections in the small intestine called villi are injured through an immune-mediated response which leads to mal-absorption of vital nutrients like iron, folic acid, calcium and fat-soluble vitamins, when exposed to gluten (Di Sabatino & Corazza, 2009) (Thompson, 2009). Due to lack of any treatment, food allergic and food intolerant people need to avoid the foods to which they are allergic or intolerant (Sollid, 2002). However, individuals who follow an allergy-free diet may be at danger of several nutrient deficiencies (Collin et al., 1994). Instead of wheat, rye, barley, or other gluten-containing cereal grains, there are numerous other edible seeds that can be consumed, including amaranth, buckwheat, proso millet, teff and wild rice, as well as cereal grains that do not contain gluten, including corn, millet, rice, and sorghum (Lee et al., 2009) (Thompson, 2009) (Pagano, 2006). For example, proso millet is a pseudo cereal and has been cultivated in the Andean region. It is an annual plant, 1–2 m tall with deep penetrating roots. It can be grown from sea level up to an altitude of 3800 m (Alandia, Rodriguez, Jacobsen, Bazile, & Condori, 2016). The plant has tolerance to frost, salinity and drought, and is cultivated on marginal soils (Jacobsen, 2003). Proso millet has outstanding protein quality and a wide range of minerals and vitamins, which makes it popular for consumption (Jones, Beckwith, Khoo, & Inglett, 1970). In addition, it is rich in amino acids like lysine and methionine that are deficient in cereals (Parameswaran & Sadasivam, 1994). Proso millet is an excellent source of fiber, iron, calcium, magnesium, and B vitamins (Navruz-Varli & Sanlier, 2016). Because of their nutritive properties, edible seeds other than the gluten-containing cereal grains and pseudocereals are viable candidates to formulate gluten-free diets.

One of the factors which limit the widespread utilization of proso millet is phytic acid. Phytic acid (myoinositol hexaphosphoric acid) reduces the availability of many minerals like iron, zinc, calcium and magnesium (Cheryan, Rackis, & Nutrition, 1980). The formation of iron-phytate complexes of low solubility, in the small intestine is the basis for the interference of phytate with iron absorption (Ravindran, 1991). Although the iron content in cereals is usually high, the iron absorption from them is often poor due to the presence of high amounts of phytates. They are also found to inhibit the proteases and amylases of the intestinal tract (Coulibaly, Kouakou, & Chen, 2011). Proso millet contains many essential minerals like calcium (874 mg/kg), phosphorus (5.3 g/kg),iron (5.3 g/kg) and zinc (36 mg/kg) and their availability is of importance in relation to its use as a basic source of nutrients in an infant food (Oatway, Vasanthan, & Helm, 2001). Soaking, sprouting and fermentation are some of the methods that can break down the phytic acid to increase the digestibility of the food as well as enhance mineral absorption (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2003).

Sprouted grains have been consumed by people who have a strict diet schedule or by ones who have a rigorous exercise pattern. Soaking and sprouting are an age-old technique however the benefits of these methods has not been completely exploited to increase the digestibility of food. Sprouted seeds are nutritionally superior to their respective seeds Germinated grains have higher levels of nutrients, lower levels of anti-nutrients such as phytic acid and flatulence-causing sugars, increased protein and starch digestibility, and increased bioavailability of some minerals (Carciochi, Manrique, & Dimitrov, 2014). The inclusion of sprouted grains in the formulation of whole-grain-rich, fiber-rich, and gluten-free baked goods may provide a natural fortification and enrichment mechanism; it may improve nutritional quality because of the increased digestibility and bioavailability of some nutrients and the reduction of some anti-nutrients. Comprehensive reviews of the effects of germination on the nutrient composition of cereals and pseudocereals have been published (Carciochi et al., 2014) (Lombardi-Boccia, Lullo, Carnovale, & Agriculture, 1991) (Lorenz & D'Appolonia, 1980). Not all people enjoy the flavour of a sprouted whole grain. Hence it is important to convert it to an interesting product before it reaches the consumer.

Twin-screw extrusion processing is a commonly used processing technology in the food industry with a wide number of applications. It is a processing system that utilizes a twin screw or a set of screws to force food materials through a die opening. The food ingredients are subjected to high shear, temperature, and pressure for a short period of time (Gopirajah & Muthukumarappan, 2018). The role of the screw is to assist in imparting shear to the ingredient mixture and forcing the dough of the mixed ingredients out from the extruder through the die (Muthukumarappan & Karunanithy, 2012). The screws are also responsible for the build-up of pressure that occurs at the end of the extruder as well as added mixing of the ingredients. This helps to transform the ingredients from solid powders to a melt state inside of the extruder (Guy, 2001). The melted ingredients are then forced through a die at the end of the extruder into the atmosphere. The melt coming out of the extruder encounters a sudden drop in pressure, resulting in rapid expansion as well as a decrease in temperature, helping it to transform into a cooked product. The final quality and texture of the extruded product depends on various factors, including the ingredient mixture and its properties, extrusion processing conditions, and post-processing conditions (Harper & Clark, 1979). Because of the great flexibility of extrusion processing, it has found very diverse applications in the food industry.

Screw extruders are divided into single screw and twin-screw extruders. Single screw extruders are most popular for their low cost, simple designs, ruggedness, and reliability. Co-rotating and counter-rotating twin-screw extruders are commonly used in the food industry for profile extrusion to compounding and mixing (Van Zuilichem, Kuiper, Stolp, & Jager, 1999). Counter-rotating twin-screw extruders, which are like gear pumps, provide the maximum positive displacement. They are the machine of choice for profile extrusion, whereas co-rotating twin-screw extruders are more suitable for other applications such as compounding and mixing (Van Zuilichem, Stolp, & Janssen, 1983). The main reason for suitability of twin-screw extruders for these applications is the complexity of the flow in the intermeshing region, which provides them good mixing and compounding characteristics (Yang & Williams, 1990). Compared to single screw extruders, twin screw extruders are more efficient in providing homogeneous mixing of different ingredients. Larger variety of twin-screw extruders are available that may vary in construction, could have parallel or conical screws that may rotate in the same direction (corotating) or opposite direction (counter-rotating), and have different degrees of intermeshing. The complexities of the flow make it difficult to predict the performance of a twin-screw extruder and difficult to design an extruder given the performance requirements.

The effect of germination on the nutrition properties of grains has been studied by various authors (Donkor, Stojanovska, Ginn, Ashton, & Vasiljevic, 2012) (Hung, Maeda, Yamamoto, & Morita, 2012) (Nelson, Stojanovska, Vasiljevic, & Mathai, 2013). Similarly, the impact of extrusion variables on the proso millet extrudates have also been studied (Kowalski, Medina-Meza, Thapa, Murphy, & Ganjyal, 2016) (Doğan & Karwe, 2003). However, the method of producing highly digestible proso millet extrudates by removing the saponins and phytic acid has not been researched. The objective of this research is to study the effect of germination on the changes in nutrients of proso millet and the impact of the twin-screw extrusion variables such as moisture content of feed, die temperature and screw speed of extruder on the physical and physico-chemical properties of extrudates made from the sprouted proso millet flour.

8.2 Materials and Methods

8.2.1 Raw materials

Proso millet seeds (white) were obtained from a national grain processor (Bob's Red Mill Natural Foods, Milwaukie, OR). For the proximate composition analysis of proso millet seeds and flour, the following AACC methods were used: Moisture -Oven drying at 135°C (method no. 44–19), Ash - calcination at 550°C (method no. 08–01), Lipids - defatting in a Soxhlet apparatus with petroleum ether (method no. 30–25), Protein - micro Kjeldahl (N 6.25) (method no. 46–13), Carbohydrate – sugars and starch (method no. 80–04, 76-13) and total fiber - (method no. 32–07). Saponin content and phytic acid content (hexaphosphate equivalents) were quantified using the method described by(Rathod & Annapure, 2017). All chemicals were purchased from Sigma–Aldrich, USA. All chemicals and reagents used were of analytical grade. All experiments were performed in triplicates.

8.2.2 Soaking and Germination

The proso millet (11 pounds) was soaked overnight (8 pm- 8 am) for 12 h in tap water (1:5 w/v) at room temperature. The soaked samples were then drained and washed. Excess water was drained, and the samples were wrapped in a moist cloth for 5 days (0 h – Day 0, 24 h - Day 1, 48 h - Day 2, 72 h – Day 3, 96 h – Day 4) at room temperature. All experiments were performed in triplicates.

8.2.3 Extrusion processing

The proso millet flour samples, each weighing around 200 g were randomly extruded using a counter rotating conical twin screw laboratory extruder (C. W. Brabender, Inc., S. Hackensack, NJ, USA) as shown in Fig 8.1. The conical screws had diameters decreasing from 43mm to 28mm along their length of 365mm from the feed end to the exit end. The compression screws were universally single flighted counter rotating intermeshing screws with interrupted flight mixing zones. The mixing screws had a mixing section, in which small portions of the screw flights were cut away. The mixing section enhanced the mixing action and increased the residence time of the sample in the barrel. A die nozzle with 3 mm diameter was used. Data was recorded by a computer for subsequent analyses. Extrusion conditions selected were based on the preliminary studies and previous experiments. The extrudates were cooled to room temperature and sealed in polyethylene bags and stored at room temperature until measurements were taken.

8.2.4 Measurement of extrudate properties

8.2.4.1 Bulk Density

The bulk density (kg/m^3) was calculated as the ratio of the mass M (kg) to the volume V (m^3) of the measured and weighed extrudate sample. It was assumed that extrudates were of cylindrical shape. The bulk density of all extrudates were measured and the measurements were done in triplicate.

$$Bulk\ Density = \frac{M}{V} \tag{8.1}$$

8.2.4.2 Expansion ratio (ER)

Expansion ratio was calculated by measuring the mean diameter of 10 randomly chosen locations on the extrudates with calipers for each extrusion process condition. Further it was divided by the die diameter of 3 mm.

$$Expansion\ ratio = \frac{Mean\ diameter\ of\ the\ extrudate\ (mm)}{Die\ diameter\ (mm)} \tag{8.2}$$

8.2.4.3 Hardness

Hardness is defined as the maximum force per unit area of the proso millet extrudate that is necessary to breakdown the extrudate into two pieces. Hardness was tested by breaking the extrudate using a cylindrical probe (TA-4, Texture Technologies Corp, NY) attached to the crosshead of texture analyser. The sample was positioned in its natural position. A vertical force was applied at a speed of 50 mm/min. The force applied vs deformation was recorded and the maximum force recorded in the curve was considered as the hardness. The hardness of all 65 extrudates were measured and the measurements were done in triplicate.

8.2.4.4 Water Solubility Index and Water Absorption Index

The water absorption index and water solubility index of all extrudates were measured and the measurements were done in triplicate. The proso millet extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was mixed in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was mixed occasionally and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminium cup and dried at 135°C for 2h (AACC, 2000). The weight of the gel in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by:

$$Water\ Solubility\ Index(\%) = \frac{W_{ss}}{W_{ds}} * 100 \tag{8.3}$$

$$Water\ Absorption\ Index = \frac{W_g}{W_{ds}} \tag{8.4}$$

where, W_{ss} is the weight of dry solids of supernatant (g), W_{ds} is the weight of dry sample (g), and W_g is the weight of gel (g)

8.2.4.5 Color

The color of proso millet extrudate samples was measured using colorimeter (LabScan XE, HunterLab, Reston, VA) in triplicate in terms of Hunter Lab values (L^*, a^*, b^*). The Hunter Lab value 'L^*' signifies lightness with 0 for dark and 100 for bright. The value 'a^*' measures the degree of green color in the range from -100 to 0 and red in the range 0 to 100. The 'b^*' value represents blue color in the range from -100 to 0 and yellow in the range from 0 to 100.The total color change (ΔE) is calculated as

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \tag{8.5}$$

where $\Delta L = L^* - L_0$, $\Delta a = a^* - a_0$, and $\Delta b = b^* - b^*_0$; the subscript ''0'' represents initial color values before processing. All readings were taken in triplicate.

8.2.4.6 Starch and Protein Digestibility

The starch digestibility and protein digestibility of all extrudates were measured and the measurements were done in triplicate. The proso millet extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The in-vitro starch digestibility was determined in the extrudate powder (50 mg/ml of 0.2 M phosphate buffer, pH 6.9) after amylolysis with 0.5 ml of pancreatic amylase (1260 U/mg) suspension (0.4 mg/ml of 0.2 M phosphate buffer, pH 6.9) at 20 °C for 2 h according to the method of (Rathod & Annapure, 2017). At the end of the incubation period, 2 ml of 3,5-dini-trosalicylic acid reagent were added and the mixture boiled for 5 min. After cooling, the absorbance of the filtered solution was measured at 550 nm with maltose used as standard.

A system consisting, 100 mg (0.1 g) dry and defatted samples were incubated at 1.5 mg pepsin in 15 ml of 0.1N HCl at 37 °C for 3 h. Further it was neutralized with 7.5

ml 0.2N NaOH. Then 4 mg pancreatin in 7.5 ml phosphate buffer (pH 8) was added and incubated for 37 °C for 24 h, further 5 mg of TCA was added, centrifuged at 5000 rpm for 10 min. After that the supernatant dry residue at 40–50 °C was discarded and the micro Kjeldahl process for protein estimation was carried out. The percent in vitro protein digestibility was calculated using the following equation (Rathod & Annapure, 2017):

$$Protein\ digestibility\ (\%) = \frac{Total\ protein - Residual\ protein}{Total\ protein} * 100 \qquad (8.6)$$

8.2.5 Experimental Design and Statistical Analysis

Experiments were conducted using the Box Benhken design (BBD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA), consisting four factors namely moisture content (X_1), temperature (X_2), screw speed (X_3) and germination time (X_4). Three replicates were taken at the design center and the total number of observations were 75 [60 (not center points) and 15 (center points)]. Second-order polynomial regression models were established for the dependent variables to fit experimental data for each response using statistical software Design-Expert 8 (Stat-Ease Inc., Minneapolis, MN). The fitness of the model was evaluated and the interactions between the independent and dependent variables were identified by using an analysis of variance (ANOVA). The goodness of fit of the second-order equation was expressed by the coefficient of determination (R^2) and its statistical significance was determined by the F-test. Three-dimensional response surfaces were used to visualize the interactive effects of the independent variables.

8.3 Results and Discussion

8.3.1 Effect of Soaking and Germination on the sprouted proso millet extrudates

The nutritional properties of the sprouted proso millet are shown in Table 8.1. After soaking for 12 h, the white color hypocotyls appeared in the proso millet seeds on Day 1. The germination ratio was more than 90%. The protein content decreased significantly on Day 4 of germination and a simultaneous increase in the hypocotyl length was observed. The change in color of the sprouted flour indicates the breakdown of phytates. The optimised soaking time was 12 h and germination time was 96 h.

In general, protein content of proso millet seeds ranges between 13.1% and 14.0% (James, 2009). The total protein content of proso millet is higher than that of grains such as rice, barley, corn, rye, sorghum and wheat (Navruz-Varli & Sanlier, 2016). The chief carbohydrate constituent of proso millet is starch, and it ranges from 62-64%. The total fiber was close to other grain products (7.0%-9.7%). Proso millet contains sugar by about 3%. It mostly contains maltose, D-galactose, and D-ribose in addition to low levels of fructose and glucose (Navruz-Varli & Sanlier, 2016).

The phytic acid concentration in proso millet seeds is comparable to whole grain rye flour (7.7 mg/g), whole grain wheat flour (8.7 mg/g), lentils (8.4 mg/g) and fava beans (8.0 mg/g). Phytic acid is not only present in the outer layers of proso millet, as in the case of rye and wheat but is also evenly distributed within the endosperm (Ahamed, Singhal, Kulkarni, Pal, & Bulletin, 1998). Phytic acid binds minerals, such as Fe, Zn, Ca and Mg, and can make the mineral content of a cereal inadequate, especially for children (Khattak, Zeb, Bibi, Khalil, & Khattak, 2007). Soaking and sprouting processes reduce the 'binding' effect of the phytic acid by activating the enzyme phytase (James, 2009). The amounts of phytic acid of the proso millet seeds obtained in this study are in concordance with the values for Cherry vanilla proso millet (Kowalski et al., 2016). A significant reduction in phytic acid content is observed with an increase in germination time. Extrusion further decreased the phytic acid content of pea flour (Alonso, Orue, & Marzo, 1998), bean flour (Anton, Fulcher, & Arntfield, 2009) and other legumes (Lombardi-Boccia et al., 1991).

8.3.2 Effect of independent variables on the twin screw extrusion process of sprouted proso millet flour

The results of the twin screw extrusion process of sprouted proso millet flour along with the experimental design is presented in Appendix F.

8.3.2.1 Bulk Density

The bulk density of the extrudates varied between 119 and 161 kg/m^3. Low density, which is a required characteristic of the extruded product, was achieved with low feed moisture content, average temperature and high screw speed combination. Fig 8.2 shows that feed moisture content had a significant effect on density followed by screw speed, whereas temperature seemed to have a minor effect. Low product density of 119 kg/m^3 was obtained at feed moisture of 15%, 115°C temperature, 220 rpm screw speed and Day 4 germinated samples.

8.3.2.2 Expansion ratio

The measured expansion ratio of proso millet extrudates ranged from 3.02 to 3.63. The expansion ratio was significantly affected by temperature and feed moisture content. High expansion ratio at low feed moisture content for expanded products is distinctive for cereals. However, Fig 8.3 shows that the temperature and screw speed also have a significant effect on the expansion ratio of the proso millet extrudates. Only the starch granules that have been gelatinised could participate in the formation of a stable expanded structure whereas excessive molecular degradation resulted in poor expansion (Anton et al., 2009). Starch gelatinisation and breakdown depend on mechanical and thermal energy inputs. The results from the study showed that extrudates from low moisture content expanded less than from high moisture content. This may be due to their high viscosity at low moisture content inside the extruder resulting in high shear rates that cause a predominant molecular disruption of amylopectin molecules, which seems to inhibit an increase in expansion (Mason & Hoseney, 1986).

8.3.2.3 Hardness

Hardness, the maximum force to break the sample, ranged between 1.13 and 1.55 N. Fig 8.4 shows the effect of moisture and screw speed on the product hardness. At all feed moisture content levels, decrease in die temperature decreased the product hardness. Screw speed did not have a significant effect on the extrudate hardness. Chemical changes occurring during extrusion cooking affect the development of textural and mechanical properties such as hardness in extrudates. The elastic swell effect and bubble growth effect equally contribute to the structural changes of starch during extrusion. Water performs as a plasticizer to the starch-based material decreasing its viscosity and the mechanical energy dissipation in the extruder. Hence the product becomes dense and bubble growth is compressed. The reduced starch conversion and compressed bubble growth result in a dense product and wetness of extrudate. Previous studies have also reported similar trends for extrudates based on rice (Ding, Ainsworth, Plunkett, Tucker, & Marson, 2006), wheat (Ding et al., 2006), oat bran and soy flour (Anuonye, Onuh, Egwim, & Adeyemo, 2010) and chickpea (Khattak et al., 2007). The coefficient of determination for hardness was high (R^2 = 0.9) and the moisture, temperature and germination period were found to have significant contributions to the hardness of the extrudates. The hardness was high of 1.55 N at about 140°C, 150 rpm screw speed, 25% feed moisture content and Day 0 sprouted samples.

8.3.2.4 Water Solubility Index and Water Absorption Index

Water solubility index is related to the quantity of soluble solids, which is related to dextrinization. The squared effects of feed moisture, screw speed and temperature had significant effect on water solubility index. In general, water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. The coefficient of determination for water solubility index was highly significant as revealed by a high value of R^2 (0.9). Fig 8.5 shows the significant effect of temperature and screw speed on water solubility index. The increase in water solubility index with increasing temperature was consistent with the results reported for oat extrudates (Gutkoski & El-Dash, 1999). The water solubility index decreased with the increase in moisture. The poor correlation between water solubility index and most of the process and product responses is explained by the fact that water solubility index includes the opposing effects of starch dextrinization and the molecular level interactions between degraded components, which may not be favoured at the same condition (Lei, Ruan, Fulcher, van Lengerich, & Engineering, 2008). An increase in the amount of dextrinized starch during extrusion cooking results in an increase in water solubility index. However, molecular interactions between degraded starch, protein, and lipid components, which in turn lead to an increase in molecular weight, may decrease the solubility, thus water solubility index (Camire, Camire, & Krumhar, 1990).

Water absorption index of extrudates ranged between 3.36 and 3.80. Water absorption index depends on the availability of hydrophilic groups and on the gel formation capacity of the macromolecules. It is a measure of damaged starch together with protein denaturation and new macromolecular complex formations. The regression analysis showed that the squared effect of moisture, screw speed and temperature were highly significant on water absorption index as shown in Fig 8.6. Similar responses were observed in rice flour (Hagenimana, Ding, & Fang, 2006), oats (Gutkoski & El-Dash, 1999), finger millet (Sawant, Thakor, Swami, Divate, & Vidyapeet, 2012) and cassava starch (Hashimoto, Grossmann, & technology, 2003). water absorption index had poor correlations with almost all process and product responses except product density. This is an expected result since it includes the effect of starch gelatinization, protein denaturation and molecular level cross-linking reactions which are not always favored at the same conditions (Doğan & Karwe, 2003).

8.3.2.5 Color

The total color change in extruded products ranged between 18.1 and 22.8. Color is an important quality parameter since it reflects the extent of chemical reactions and degree of cooking or degradation that take place during extrusion cooking. In this study, ΔE represents the total color difference compared to the color of unsoaked and unsprouted proso millet. Higher ΔE means darker products with more intense yellow and red color. Squared effects of temperature and moisture content were found to have the highest contribution to total color change. As depicted in Fig 8.7, low feed moisture content and high temperature increased the total color change possibly due to increased extent of browning reactions under this condition. At low screw speeds a slight increase in color change observed due to longer residence times which might increase the extent of chemical reactions.

8.3.2.6 Starch Digestibility (SD) and Protein Digestibility (PD)

Fig 8.8 and Fig 8.9 illustrate the effect of the independent variables on the protein and starch digestibility respectively. The results show that both protein digestibility and starch digestibility of the proso millet extrudates were increased significantly by soaking, germination and extrusion. The squared effects of all the variables had a significant effect on the protein and starch digestibility. The increases of digestibility produced by the different treatments were higher in protein than in starch. Improvement of protein digestibility after processing could be attributable to the reduction or elimination of different anti-nutrients. Phytic acid, as well as condensed tannins and polyphenols, are known to interact with protein to form complexes. This interaction increases the degree of cross-linking, decreasing the solubility of proteins and making protein complexes less susceptible to proteolytic attack than the same protein alone (Alonso et al., 1998). Extrusion produced a higher increase in protein digestibility than the other processing methods. This can be related to the higher efficiency of this thermal treatment in reduction of anti-nutrient activities in addition to soaking and germination.

The degree of starch gelatinisation of germinated samples Day 4 is higher than in Day 0 and it is thus more readily hydrolysed. Enhancement of starch digestibility during soaking and germination may be attributed to the loss of phytic acid, condensed tannins and polyphenols, which inhibit activity of α-amylase and thus decrease the starch digestibility (Linko, Linko, & Olkku, 1983). The rupture of starch granules of sprouted proso millet, making the substrate more accessible, facilitated the amylolysis. On the other hand, reduction of phytates, condensed tannins and polyphenols and total inactivation of α-amylase inhibitors increased the starch digestibility (Egli et al., 2003).

8.3.3 Box-Behnken design and analysis

In the present study, the Box Behnken Design was used to obtain a proper model for the optimization of the extrusion process variables. Experiments were carried out according to the experimental design in order to explore the optimum conditions and study the influence of process variables on the physical and physico-chemical properties of the extrudates. This response surface methodology is an empirical modeling technique used to evaluate the relationship between the experimental and the predicted results (Muthukumarappan & Swamy, 2018). The predicted values were attained by the model fitting technique in Design Expert software version 8.0.7.1 and the values correlated well with the experimental values. Fitting of the data to various models such as the linear, interactive, quadratic and cubic models was performed to acquire the regression equations. Sequential model sum of squares and model summary statistics tests were carried out to decide the adequacy of models in the present study and the results are presented in Table 8.2. From Table 8.2, it is observed that the quadratic model is highly significant for the extrusion of sprouted proso millet. The model summary statistics demonstrated that the quadratic model had maximum R- Squared, Adjusted R-Squared and Predicted R-Squared values. The cubic model was not suggested for the extrusion process since the BBD matrix had enough data to understand the outcome of the system. Hence, quadratic model was chosen for further analysis of data.

8.3.4 Fitting of polynomial equations and statistical analysis

Based on the Box-Behnken experimental design model, the empirical relationship between the input variables and experimental results obtained were expressed by a second-order polynomial equation with interaction terms (Swamy, Sangamithra, & Chandrasekar, 2014). The equations generated in coded factors are presented in Table 8.3.

The investigation and optimization of a fitted response surface might generate poor or disingenuous results. Therefore, it was essential to ensure the fitness of the model. Regression analysis and Pareto analysis of variance (ANOVA) were used to test the adequacy and fitness of the models. The results pointed out that the equation sufficiently presented the actual relationship between the input parameters and responses. ANOVA is a statistical technique that subdivides the total variation in a dataset into component parts allied with sources of variation for testing hypotheses on the variables of the model (Swamy & Muthukumarappan, 2017).

ANOVA followed by Fisher’s statistical test (F-test) was conducted to analyse the significance of each independent variable. The F-value is the ratio of the regression mean square and the real error mean. It indicates the influence of each controlled factor on the tested model. The ANOVA results in Table 8.4 shows the F-value for the responses, implying that the model is highly significant. The large Fisher value signifies that the variation in the responses can be explicated by the regression equation (Asokapandian, Venkatachalam, Swamy, & Kuppusamy, 2016). The associated p value estimates if F value is large enough to show statistical significance. The p values that are lower than 0.05 indicate that the model and the associated terms are statistically significant. Coefficient of determination (R^2) and adjusted-R^2 were computed to verify the sufficiency and fitness of the model. The R^2 signifies the proportion of the total variation in the response expected by the model, thus indicating the ratio of the regression sum of squares (SSR) and total sum of squares (SST) (Sangamithra, Sivakumar, Kannan, & John, 2015). The values of R^2 imply that 95% of experimental data was well-suited. The high R^2 coefficient gives a satisfactory adjustment of the quadratic model to the experimental data. The purpose of the adjusted-R^2 is to analyse the model adequacy and fitness. The adjusted-R^2 value adjusts the R^2 value for the sample size and for the number of terms in the model (Albarracín, De Greef, González, & Drago, 2015). The high value of adjusted-R^2 supports a high correlation between the experimental and the predicted values. The high coefficient of determination and very small p-value (<0.0001) demonstrate that the quadratic polynomial model is significant and adequate to characterize the actual relationship between the response and input. The coefficient of variation (CV) shows the scattering of the experimental points from the predicted values of the second order polynomial model (Swamy & Muthukumarappan, 2017). A high coefficient of variation points out that there is extreme variation in the mean value and does not adequately develop a sufficient model. The very low coefficient of variation value undoubtedly represents a high degree of precision and reliability of experiments conducted.

8.3.5 Selection of optimum conditions

Optimum conditions for the extrusion process were deduced to get desired extrudate properties. Second order polynomial models attained in the study were used for each response to acquire specified optimum conditions. To optimize the independent variables, the Derringer's desirability function method was utilized. After satisfying the necessities for each response in the design, the Derringer's desirability function seeks for a combination of factor levels that together optimize a set of responses. By the conversion of each into a dimensionless desirability scale which defines a partial desirability function, combining the discrete desirability values to attain the global desirability function and then maximizing the global function and recognizing the optimal factor settings, the optimization process is accomplished. The desirability function scale operates between 0 and 1 with 0 signifying a completely undesirable response and 1 representing a fully desired response. The individual desirability for each response is achieved by stating the goals, i.e., minimize, maximize or target the response, and boundaries essential for each response. A weight factor (defines the shape of the desirability function for each response) is then allocated. The weights range between 0.1 and 10 with the larger ones relating to more significant responses. A weight factor of 1 was selected for the individual desirability in this research. The "importance" of the goals can be arranged sequentially according to increasing importance. It can vary from 1 (least importance) to 5 (most important). To optimize the process the following parameters (1) Moisture (15-25%) (2) Temperature (90-140°C) (3) Screw speed (80-220 rpm) and (4) Germination period (Day 0- Day 4) respectively, were set for maximum desirability. By applying the methodology of the desired function, the optimized process variables were obtained. It indicated that a moisture 16%, temperature 115°C, screw speed 140 rpm and Day 3 germinated flour input will produce an extrudate with bulk density=123.9 kg/m^3, hardness=1.17 N, water solubility index=20.5, water absorption index=3.7, ΔE=18.6, ER=3.5, protein digestibility =85% and starch digestibility =84.5% respectively with overall desirability value of 0.71.

8.3.6 Verification of optimized conditions and predictive model

The fitness of the model equations for predicting optimum response values was investigated under the conditions: a moisture 16%, temperature 115°C, screw speed

140 rpm and Day 3 germinated flour. These conditions were optimum by the RSM optimization approach. To verify the validity of the optimized conditions, experiments were performed to evaluate the experimental results vs predicted values of the output using the model equation. The experiments were carried out thrice and the average values were presented in Table 8.5. The results expected from the optimum solution was bulk density 123.9 kg/m^3, hardness 1.17 N, water solubility index 20.5, water absorption index= 3.7, ΔE=18.6, expansion ratio=3.5, protein digestibility =85% and starch digestibility =84.5% and the experimental value was bulk density=123.9±0.07 kg/m^3, hardness=1.17±0.05N, water solubility index =20.5±0.04, water absorption index =3.7±0.11, ΔE=18.6±0.09, expansion ratio=3.5±0.06, protein digestibility =85±0.04% and starch digestibility =84.5±0.05%. The mean values obtained through the experiments were compared with the predicted values. The values obtained through confirmation experiments are within 95% of predicted values. This shows that the developed quadratic models are well-suited. It is also evident that the optimal values are valid within the specified range of process variables.

8.4 Conclusions

The effects of processing variables on the physicochemical and textural properties of sprouted proso millet extrudates prepared by twin-screw extrusion were investigated using response surface methodology. The influence of feed moisture (15-25% w.b.), temperature (80-140°C), screw speed (90-220 rpm) and germination time (Days 0-4) on extrudates were studied. Both feed moisture and extruder temperature had significant effects on the extrudate characteristics. Changing the level of moisture content and temperature had significant effect ($p<0.01$) on all physical properties. The total color change in extruded products ranged between 18.1 and 22.8. Water solubility index increased with increasing temperature and screw speed and decreasing feed moisture content because of high thermal and mechanical energy inputs favor starch dextrinization. The regression analysis showed that the squared effect of moisture, screw speed and temperature were highly significant on water absorption index. Hardness ranged between 1.13 and 1.55 N and the coefficient of determination for hardness was high ($R^2 = 0.9$). Extruder screw speed had minimal effect on the extrudate properties. In addition to soaking and germination, extrusion was most effective in improving protein and starch digestibility. By applying the methodology of the desired function, the optimized process variables were moisture 16%, temperature 115°C, screw speed 140 rpm and Day 3 germinated flour with bulk density=123.9 kg/m^3, hardness=1.17 N, water solubility index=20.5, water absorption index 3.7, ΔE=18.6, expansion ratio=3.5, protein digestibility =85% and starch digestibility=84.5% respectively with overall desirability value of 0.710. Overall, the proso millet studied in the range extrusion conditions showed average expansion characteristics as compared to widely used cereal grains, suggesting that a suitable blend can help in the creation of puffed snacks.

Table 8.1: Impact of soaking and germination on the nutritional properties of proso millet

	Raw proso millet	Day 0	Day 1	Day 2	Day 3	Day 4
Total Protein[a]	13.1±0.5	13.1±0.15	13.2±0.03	13.3±0.05	13.4±0.01	13.1±0.07
Carbohydrates[a]	63.2±0.6	63.2±0.16	63.3±0.03	63.4±0.07	63.4±0.09	63.6±0.02
Moisture[a]	11.1±0.2	13.2±0.09	13.1±0.04	12.8±0.06	12.6±0.03	12.5±0.02
Phytic acid[b]	1.1±0.07	0.69±0.05	0.55±0.05	0.46±0.02	0.33±0.04	0.21±0.02
Saponin[b]	0.80±0.04	0.40±0.11	0.40±0.06	0.03±0.04	0.20±0.02	0.10±0.02

[a] g/100 g edible material

[b] g/100 dry basis

Table 8.2: Results of model adequacy tested in the Box-Behnken design

Source	Sum of squares	DF	Mean square	F value	Prob > F	Remarks
Sequential model sum of squares for ***Bulk Density***						
Quadratic	497.06	3	165.69	283.59	< 0.0001	Suggested
Sequential model sum of squares for ***Hardness***						
Quadratic	0.035	3	0.012	198.37	< 0.0001	Suggested
Sequential model sum of squares for ***Water Solubility Index***						
Quadratic	3.98	3	1.33	126.91	< 0.0001	Suggested
Sequential model sum of squares for ***Water Absorption Index***						
Quadratic	0.038	3	0.013	166.37	< 0.0001	Suggested
Sequential model sum of squares for ***Total Color Difference ΔE***						
Quadratic	4.25	3	1.42	224.23	< 0.0001	Suggested
Sequential model sum of squares for ***Expansion Ratio***						
Quadratic	0.063	3	0.021	166.39	< 0.0001	Suggested
Sequential model sum of squares for ***Protein Digestibility***						
Quadratic	7.20	3	2.40	19.28	< 0.0001	Suggested
Sequential model sum of squares for ***Starch Digestibility***						
Quadratic	1.16	3	0.39	22.92	< 0.0001	Suggested

Source	**Std. Dev.**	**R-squared**	**Adjusted R-squared**	**Predicted R-squared**	**PRESS**	
Model summary statistics for ***Bulk Density***						
Quadratic	0.76	0.9329	0.9939	0.9885	93.03	Suggested
Model summary statistics for ***Hardness***						
Quadratic	0.007	0.9957	0.9939	0.9887	0.009	Suggested
Model summary statistics for ***Water Solubility Index***						
Quadratic	0.10	0.9937	0.9910	0.9828	1.67	Suggested
Model summary statistics for ***Water Absorption Index***						
Quadratic	0.008	0.9946	0.9923	0.9857	0.012	Suggested
Model summary statistics for ***Total Color Difference ΔE***						
Quadratic	0.079	0.9964	0.9948	0.9904	0.98	Suggested
Model summary statistics for ***Expansion Ratio***						
Quadratic	0.011	0.9959	0.9941	0.9889	0.020	Suggested
Model summary statistics for ***Protein Digestibility***						
Quadratic	0.35	0.9672	0.9533	0.9108	19.98	Suggested
Model summary statistics for ***Starch Digestibility***						
Quadratic	0.13	0.9892	0.9846	0.9730	2.48	Suggested

+ case(s) with leverage of 1.0000: PRESS statistic not defined

Table 8.3: Second order polynomial equations representing the relationship between independent and dependent variables

Bulk Density	$133.00 +12.25A -5.35B -2.20C +2.34D_1 +1.05D_2 +0.047D_3 -1.13D_4 -0.25AB -0.35AC -0.35BC -0.52BD_1 -0.15BD_2 -0.15BD_3 +0.22BD_4 -0.17CD_1 -0.050CD_2 -0.050CD_3 +0.075CD_4 +3.12A^2 +2.92B^2 +1.73C^2$
Hardness	$1.28 +0.12A -0.053B -0.023C +0.026D_1 +0.010D_2 +1.176E\text{-}004D_3 -0.012D_4 -2.500E\text{-}003AB +5.000E\text{-}004AC +1.000E\text{-}003AD_1 -2.500E\text{-}004AD_2 -2.500E\text{-}004AD_3 -2.500E\text{-}004AD_4 -9.750E\text{-}003BD_1 -1.000E\text{-}003BD_2 -1.000E\text{-}003BD_3 +4.000E\text{-}003BD_4 -2.250E\text{-}003CD_1 +2.500E\text{-}004CD_2 +2.500E\text{-}004CD_3 +2.500E\text{-}004CD_4 +0.014A^2 +0.032B^2 +0.017C^2$
Water Solubility Index	$19.50 -1.37A +0.55B +0.24C -0.24D_1 -0.11D_2 +5.882E\text{-}003D_3 +0.12D_4 +0.095AB +5.000E\text{-}003AC -0.012AD_1 +0.020BC +0.078BD_1 +0.027BD_2 -0.010BD_3 -0.035BD_4 +0.020CD_1 +7.500E\text{-}003CD_2 -5.000E\text{-}003CD_3 -5.000E\text{-}003CD_4 -0.24A^2 -0.32B^2 -0.12C^2$
Water Absorption Index	$3.65 -0.12A +0.057B +0.024C -0.026D_1 -0.011D_2 +5.882E\text{-}004D_3 +0.012D_4 +5.000E\text{-}003AB +1.500E\text{-}003AC -1.750E\text{-}003AD_1 -5.000E\text{-}004AD_2 +7.500E\text{-}004AD3 +7.500E\text{-}004AD_4 +3.500E\text{-}003BC +0.011BD_1 +2.000E\text{-}003BD_2 -5.000E\text{-}004BD_3 -4.250E\text{-}003BD_4 +3.500E\text{-}003CD_1 +1.000E\text{-}003CD_2 -2.500E\text{-}004CD_3 -1.500E\text{-}003CD_4 -0.020A^2 -0.031B^2 -0.016C^2$
Total Color Difference ΔE	$19.80 +1.42A -0.56B -0.25C +0.25D_1 +0.11D_2 -4.706E\text{-}003D_3 -0.12D_4 -0.025AB +0.020AD_1 -5.000E\text{-}003AD_2 -5.000E\text{-}003AD_3 -5.000E\text{-}003AD_4 -1.000E\text{-}002BC -0.075BD_1 -0.038BD_2 +0.037BD_4 -$

	$0.025CD_1 -0.012CD_2 +0.013CD_4 +0.21A^2 +0.31B^2 +0.20C^2$
Expansion Ratio	3.41 -0.19A +0.069B +0.027C -0.026D1 -0.011D2 +1.176E-004D3 +0.012D4 +3.500E-003AB -2.000E-003AC -1.750E-003AD1 -5.000E-004AD2 +7.500E-004AD3 +7.500E-004AD4 +4.000E-003BC +0.011BD1 +2.500E-003BD2 -5.000E-003BD4 +3.000E-003CD1 +5.000E-004CD2 -7.500E-004CD3 -7.500E-004CD4 -0.025A2 -0.042B2 -0.018C 2
Protein Digestibility	$83.48 -1.60A +0.60B +0.26C -2.01D_1 +0.31D_2 +0.42D_3 +0.59D_4 +0.23AB +0.12AC -0.27AD_1 +0.012AD_2 +0.12AD_4 +0.090BC +0.080BD_1 +0.017BD_2 -7.500E-003BD_3 -0.033BD_4 +0.12CD_1 +0.045CD_2 +0.032CD_3 -0.092CD_4 -0.37A^2 -0.38B^2 -0.17C^2$
Starch Digestibility	$83.50 -0.84A +0.37B +0.22C -1.56D_1 +0.21D_2 +0.34D_3 +0.45D_4 -0.050AB -0.040AC -0.21AD_1 +0.052AD_2 +0.065AD_3 +0.052AD_4 +0.015BC +0.25BD_1 -0.035BD_2 -0.048BD_3 -0.073BD_4 +0.083CD_1 +7.500E-003CD_2 -0.017CD_3 -0.030CD_4 -0.048A^2 -0.18B^2 -0.12C^2$

A – Moisture Content (%w.b.); B – Screw Speed (rpm); C – Die Temperature; D_1, D_2, D_3, D_4 – Germination time (Days)

Table 8.4: Pareto analysis of variance (ANOVA) analysis and significant statistical parameters of the model

Source	Sum of squares	DF	Mean square	F-value	p-value
Bulk Density					
Model	8074.64	25	322.99	552.82	< 0.0001
A-moisture content	6002.50	1	6002.50	10273.90	< 0.0001
B- die temperature	1144.90	1	1144.90	1959.62	< 0.0001
C- screw speed	193.60	1	193.60	331.37	< 0.0001
D – germ period	223.93	4	55.98	95.82	< 0.0001
A^2	205.59	1	205.59	351.89	< 0.0001
B^2	180.12	1	180.12	308.29	< 0.0001
C^2	62.64	1	62.64	107.22	< 0.0001
C.V. %	0.56				
Hardness					
Model	0.81	25	0.032	547.24	< 0.0001
A-moisture content	0.62	1	0.62	10395.77	< 0.0001
B- die temperature	0.11	1	0.11	1881.30	< 0.0001
C- screw speed	0.021	1	0.021	349.93	< 0.0001
D – germ period	0.026	4	0.006	108.05	< 0.0001
A^2	0.004	1	0.004	74.82	< 0.0001
B^2	0.022	1	0.022	364.38	< 0.0001
C^2	0.006	1	0.006	102.84	< 0.0001
C.V. %	0.59				

Water Solubility Index					
Model	96.57	25	3.86	369.82	< 0.0001
A-moisture content	75.62	1	75.62	7240.54	< 0.0001
B- die temperature	11.99	1	11.99	1147.98	< 0.0001
C- screw speed	2.35	1	2.35	225.21	< 0.0001
D – germ period	2.33	4	0.58	55.81	< 0.0001
A^2	1.16	1	1.16	111.31	< 0.0001
B^2	2.16	1	2.16	206.40	< 0.0001
C^2	0.30	1	0.30	29.03	< 0.0001
C.V. %	0.53				
Water Absorption Index					
Model	0.83	25	0.033	433.75	< 0.0001
A-moisture content	0.61	1	0.61	7975.47	< 0.0001
B- die temperature	0.13	1	0.13	1690.88	< 0.0001
C- screw speed	0.023	1	0.023	302.42	< 0.0001
D – germ period	0.026	4	0.006	85.32	< 0.0001
A^2	0.008	1	0.008	110.53	< 0.0001
B^2	0.020	1	0.020	265.55	< 0.0001
C^2	0.005	1	0.005	75.23	< 0.0001
C.V. %	0.24				

Total Color Difference ΔE					
Model	102.47	25	4.10	648.70	< 0.0001
A-moisture content	80.37	1	80.37	12720.06	< 0.0001
B- die temperature	12.66	1	12.66	2003.03	< 0.0001
C- screw speed	2.50	1	2.50	395.66	< 0.0001
D – germ period	2.55	4	0.64	100.84	< 0.0001
A^2	0.95	1	0.95	150.46	< 0.0001
B^2	2.06	1	2.06	325.38	< 0.0001
C^2	0.82	1	0.82	129.96	< 0.0001
C.V. %	0.39				
Expansion Ratio					
Model	1.80	25	0.072	569.03	< 0.0001
A-moisture content	1.49	1	1.49	11779.58	< 0.0001
B- die temperature	0.19	1	0.19	1490.86	< 0.0001
C- screw speed	0.029	1	0.029	229.94	< 0.0001
D – germ period	0.026	4	0.006	51.24	< 0.0001
A^2	0.013	1	0.013	105.84	< 0.0001
B^2	0.038	1	0.038	296.34	< 0.0001
C^2	0.006	1	0.006	52.30	< 0.0001
C.V. %	0.34				

Protein Digestibility					
Model	216.71	25	8.67	69.64	< 0.0001
A-moisture content	102.40	1	102.40	822.72	< 0.0001
B- die temperature	14.16	1	14.16	113.78	< 0.0001
C- screw speed	2.60	1	2.60	20.90	< 0.0001
D – germ period	87.57	4	21.89	175.89	< 0.0001
A^2	2.88	1	2.88	23.16	< 0.0001
B^2	3.04	1	3.04	24.42	< 0.0001
C.V. %	0.42				
Starch Digestibility					
Model	90.78	25	3.63	215.68	< 0.0001
A-moisture content	28.22	1	28.22	1676.46	< 0.0001
B-die temperature	5.55	1	5.55	329.68	< 0.0001
C- screw speed	1.89	1	1.89	112.40	< 0.0001
D – germ period	52.69	4	13.17	782.41	< 0.0001
BD	0.66	4	0.16	9.74	< 0.0001
B^2	0.70	1	0.70	41.65	< 0.0001
C^2	0.32	1	0.32	18.77	< 0.0001
C.V. %	0.16				

Table 8.5: Predicted and experimental values of the responses at optimum conditions

Moisture Content (%) = 15; Die Temperature (°C) = 125; Screw Speed (rpm) = 179; Germination = Day 3

	Optimized values[a] (predicted values)	Experimental values[b]
Bulk density(kg/m^3)	123.9	123.9±0.07
Hardness (N)	1.17	1.17±0.05
Water Solubility Index (%)	20.5	20.5±0.04
Water absorption Index	3.7	3.7±0.11
Total Color Difference ΔE	18.6	18.6±0.09
Expansion Ratio	3.5	3.5±0.06
Protein Digestibility (%)	85	85±0.04
Starch Digestibility (%)	84.5	84.5±0.05

a Predicted using response surface quadratic model

b Mean ± standard deviation of triplicate determinations from experiments

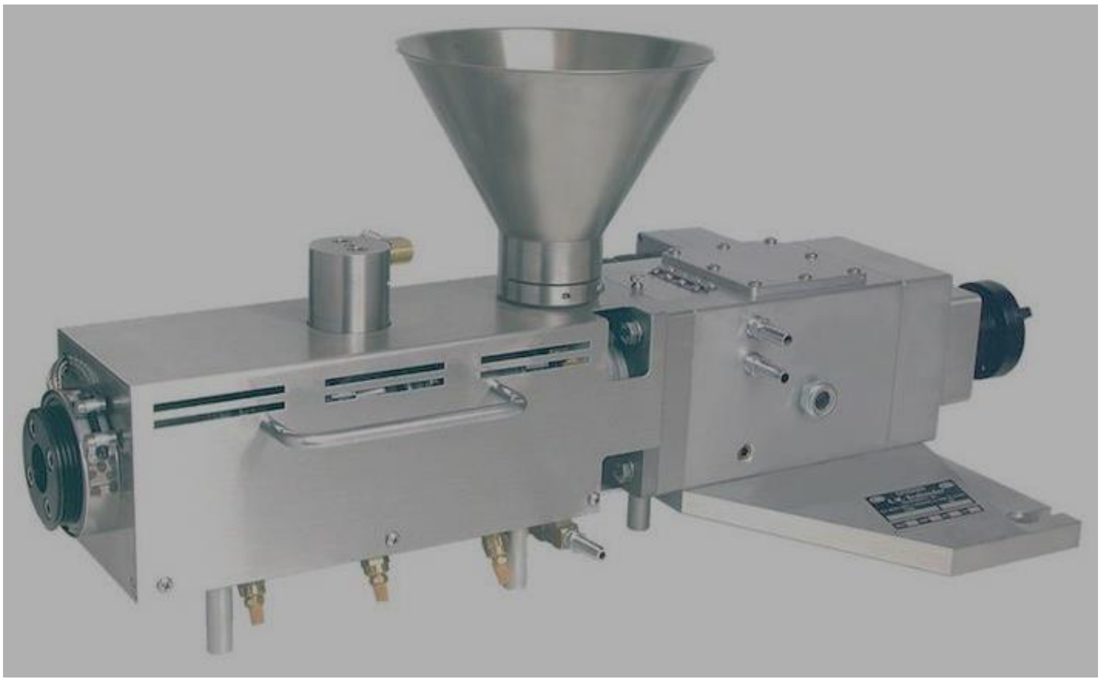


Figure 8.1: Counter rotating conical twin screw laboratory extruder

(Brabender CTSE, NJ)

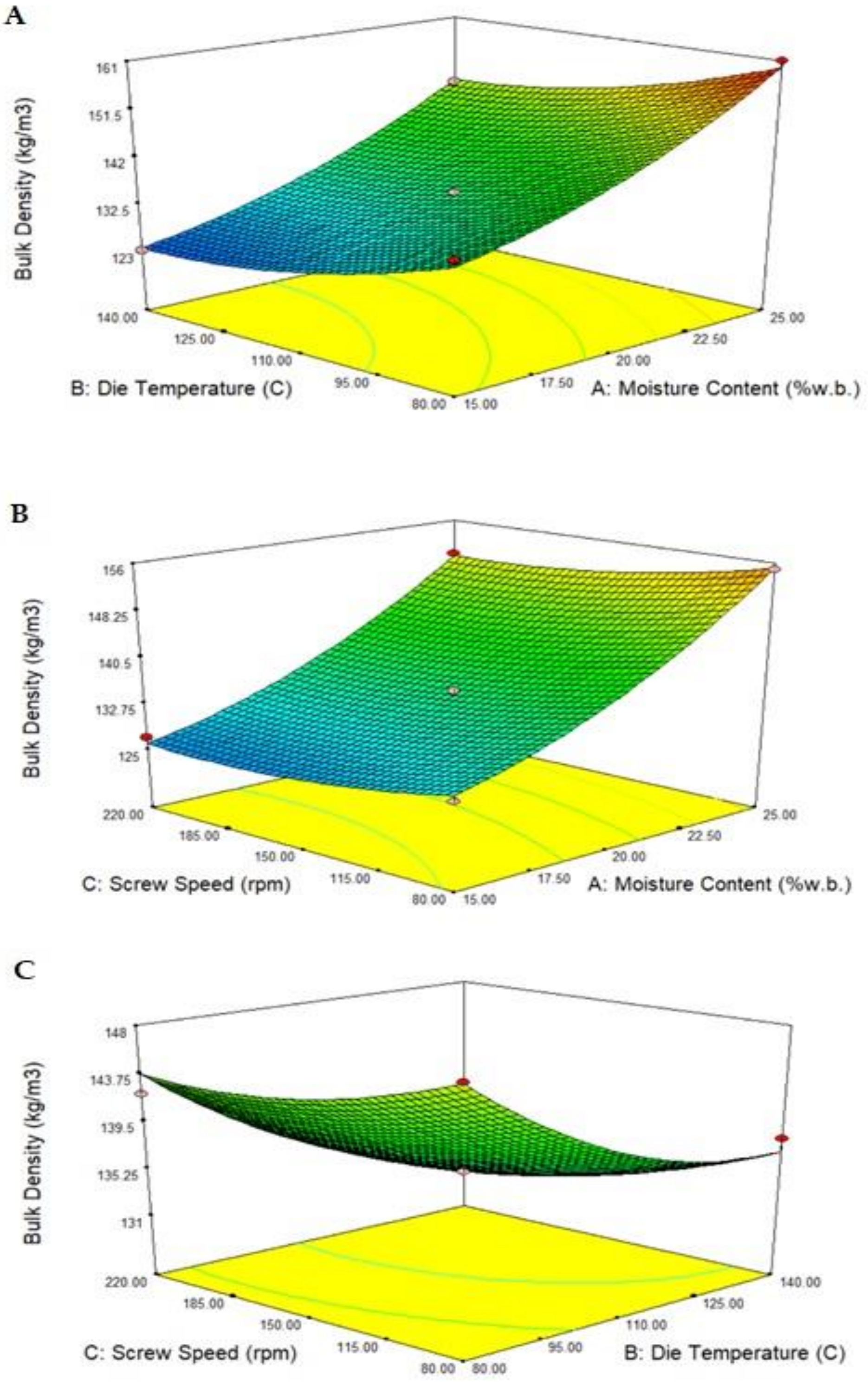


Figure 8.2: Response surface plots (3D) showing the effects on the Bulk Density (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

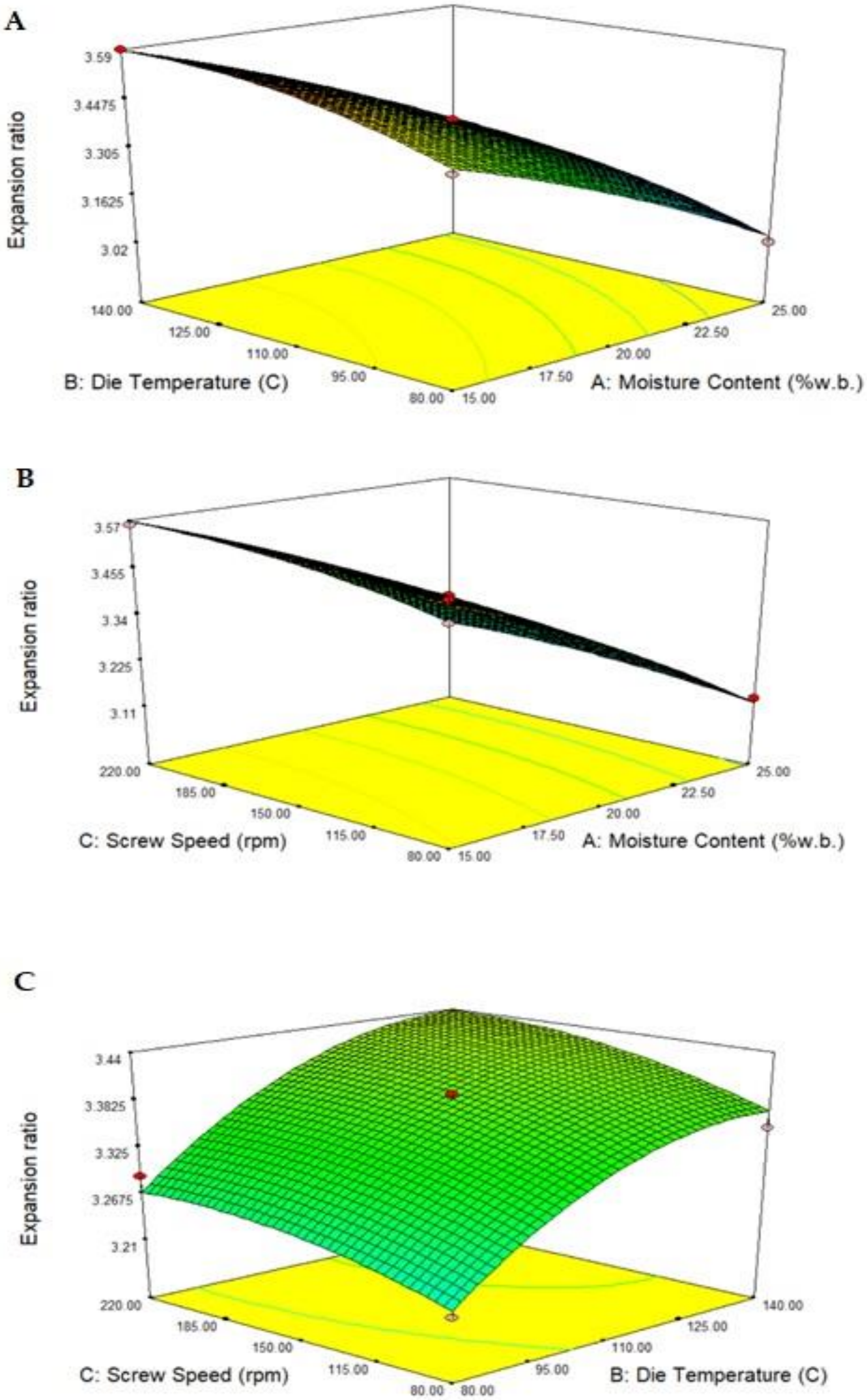


Figure 8.3: Response surface plots (3D) showing the effects on the Expansion Ratio (A) Moisture Content and Screw speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

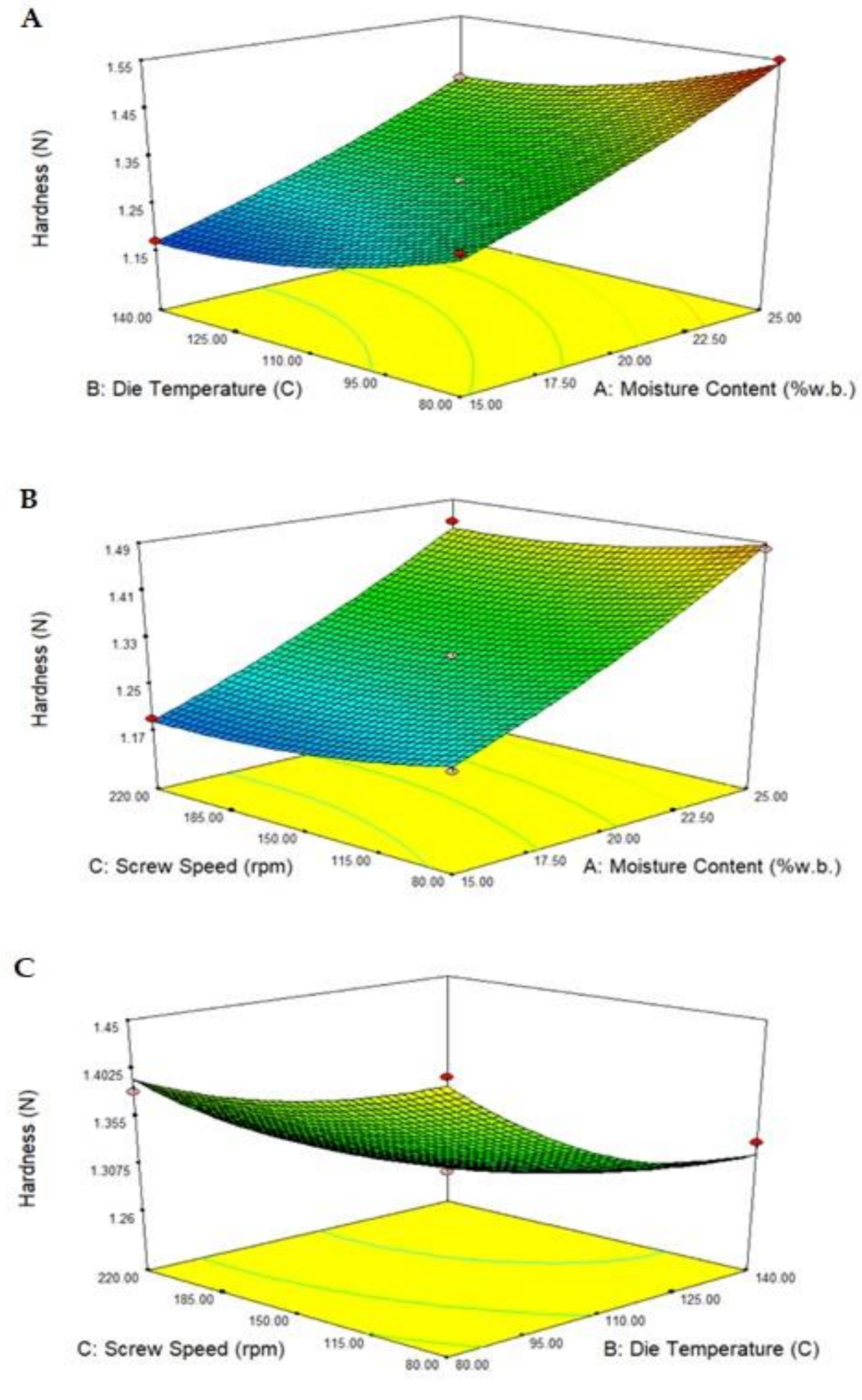


Figure 8.4: Response surface plots (3D) showing the effects on the Hardness (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

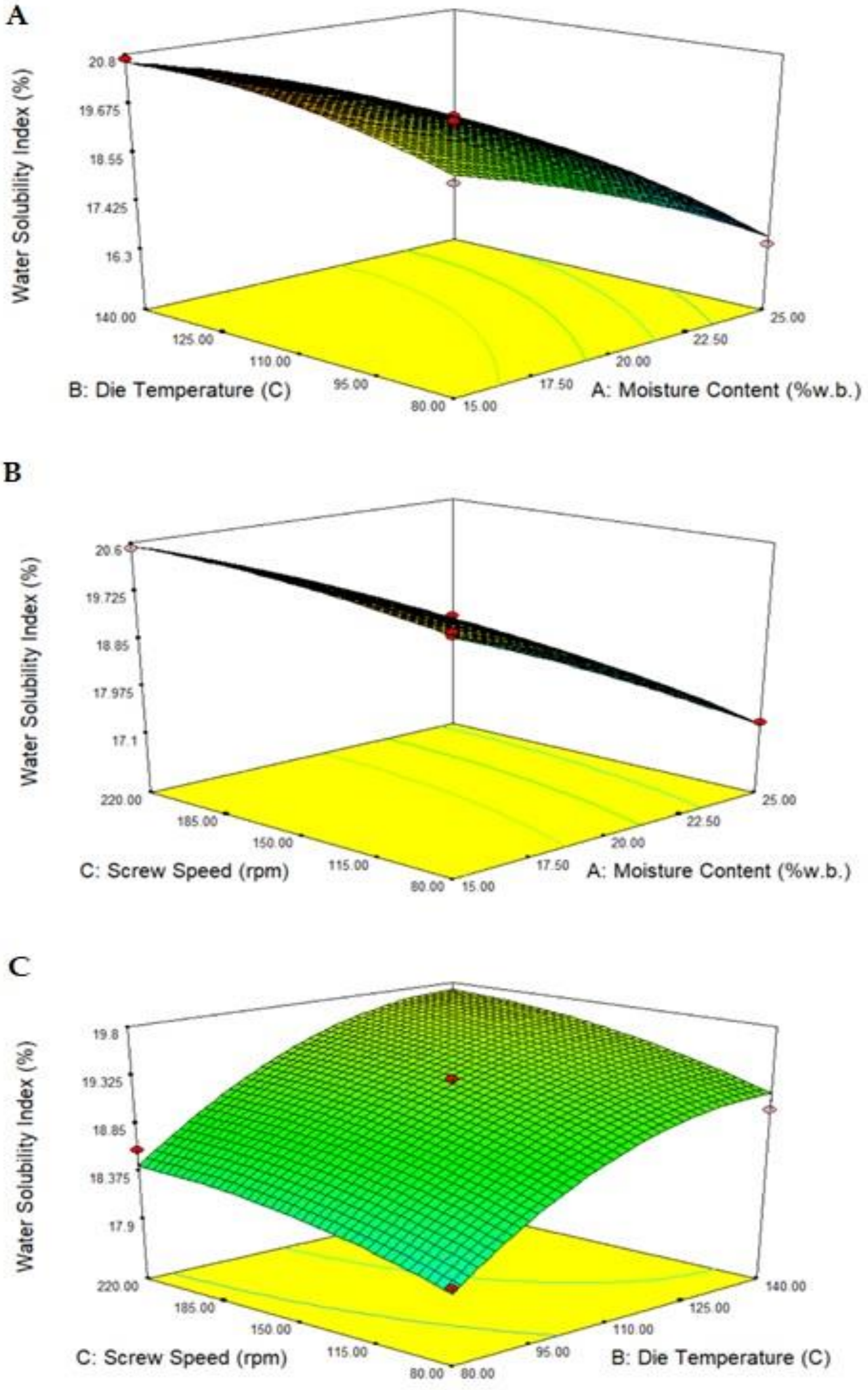


Figure 8.5: Response surface plots (3D) showing the effects on the Water Solubility Index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

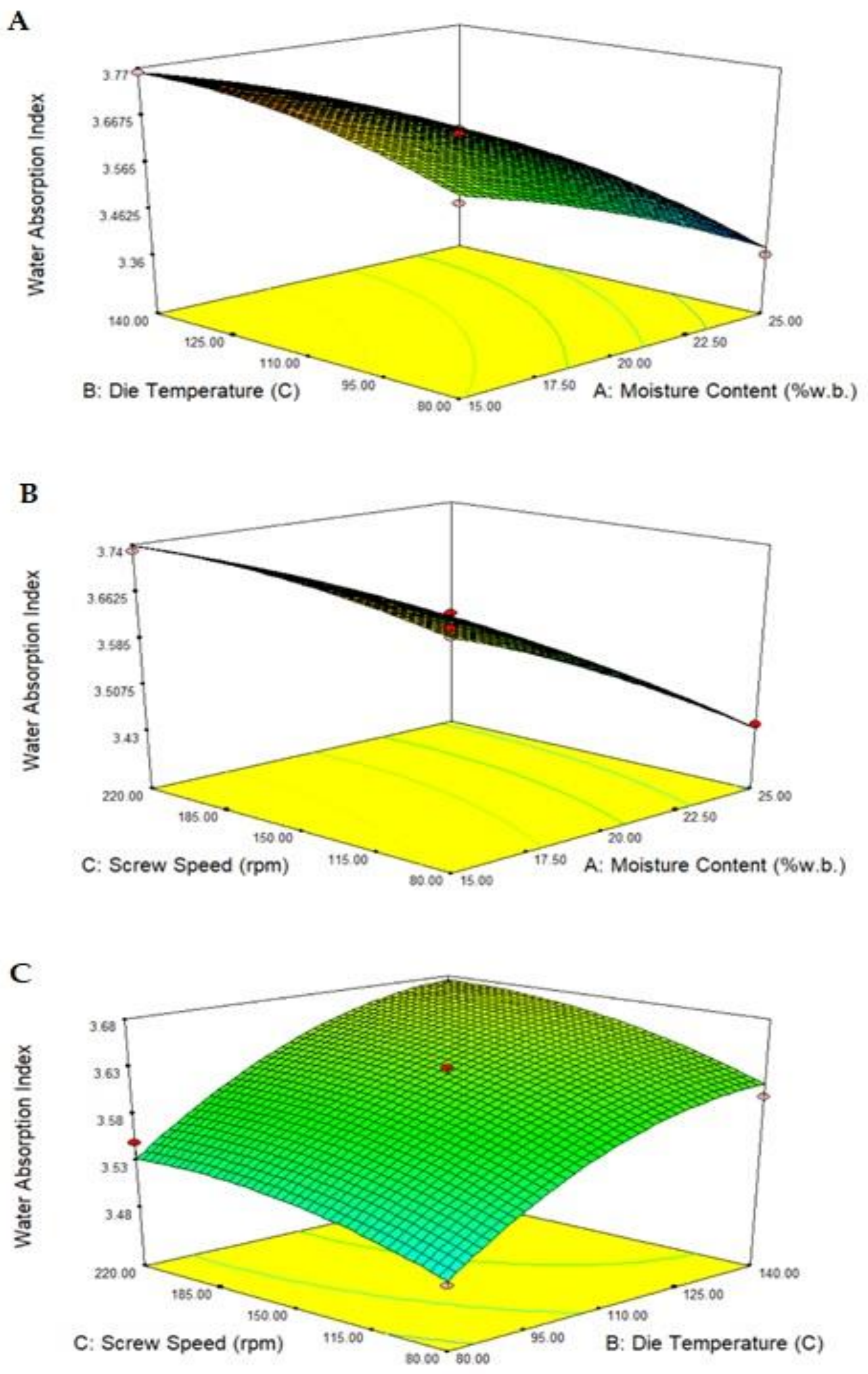


Figure 8.6: Response surface plots (3D) showing the effects on the Water Absorption Index (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

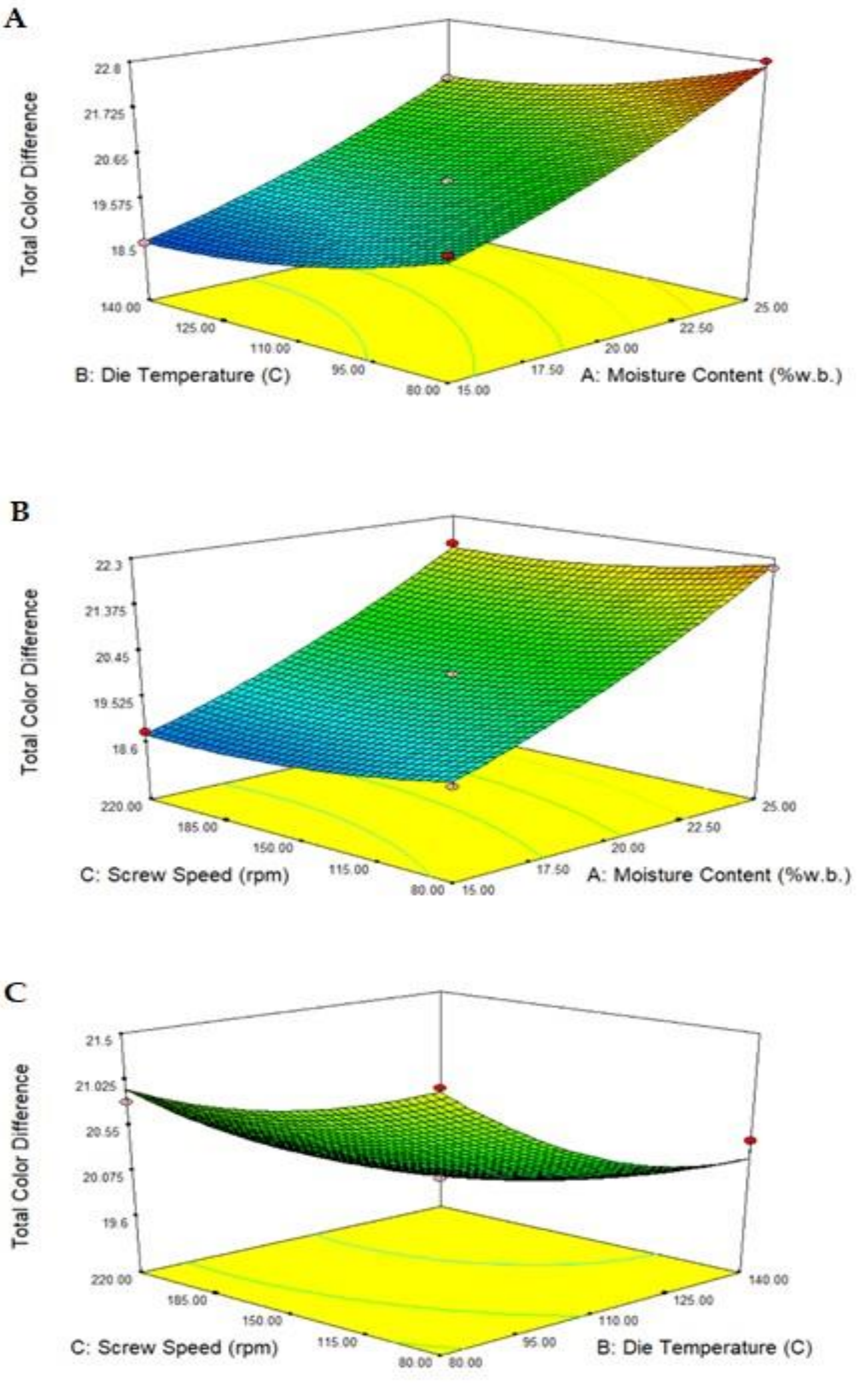


Figure 8.7: Response surface plots (3D) showing the effects on the Total Color Difference (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

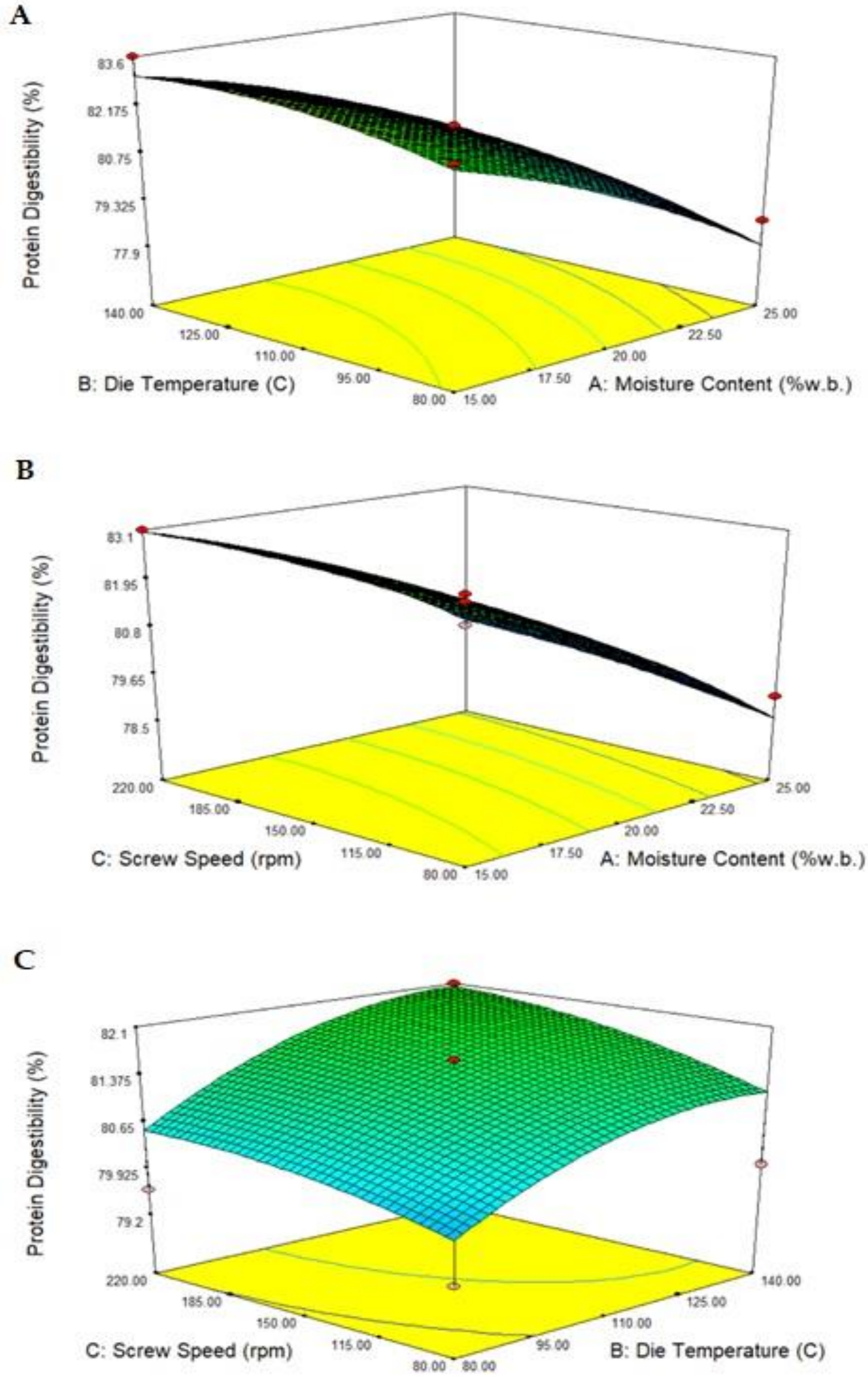


Figure 8.8: Response surface plots (3D) showing the effects on the Protein Digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

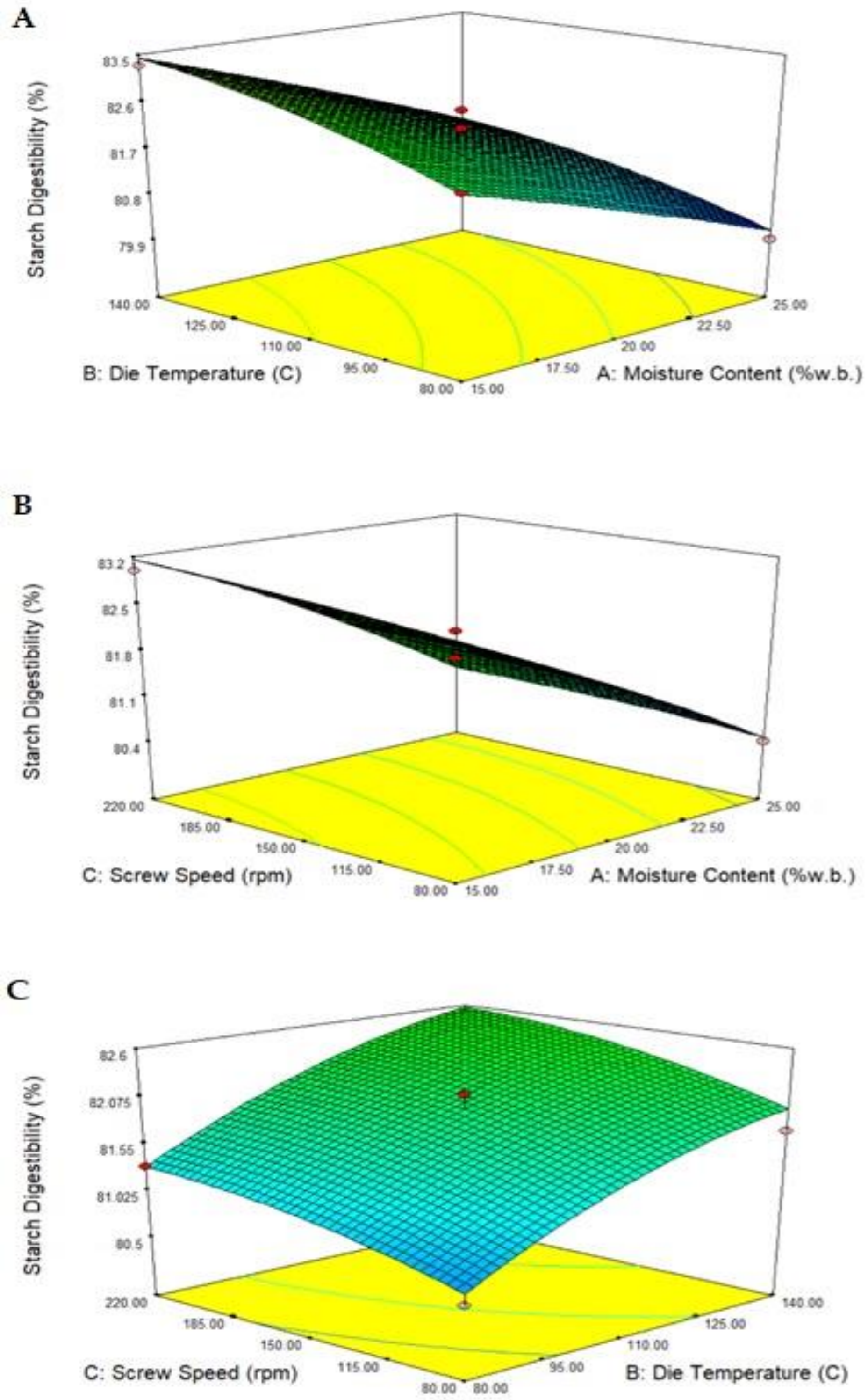


Figure 8.9: Response surface plots (3D) showing the effects on the Starch Digestibility (A) Moisture Content and Screw Speed (B) Die Temperature and Moisture Content (C) Die Temperature and Screw Speed

CHAPTER 9: ARTIFICIAL NEURAL NETWORK MODELLING OF SINGLE AND TWIN-SCREW EXTRUSION OF SPROUTED QUINOA AND PROSO MILLET

Abstract

Extrusion cooking is used to manufacture food products like breakfast cereals, expanded snacks, pasta, or bases for drinks and soups. The properties of food materials are more complex which makes the mathematical modeling of extrusion cooking task more challenging. The functions of an extruder include pumping, mixing, shaping, and working both as a heat exchanger and as a biochemical reactor. Because of the large number of variables involved in these models and because of non-linear relationships among them, several approximations are required. A comparison of response surface methodology (RSM) and artificial neural network (ANN) predictions for the single and twin-screw extrusion of sprouted quinoa, sprouted proso millet and their respective blends with corn meal has been carried out. Results clearly point out that ANN is a better methodology for prediction as it considers the non-linearity of the responses compared to RSM predictions. RSM predictions have more deviations as it is usually limited to linear and static systems. The regression and root mean square results from RSM and ANN have been discussed in detail in this chapter.

Keywords: Artificial Neural Network, Neuron, Quinoa, Proso Millet, Germination, Extrusion

9.1 Introduction

The basic idea of neural networks is to simulate the function of the human brain that has a basic unit called a neuron (Youssefi, Emam-Djomeh, & Mousavi, 2009). A simplified biological neuron consists of four parts: dendrites, soma, axon, and synapses as shown in Fig 9.1A. Dendrites are chemical receptors that receive signals from other neurons (Ameer et al., 2017). The soma is the cell body of a neuron that processes the input signals. An axon is a chemical emitter that sends out the processed signals to nearby neurons (Guo et al., 2010). Synapses are the junctions connecting neurons and regulate the signal transmittance between neurons. Although the structure and function of actual biological neurons is much more complicated, this simplified biological neuron serves as the basis of artificial neural networks (Sinha, Chowdhury, Saha, & Datta, 2013).

Like a biological neuron, an artificial neuron receives a series of input information (x_i) connected to a weight factor (w_i) as shown in Fig 9.1B. In a simple case, the neuron sums the weighed inputs and passes the result to a transfer function to generate an output. The output information is then sent to another neuron as input or is used directly as a network result. The weights are connection strengths between neurons. Because some input signals may be more important than others, the use of weights corresponding to the importance of each input signal provides an efficient way to generate ideal outputs. Weights are adjustable during network training and there are various algorithms to adjust the weights during network training (Winiczenko, Górnicki, Kaleta, & Janaszek-Mańkowska, 2018).

Neurons form layers with different types of connections among neurons. A neuron of one layer is always connected with neurons of at least one other layer. There are different types of connections between neurons. For inter-layer connections, a neuron in one layer is connected to all the neurons in the next layer, producing a fully connected network; if the neurons are connected to only some of the neurons in the next layer then the network is partially connected (Betiku et al., 2016). Neurons in one layer send output information to the next layer, and they may (in feedback networks) or may not (in feedforward networks) receive information back from the next layer (Bourquin, Schmidli, van Hoogevest, & Leuenberger, 1998). Also, neurons may or may not relate to each other in the same layer. In the so-called recurrent intra-layer connections, after receiving input information from a previous layer, neurons within one layer communicate with each other several times before they send their output to another layer (Jeyamkondan, Jayas, & Holley, 2001).

One of the most used models is a three-layer feedforward network. In this type of network, the input layer receives input information from an input file or from electrical sensors in an on-line application and passes this information to the next layer of neurons (Muthusamy, Manickam, Murugesan, Muthukumaran, & Pugazhendhi, 2019). The input layer normally does not conduct signal processing. The third layer, the output layer, processes the input information from the previous layer and then transfers information out from the network. Layers between the input and output layer are called hidden layers. Generally, one hidden layer is enough for a network, although more than one hidden layer may be used for complicated applications (Jeyamkondan et al., 2001). The required number of hidden neurons in the hidden layer was calculated by (Muthusamy et al., 2019) for quantitative applications. Increasing the number of hidden layer neurons greatly increases the possibility for a network to memorize input patterns that leads to poor generalization of the network (Therdthai & Zhou, 2001).

The learning process for developing a neural network can be either supervised or unsupervised. Supervised learning is a learning process that requires target outputs and is the most used learning process. Unsupervised learning does not require any target output but uses certain mathematical methods to organize data by itself. Since feedforward network, normally trained by a backpropagation algorithm, is the most used network, the network used in this research is a feed forward network trained by backpropagation unless otherwise stated (Youssefi et al., 2009).

Backpropagation (BP) is the most used algorithm to train feedforward neural networks. The back-propagation algorithm is normally used to train neural networks that have only inter-layer connections and no intra-layer connections. The neural networks can be either fully connected or partially connected. The BP algorithm has been studied extensively. In a backpropagation trained neural network, information from the input layer is feedforward through zero or more hidden layer to the output layer. The output errors are backpropagated from the output layer through the hidden layers to the input layer. The connection weights are adjusted to minimize an error function such as the mean square error at the output. The sum of weighted input signals is passed to a transfer function to generate an output signal. The transfer functions of backpropagation neural networks may be linear or non-linear, but the most used is a sigmoidal function.

The settings of the learning rate and the momentum factor are important for network performance and computational speed (Bourquin et al., 1998). Use of a smaller learning rate usually yields acceptable results but needs a longer training time because of the requirement for a larger amount of updating steps. Setting a momentum factor below 1.0 is normally recommended so that the current weight change always has a greater influence than the previous one (Popescu, Popescu, Wilder, & Karwe, 2001). The ideal settings of the learning rate and the momentum factor depend upon the specific application and the learning algorithm. A trial-and-error strategy is normally used to determine the settings of the learning rate and the momentum factor.

There are various derivatives of the delta rule for the BP training algorithm. The delta bar delta rule allows the use of different learning rates for each connection weight, which increases the learning rate but also has a greater tendency to be stuck in a local minimum than when the general delta rule is used. Like linear regression analysis, the sample size (number of input patterns) should be larger than or equal to the number of independent variables (sum of the number of weights and other possible variables) to obtain a unique solution for the network system (Popescu et al., 2001). The optimal number of hidden nodes is related to the number of patterns, the number of input and output nodes, and the complexity of the problem or application. Without an exact rule to determine the optimal number of hidden nodes, a trial and-error strategy is normally employed. The network training starts with either a small number of hidden nodes and then increases in size, or instead starts with many hidden nodes and then decreases in size, until the output error is acceptable (García-Gimeno, Hervas-Martinez, Rodriguez-Perez, & Zurera-Cosano, 2005).

The design of network architecture for a specific application is important, although this is simply a matter of network size for a three-layer feedforward network. A network that is too small cannot account for the complexity of the data, while one that is too large often over-fits the data and has a poor generalization. For most applications, since the input signals and output signals are used directly for the network, a network size is only a concern about the number of hidden nodes (Jeyamkondan et al., 2001).

Artificial Neural Networks have been used successfully as a modelling tool in several food processing applications like sensory analysis and quality control (color, texture, human preferences), classifications, microbiology and drying applications (Torrecilla, Otero, & Sanz, 2004). Researchers (references??) have employed artificial neural networks to predict various extrudate characteristics while even some them have compared it to response surface methodology as done in this research. Texture and color characteristics were predicted in gummies using the multilayer, feed-forward neural network (Fan et al., 2013). The simulation processing in ANN showed higher correlation coefficient of 0.9671 and 0.9856 than linear fitting model. Rice flour and rice starch were extruded in single-screw and torque, specific mechanical energy, and pressure were determined (G Ganjyal, Hanna, Supprung, Noomhorm, & Jones, 2006). Multiple input and multiple output (MIMO) models were developed to simultaneously predict properties from three input parameters. ANN performed extremely efficient in predictions with values of $R^2 > 0.95$. The researchers also extruded cross-linked waxy maize starches at initial moisture contents of 20, 24, and 28% (dry basis) and screw speeds of 110, 150, and 190 rpm (GM Ganjyal, Hanna, & Jones, 2003). Multiple input, single output neural network models were developed for predicting individual product properties, and multiple input, multiple output models were developed for predicting selected product properties at one time from selected input process parameters. Models varied only in the number of hidden neurons, ranging between 8 and 14. All ANN models had R^2 values of 0.89 and greater. Spectral stress strain analysis was used in combination with partial least squares (PLS) regression and artificial neural networks (ANN) to predict nine sensory texture attributes of cooked rice (Sitakalin & Meullenet, 2001). Improvements offered by ANN models over PLS regression varied from attribute to attribute between 10.9 and 41.6%. In addition, the ratio between root mean square error of prediction and Root mean square error of calibration, an indication of model robustness, were, as a rule, closer to 1.0 when using ANN.

A ranged of blends were also extruded and modelled using response surface methodology and artificial neural networks. Wheat flour and wheat– black soybean blend (95:5) were extruded in a single screw extruder with varying temperature (120 and 140°C), dry basis moisture content (18 and 20%) and screw speed (156, 168, 180,192 and 204 rpm). A four-layer feed forward network (3-8-6-4) consisting one input layer, one output layer and two hidden layers, satisfactorily described the experimental data. The results showed that artificial neural network (ANN) models performed better than the response surface methodology (RSM) models in describing the characteristics of the extruded product in terms of specific mechanical energy requirement, expansion ratio, water absorption index, water solubility index as well the sensory characteristics. An ingredient blend containing 40% DDGS (distillers dried grains with solubles), along with soy flour, corn flour, fish meal, vitamin mix, and mineral mix, with the net protein content adjusted to 28% was extruded in a single screw extruder (Chevanan, Muthukumarappan, & Rosentrater, 2007). The variables controlled in the first experiment included seven levels of die size, three levels of moisture content, three levels of temperature gradient in the barrel, and one screw speed. The variables altered in the second experiment included three levels of moisture content, three levels of temperature gradient in the barrel, five levels of screw speed, and one die size. Regression models and neural network models were then developed using the data pooled from the two experiments to predict extrudate properties and extrusion processing parameters. The neural network models predicted the extrusion processing parameters using three, five, and six input variables with R^2 values of 0.819 to 0.984, 0.860 to 0.988, and 0.901 to 0.991, respectively. With the regression modeling, even though increasing the number of input variables from three to six resulted in better R^2 values, there was no decrease in the coefficient of variation (CV) between the measured and predicted variables.

The objective of this work is to optimize the neural network topology to predict the error and regression coefficient and root mean square error using artificial neural network and compare the results with response surface methodology for single screw (sprouted quinoa, sprouted quinoa-corn meal blend, sprouted proso millet, sprouted proso millet-corn meal blend) and twin screw (sprouted quinoa and sprouted proso millet) extrusion processes.

9.2 Methodology

Response surface methodology (RSM) is an empirical statistical modeling technique used for multiple regression analysis by quantitative data obtained from experiments (Swamy & Muthukumarappan, 2017). Experiments were conducted using the Box Behnken design (BBD) which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN, USA). In the present study, the Box Behnken Design was used to obtain a proper model for the optimization of the extrusion process variables. The experimental design used for the single screw extrusion of sprouted quinoa are presented in Table 3.2 of Chapter 3. Experiments consisting four factors namely moisture content (X_1), temperature (X_2), screw speed (X_3) and germination time (X_4). The detailed methodology is discussed in Chapter 3. For the artificial neural network, feed-forward back-propagation network was selected to predict the bulk density, hardness, water solubility index, water absorption index, expansion ratio, starch digestibility and protein digestibility. The whole dataset was randomly divided into training, validation and test subsets during development of ANN models. The numbers of samples used for training, validation and test subsets were 55, 10 and 10 respectively. In this study, the optimization of the topology of the ANN models was limited to the optimal number of the neurons in the hidden layer. A series of topologies was examined in order to determine the optimum number of neurons in the hidden layer. The optimal topologies for predicting the product responses were obtained according to the minimized performance function based on Mean Square

Error (MSE). A similar process in carried out for single screw extrusion of proso millet. A detailed explanation is provided in Chapter 6.

For the single screw extrusion of sprouted quinoa – corn meal blend experiment, the design of experiments, which is used for training the network and respective experimental yields are presented in Table 4.2 of Chapter 4. Experiments consisting five factors namely moisture content (X_1), temperature (X_2), screw speed (X_3), corn meal ratio (X_4) and germination time (X_5). The product responses analyzed include bulk density, hardness, water solubility index, water absorption index, expansion ratio, starch digestibility and protein digestibility and the data are presented in Chapter 4. The whole dataset was randomly divided into training, validation and test subsets during development of ANN models. The numbers of samples used for training, validation and test subsets were 115, 10 and 10 respectively. Table 7.2 in Chapter 7 discusses the design and results of proso millet – corn meal blend extrusion in a similar fashion. The counter-rotating twin screw experiments for sprouted quinoa flour consisted four factors namely moisture content (X_1), temperature (X_2), screw speed (X_3), and germination time (X_4). The product responses analyzed in detail in Table 5.2 of Chapter 5 include bulk density, hardness, water solubility index, water absorption index, expansion ratio, starch digestibility and protein digestibility. The experimental design created for the experimented using a Box-Behnken design is shown in Table 9.13. Detailed experimental design and extrusion responses are described in Table 8.2 of Chapter 8 for twin screw extrusion of proso millet. The RSM and ANN prediction methodology has been like the one adopted for quinoa.

The experimental data were analyzed by the response surface regression procedure and the parameters obtained from the RSM analysis were substituted into the following second-order polynomial model equation (Swamy & Muthukumarappan, 2017).

$$Y = \beta_0 + \sum_{a=1}^{c} \beta_a z_a + \sum_{a=1}^{c} \beta_{aa} z_a^2 + \sum_a \sum_{b\leq 2}^{c} \beta_{ab} z_a z_b + e_a \tag{9.1}$$

where Y is the response; z_a and z_b – variables, where a and b range from 1 to c; β_0 - intercept β_a, β_{aa} and β_{ab} - interaction coefficients; c - independent factors; e_a – error.

Artificial neural network (ANN) can be used as an alternative to the polynomial regression-based modeling tool, which provides the modeling of complex non-linear relationships. Artificial Neural Network modeling was performed using commercial software Matlab, R2016a (The MathWorks, Inc., MA, USA). The criteria used for the neural network model evaluation was the regression coefficient R^2. Data sets were divided randomly, 70% as training and 20% as testing sets. Three-layered feedforward networks were used with a backpropagation algorithm. The networks were trained rigorously varying the number of neurons in the hidden layer, learning rates, momentum, and initial weights to arrive at optimum values of all the parameters when the error was lowest. The methodology adopted for developing the neural network programming in presented in Fig 9.3. The coding for the neural network is provided in Program 9.1. The same methodology and coding were used for all extrusion processes. In this study, a tan-sigmoid transfer function (tansig) at hidden layer and a linear transfer function (purelin) at output layer were applied. The Levenberg–Marquardt back-propagation algorithm was used for network training. The product responses predicted by ANN can be written as (Wanto, Windarto, Hartama, & Parlina, 2017):

$$Z_n(y_n) = purelin\left(LW^{(m,1)} \log sig\left(IW^{(1,1)} y_n + a^{(1)}\right) + a^{(2)}\right) \tag{9.2}$$

where Zn denotes the product response predicted by ANN models, y_n are the input variables, $IW^{(1,1)}$ is the input weight matrix, $LW^{(m,1)}$ is the layer weight matrix, a(1) and a(2) are the biases, whose destinations are the hidden layer and output layer, respectively.

9.3 Results and Discussion

9.3.1. Single screw extrusion of sprouted quinoa flour

The product responses of the single screw extrusion of sprouted quinoa flour has been presented in Appendix A. Table 9.1 and Table 9.2 presents the RSM predictions and optimal topologies of ANN models for the predictions of product responses and the corresponding weights and biases values. For predicting the product responses, the numbers of neurons in the hidden layers of ANN models were 10.

Statistical parameters R^2 and RMSE were employed to evaluate the performance of the developed artificial neural network models for the product responses. The results were calculated using the experimental data derived from the Box Behnken Design. The developed Artificial Neural Network models fitted the experimental data with satisfactory accuracy, since both ANN models of bulk density, hardness, water solubility index, water absorption index, expansion ratio, starch digestibility and protein digestibility had high values of R^2 and low values of RMSE as shown in Table 9.3. Similar results were reported for rice flour and rice starch (G Ganjyal et al., 2006), dry distiller’s grains with solubles (Bhadra, Muthukumarappan, & Rosentrater, 2006) and surimi (J. Zhang, Wang, Lv, & Ding, 2012).

The response surface models, and artificial neural network model were further compared with random values. In order to validate and test the extrapolative capability of both the ANN and RSM models, experiments were conducted for 10 new trials, consisting of combinations of random experimental factors from the training data sets. The experimental and predicted values of the response for both the ANN and RSM models are given in Table 9.4 and Table 9.5. These results indicated that the RSM prediction had a greater deviation than of ANN for all product responses. Both RSM and ANN models provided good quality predictions in this study, nevertheless the ANN showed a superiority over RSM for both data fitting and estimation capabilities. RSM has the advantage of giving a regression equation for prediction and showing the effect of experimental factors and their interactions on responses in comparison with ANN. However, the main limitation of RSM that it assumes only quadratic non-linear correlation, but ANN can inherently capture almost any form of non-linearity, it can easily overcome the limitation of RSM and this methodology does not require a standard experimental design to build the model.

9.3.2. Single screw extrusion of sprouted quinoa-corn meal blend

The product responses are shown in Appendix B and RSM predictions are discussed in Chapter 4. A feed-forward back-propagation network was selected to predict the product responses in artificial neural network. The whole dataset was randomly divided into training, validation and test subsets during development of ANN models. In this study, the optimization of the topology of the neural network models was limited to the optimal number of the neurons in the hidden layer. A series of topologies was examined in order to determine the optimum number of neurons in the hidden layer. Table 9.6 and Table 9.7 presents the response surface methodology predictions and optimal topologies of artificial neural network models for the predictions of product responses and the corresponding weights and biases values. The developed artificial neural network models could fit the experimental data with satisfactory accuracy, since both ANN models of bulk density, hardness, water solubility index, water absorption index, expansion ratio, starch digestibility and protein digestibility had high values of R^2 and low values of RMSE as shown in Table 9.8. The highest R^2 square values of 0.9923 was obtained by ANN model for expansion ratio while the RSM value was 0.9629. Similarly, the lowest RSME value of 0.32 was obtained for expansion ratio in the ANN model. Similar results were reported for soy protein isolate-corn flour blends (Yu et al., 2012), rice kernel extrudates (Dalbhagat & Mishra, 2019) and fish muscle-rice flour blend (Shankar & Bandyopadhyay, 2007)

In order to validate and test the extrapolative capability of both the ANN and RSM models, the response surface models, and artificial neural network model were further compared with random values. Experiments were conducted for 10 new trials, consisting of combinations of random experimental factors from the training data sets. The experimental and predicted values of the response for both the artificial neural network and response surface methodology models are given in Table 9.9 and Table 9.10. These results indicated that the RSM prediction had a greater deviation than of ANN for all product responses. Response surface methodology models have the advantage of giving a regression equation for prediction and showing the effect of experimental factors and their interactions on responses in comparison with artificial neural network. However, the main limitation of RSM that it assumes only quadratic non-linear correlation. Both RSM and ANN models provided good quality predictions in this study, nevertheless the ANN showed a superiority over RSM for both data fitting and estimation capabilities. Artificial neural networks capture almost any form of non-linearity, it can easily overcome the limitation of response surface models.

9.3.3. Twin screw extrusion of sprouted quinoa

The individual product responses were predicted by equation 9.1 and 9.2. The response surface methodology predictions and optimal topologies of artificial neural network models for the predictions of product responses and the corresponding weights and biases values are presented in Table 9.11 and Table 9.12. The neural network models had almost the same architecture, with the only variation being in the number of neurons in the hidden layer. All the models performed well with the testing data with R^2 values of greater than 0.9 as shown in Table 9.13. The regression models also performed well, with R^2 values greater than 0.88. The variables and their combinations were very different for the regression models. The neural network models had higher R^2 values and lower standard error values of prediction than did the regression models. The optimized network indicated that one hidden layer with a learning rate of 0.1 steep descent learning rule, 1000 epochs and a logistic sigmoid transfer function predicted the extrudate properties better than RSM. The extrapolative capability of both the ANN and RSM models, the response surface models, and artificial neural network model were further compared with random values. Experiments were conducted for 10 new trials, consisting of combinations of random experimental factors from the training data sets. The experimental and predicted values of the response for both the artificial neural network and response surface methodology models are given in Table 9.14 and Table 9.15.

The results demonstrate that ANN can solve real multivariate complex problems like extrusion cooking, where it is difficult to develop a robust model using statistical methods. ANN showed a superiority over RSM for both data fitting and estimation capabilities. RSM prediction had a greater deviation as it assumes only quadratic non-linear correlation. Both RSM and ANN models provided good quality predictions for twin screw extrusion ($R^2 > 0.9$), nevertheless the neural networks capture almost any form of non-linearity and it easily overcomes the limitation of response surface models.

9.3.4. Single screw extrusion of sprouted proso millet

The detailed discussion on the RSM responses and predictions is in Appendix D and Chapter 6 respectively. The RSM and ANN predictions of bulk density, hardness, water solubility index and water absorption index in the single screw extrusion of proso millet is presented in Table 9.16. The ANN predictions of product responses and the corresponding weights and biases values are presented in Table 9.17. For predicting the product responses, the numbers of neurons in the hidden layers of ANN models were 10. The regression coefficient and root mean square error were employed to evaluate the performance of the developed artificial neural network models for the product responses. The results were calculated using the experimental data derived from the Box Behnken Design. All product responses had high values of R^2 and low values of RMSE as shown in Table 9.18. The regression coefficient was greater than 0.99 for all product responses from RSM and ANN. In this experiment, the RSM and ANN predictions were closer as compared to the quinoa extrusions. The validation tests were further conducted with a random data set with ten runs. The experimental and predicted values of the response for both the ANN and RSM models are given in Table 9.19 and Table 9.20. These results indicated that the RSM prediction was more precise than ANN predictions. Similar results were reported for rice flour and rice starch (G Ganjyal et al., 2006), dry distiller’s grains with solubles (Bhadra et al., 2006) and surimi (J. Zhang et al., 2012). RSM generated regression equation for prediction and showing the effect of experimental factors and their interactions on responses. However, it assumes only quadratic non-linear correlation, whereas ANN considers the non-linearity.

9.3.5. Single screw extrusion of sprouted proso millet-corn meal blend

Food extrusion process modeling has been a difficult task due to its complexities. Analyzing the product responses such as bulk density, hardness, water solubility index, water absorption index, total color difference, expansion ratio, protein digestibility and starch digestibility from blends of sprouted proso millet-corn meal using RSM and ANN a further challenging process. The product responses and RSM predictions are presented in Appendix E and also discussed in Chapter 7. Table 9.21 and Table 9.22 presents the response surface methodology predictions and optimal topologies of artificial neural network models for the predictions of product responses and the corresponding weights and biases values. The developed artificial neural network models could fit the experimental data with satisfactory accuracy, since both ANN models of bulk density, hardness, water solubility index, water absorption index, expansion ratio, starch digestibility and protein digestibility had high values of R^2 and low values of RMSE as shown in Table 9.23. The highest R^2 square values of 0.9992 was obtained for total color difference while the lowest value was obtained for the water absorption index in the RSM predictions. The lowest RSME value of 0.91 was obtained for bulk density in the ANN model. A similar prediction was obtained from ANN but the values for total color difference was 0.9999 and lowest scare for water absorption index was 0.9368. Similarly, the lowest RSME value of 0.32 was obtained for expansion ratio in the ANN model. Similar results were reported for soy protein isolate-corn flour blends (Yu et al., 2012), rice kernel extrudates (Dalbhagat & Mishra, 2019) and fish muscle-rice flour blend (Shankar & Bandyopadhyay, 2007). Experiments were conducted for ten random trials consisting of combinations of random experimental factors from the data sets. The experimental and predicted values of the response for both the artificial neural network and response surface methodology models are given in Table 9.24 and Table 9.25. Though regression techniques are commonly used, difficulties arise when dealing with the complex characteristics of some systems. Regression is usually limited to linear and static systems, and conventional nonlinear regression algorithms are clumsy when handling systems like the extrusion process with multiple inputs and outputs. For non-linear problems, neural networks are a promising alternative technique as they learn from examples through iteration, without requiring a prior knowledge of relationships between variables under investigation. The advantage of neural network over a rule-based model is that, if the process under analysis changes, new examples can be added, and the neural network can be retrained.

9.3.6. Twin screw extrusion of sprouted proso millet

Comparing twin screw extrusion versus single screw extrusion is an important exercise for most researchers who want to create new products through extrusion technology. The impact of twin-screw extrusion process parameters on the product responses has been discussed in detail in Appendix F and Chapter 8. The response surface methodology predictions and optimal topologies of artificial neural network models for the predictions of product responses and the corresponding weights and biases values are presented in Table 9.26 and Table 9.27. The responses clearly showed that ANN had a close prediction to the actual responses when compared to the RSM predictions. All the models performed well with the testing data with R^2 values of greater than 0.9 as shown in Table 9.28. The root mean square error was less than 1 for all responses predicted by ANN whereas RSM has RMSE values greater than 1 for all product responses. RSM prediction had a greater deviation as it assumes only quadratic non-linear correlation. The extrapolative capability of both the ANN and RSM models, the response surface models, and artificial neural network model were further compared with ten random runs. The experimental and predicted values of the response for both the artificial neural network and response surface methodology models are given in Table 9.29 and Table 9.30. The results demonstrate that ANN can solve real multivariate complex problems like extrusion cooking, where it is difficult to develop a robust model using statistical methods. ANN showed a superiority over RSM for both data fitting and estimation capabilities.

9.3.6. Comparison of prediction performances

Although neural networks have become one of the key research objects within artificial intelligence, relatively little information is available on neural networks related to food process control. The interest in such areas as dynamic modelling of food processes has increased, not least due to dramatic improvement and availability of the calculation methods and hardware. Fig 9.4 and Fig 9.5 show the prediction performances of ANN for sprouted quinoa-based extrusion processes and sprouted proso millet extrusion processes. Both results predict that the respective single screw extrusion processes have a better performance suggesting that the single screw extrusion process is more applicable to manufacture extrudates of high quality especially with increased starch and protein digestibility. The greatest deviation is observed in the twin screw extrusion process. The counter-rotating twin screw process does not suit production of high quality extrudates with higher expansion ratio, low bulk density and increased starch and protein digestibility.

9.4 Conclusion

A comparison of the response surface methodology and artificial neural network reveals that the ANN algorithm has the capability of relating the input and output parameters, learning from examples through iteration, without requiring a prior knowledge of the relationships of the process parameters. Its structure is relatively simple, with connections in parallel and sequence between neurons. This means a short computing time and a high potential of robustness and adaptive performance. Irrespective of the ingredient composition and blend for all extrusion processes, ANN predictions have regression coefficients greater than 0.9 and RSM predictions are greater than 0.8. Similarly, the root mean square error values were low in all ANN predictions are compared to RSM. Based on error analysis results, the prediction capability of ANN model is found to be the best of all the prediction models investigated, irrespective of food composition and extrusion processes.

Table 9.1: Actual and predicted values of Bulk Density, Hardness, Water Solubility Index and Water Absorption Index of sprouted quinoa flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	**Bulk Density kg/m^3**			**Hardness N**			**Water Solubility Index %**			**Water Absorption Index**		
	Actual	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**	
		RSM	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**
1	138	136.8	137.4	1.16	0.93	1.09	15.2	12.4	14.04	2.80	1.64	2.22
2	149	147.4	148.2	1.63	1.53	1.60	11.5	9.10	9.87	2.36	0.73	1.55
3	128	126.9	127.5	1.10	0.77	1.01	16.2	12.8	15.10	3.41	2.31	2.86
4	149	147.3	148.2	1.69	1.57	1.66	13.0	10.5	11.31	2.51	0.82	1.67
5	130	128.9	129.4	1.13	0.87	1.06	15.8	12.9	14.67	2.94	1.81	2.38
6	146	144.5	145.3	1.50	1.37	1.46	12.0	9.6	10.50	2.42	0.92	1.67
7	132	130.7	131.4	1.27	0.99	1.19	16.1	12.9	14.83	3.22	1.95	2.59
8	154	152.2	153.1	1.80	1.70	1.77	12.6	10.1	10.80	2.50	0.70	1.60
9	145	143.7	144.4	1.30	1.13	1.25	12.4	9.9	11.10	2.49	1.19	1.84

10	136	134.7	135.4	1.29	1.09	1.23	14.4	11.7	13.11	2.69	1.40	2.05
11	143	141.6	142.3	1.40	1.24	1.36	12.9	10.4	11.50	2.51	1.11	1.81
12	141	139.5	140.3	1.47	1.29	1.42	15.1	12.4	13.63	2.74	1.27	2.01
13	140	138.6	139.3	1.36	1.17	1.31	13.6	10.9	12.24	2.72	1.36	2.04
14	140	138.6	139.3	1.36	1.17	1.31	13.6	10.9	12.24	2.72	1.36	2.04
15	140	138.6	139.3	1.36	1.17	1.31	13.6	10.9	12.24	2.72	1.36	2.04
16	136	134.9	135.4	1.13	0.89	1.06	15.4	12.6	14.27	2.83	1.70	2.27
17	148	146.4	147.2	1.60	1.49	1.57	11.7	9.30	10.10	2.36	0.76	1.56
18	125	123.3	124.2	1.70	1.45	1.63	16.3	12.9	14.6	3.43	1.73	2.58
19	145	143.4	144.2	1.65	1.53	1.62	13.2	10.7	11.55	2.52	0.87	1.70
20	129	127.9	128.5	1.10	0.83	1.03	15.9	12.9	14.8	2.96	1.86	2.41
21	144	142.5	143.3	1.46	1.32	1.42	12.2	9.8	10.74	2.43	0.97	1.70
22	131	129.8	130.4	1.22	0.93	1.14	16.2	12.9	14.98	3.26	2.04	2.65
23	151	149.2	150.1	1.77	1.67	1.74	12.7	10.2	10.93	2.50	0.73	1.62
24	143	141.8	142.4	1.25	1.07	1.20	12.6	10.1	11.35	2.49	1.24	1.87

25	133	131.8	132.4	1.25	1.04	1.19	14.6	11.9	13.35	2.69	1.44	2.07
26	142	140.6	141.3	1.36	1.19	1.31	13.1	10.6	11.74	2.53	1.17	1.85
27	141	139.6	140.3	1.45	1.26	1.40	15.1	12.3	13.65	2.76	1.31	2.04
28	138	136.7	137.3	1.35	1.15	1.29	13.8	11.1	12.45	2.73	1.38	2.06
29	138	136.7	137.3	1.35	1.15	1.29	13.8	11.1	12.45	2.73	1.38	2.06
30	138	136.7	137.3	1.35	1.15	1.29	13.8	11.1	12.45	2.73	1.38	2.06
31	135	133.9	134.5	1.10	0.85	1.03	15.6	12.7	14.5	2.86	1.76	2.31
32	145	143.4	144.2	1.56	1.44	1.53	11.8	9.4	10.24	2.37	0.81	1.59
33	122	120.9	121.4	1.15	0.82	1.06	16.4	12.9	15.25	3.46	2.31	2.89
34	143	141.4	142.2	1.63	1.50	1.59	13.3	10.8	11.67	2.52	0.89	1.71
35	127	125.9	126.5	1.08	0.81	1.00	16.0	13.0	14.92	2.99	1.91	2.45
36	144	142.6	143.3	1.43	1.29	1.39	12.4	10.0	10.97	2.43	1.00	1.72
37	129	127.8	128.4	1.19	0.89	1.11	16.2	12.9	15.01	3.28	2.09	2.69
38	148	146.3	147.1	1.73	1.62	1.70	12.9	10.4	11.17	2.51	0.78	1.65
39	142	140.8	141.4	1.22	1.04	1.17	12.7	10.2	11.48	2.50	1.28	1.89

40	130	128.8	129.4	1.23	1.02	1.17	14.8	12.1	13.57	2.70	1.47	2.09
41	140	138.7	139.3	1.33	1.16	1.28	13.3	10.8	11.97	2.53	1.20	1.87
42	140	138.6	139.3	1.43	1.24	1.38	15.2	12.4	13.77	2.77	1.34	2.06
43	137	135.7	136.3	1.31	1.11	1.25	13.9	11.2	12.59	2.74	1.43	2.09
44	137	135.7	136.3	1.31	1.11	1.25	13.9	11.2	12.59	2.74	1.43	2.09
45	137	135.7	136.3	1.31	1.11	1.25	13.9	11.2	12.59	2.74	1.43	2.09
46	132	130.9	131.5	1.09	0.83	1.02	15.7	12.8	14.61	2.88	1.79	2.34
47	144	142.5	143.2	1.55	1.43	1.52	11.9	9.5	10.35	2.39	0.84	1.62
48	120	118.9	119.4	1.15	0.82	1.06	16.4	12.9	15.25	3.48	2.33	2.91
49	142	140.4	141.2	1.60	1.47	1.56	13.4	10.9	11.80	2.52	0.92	1.72
50	126	124.9	125.5	1.07	0.79	0.99	16.1	13.1	15.03	3.03	1.96	2.50
51	143	141.6	142.3	1.41	1.26	1.37	12.5	10.1	11.09	2.45	1.04	1.75
52	126	124.8	125.4	1.17	0.86	1.08	16.3	13.0	15.13	3.33	2.16	2.75
53	146	144.3	145.2	1.69	1.57	1.66	13.1	10.6	11.41	2.51	0.82	1.67
54	140	138.8	139.4	1.21	1.02	1.16	12.7	10.2	11.49	2.51	1.3	1.91

55	127	125.8	126.4	1.22	1.01	1.16	14.8	12.1	13.58	2.72	1.5	2.11
56	140	138.7	139.4	1.29	1.11	1.24	13.3	10.8	12.01	2.54	1.25	1.90
57	137	135.6	136.3	1.41	1.22	1.36	15.3	12.5	13.89	2.77	1.36	2.07
58	135	133.7	134.4	1.28	1.07	1.22	14.0	11.2	12.72	2.76	1.48	2.12
59	135	133.7	134.4	1.28	1.07	1.22	14.0	11.2	12.72	2.76	1.48	2.12
60	135	133.7	134.4	1.28	1.07	1.22	14.0	11.2	12.72	2.76	1.48	2.12
61	129	127.9	128.5	1.07	0.80	1.00	15.8	12.9	14.73	2.94	1.87	2.41
62	144	142.5	143.2	1.53	1.40	1.49	12.1	9.7	10.57	2.41	0.88	1.65
63	116	114.9	115.4	1.13	0.79	1.03	16.5	13.0	15.37	3.51	2.38	2.95
64	140	138.4	139.2	1.58	1.44	1.54	13.4	10.9	11.82	2.54	0.96	1.75
65	122	121.0	121.5	1.05	0.76	0.97	16.2	13.2	15.15	3.05	2.00	2.53
66	141	139.6	140.3	1.37	1.21	1.33	12.7	10.2	11.33	2.47	1.10	1.79
67	125	123.9	124.4	1.15	0.84	1.06	16.4	13.1	15.25	3.35	2.20	2.78
68	146	144.4	145.2	1.64	1.51	1.60	13.2	10.7	11.56	2.53	0.89	1.71
69	140	138.8	139.4	1.19	1.00	1.14	12.8	10.3	11.61	2.51	1.32	1.92

70	123	121.8	122.4	1.20	0.98	1.14	14.9	12.2	13.7	2.73	1.53	2.13
71	138	136.7	137.4	1.27	1.09	1.22	13.4	10.9	12.13	2.54	1.27	1.91
72	135	133.6	134.3	1.37	1.17	1.31	15.4	12.6	14.03	2.79	1.42	2.11
73	132	130.8	131.4	1.25	1.03	1.19	14.2	11.4	12.95	2.77	1.52	2.15
74	132	130.8	131.4	1.25	1.03	1.19	14.2	11.4	12.95	2.77	1.52	2.15
75	132	130.8	131.4	1.25	1.03	1.19	14.2	11.4	12.95	2.77	1.52	2.15

Table 9.2: Actual and predicted values of Total Color Difference, Expansion Ratio, Starch Digestibility and Protein Digestibility of sprouted quinoa flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	21.1	20.2	20.9	3.42	2.93	3.31	83.5	82.0	83.4	81.1	79.6	81.0
2	16.5	15.9	16.4	2.48	2.13	2.40	80.5	79.4	80.4	80.9	79.8	80.8
3	21.7	20.8	21.5	3.71	3.18	3.59	83.0	81.4	82.9	82.0	80.4	81.9
4	18.4	17.6	18.2	3.04	2.61	2.94	81.9	80.6	81.8	81.4	80.1	81.3
5	20.5	19.6	20.3	3.57	3.06	3.45	83.9	82.4	83.8	81.9	80.4	81.8
6	15.8	15.1	15.7	2.68	2.30	2.59	81.5	80.4	81.4	80.5	79.4	80.4
7	21.4	20.5	21.2	3.68	3.15	3.56	83.0	81.4	82.9	82.1	80.5	82.0
8	17.1	16.4	17.0	2.81	2.41	2.72	81.7	80.5	81.6	81.0	79.8	80.9
9	18.4	17.7	18.3	2.92	2.50	2.82	81.0	79.7	80.9	80.7	79.4	80.6

10	17.4	16.6	17.2	3.03	2.60	2.93	82.0	80.7	81.9	80.9	79.6	80.8
11	20.9	20.1	20.7	3.18	2.73	3.07	82.5	81.1	82.4	81.0	79.6	80.9
12	21.4	20.5	21.2	3.51	3.01	3.39	82.1	80.6	82.0	81.9	80.4	81.8
13	19.8	19.0	19.6	3.32	2.85	3.21	83.0	81.6	82.9	81.6	80.2	81.5
14	19.8	19.0	19.6	3.32	2.85	3.21	83.0	81.6	82.9	81.6	80.2	81.5
15	19.8	19.0	19.6	3.32	2.85	3.21	83.0	81.6	82.9	81.6	80.2	81.5
16	20.8	19.9	20.6	3.44	2.95	3.33	85.5	84.0	85.4	83.8	82.3	83.7
17	16.2	15.6	16.1	2.52	2.16	2.44	83.6	82.5	83.5	83.5	82.4	83.4
18	21.6	20.7	21.4	3.72	3.19	3.60	86.1	84.5	86.0	85.3	83.7	85.2
19	18.1	17.3	17.9	3.06	2.62	2.96	85.1	83.8	85.0	84.3	83.0	84.2
20	20.3	19.4	20.1	3.58	3.07	3.46	85.6	84.1	85.5	84.6	83.1	84.5
21	15.5	14.8	15.4	2.72	2.33	2.63	84.3	83.1	84.2	83.3	82.1	83.2
22	21.2	20.3	21.0	3.69	3.16	3.57	85.8	84.2	85.7	85.1	83.5	85.0
23	17	16.3	16.9	2.85	2.44	2.76	85.1	83.9	85.0	83.9	82.7	83.8
24	18.2	17.5	18.1	2.94	2.52	2.84	84.1	82.8	84.0	83.5	82.2	83.4

25	17.3	16.5	17.1	3.07	2.63	2.97	84.8	83.5	84.7	83.5	82.2	83.4
26	20.7	19.9	20.5	3.21	2.75	3.10	85.0	83.6	84.9	84.2	82.8	84.1
27	21.3	20.4	21.1	3.47	2.97	3.35	85.7	84.2	85.6	84.8	83.3	84.7
28	19.6	18.8	19.4	3.35	2.87	3.24	85.3	83.9	85.2	84.5	83.1	84.4
29	19.6	18.8	19.4	3.35	2.87	3.24	85.3	83.9	85.2	84.5	83.1	84.4
30	19.6	18.8	19.4	3.35	2.87	3.24	85.3	83.9	85.2	84.5	83.1	84.4
31	20.7	19.8	20.5	3.47	2.97	3.35	85.7	84.2	85.6	84.0	82.5	83.9
32	16.1	15.5	16.0	2.56	2.19	2.47	83.7	82.6	83.6	83.6	82.5	83.5
33	21.4	20.5	21.2	3.74	3.21	3.62	86.2	84.6	86.1	85.5	83.9	85.4
34	17.9	17.1	17.7	3.08	2.64	2.98	85.2	83.9	85.1	84.5	83.2	84.4
35	20.2	19.3	20.0	3.6	3.09	3.48	85.7	84.2	85.6	84.8	83.3	84.7
36	15.4	14.7	15.3	2.75	2.36	2.66	84.4	83.2	84.3	83.5	82.3	83.4
37	21.1	20.2	20.9	3.7	3.17	3.58	86	84.4	85.9	85.3	83.7	85.2
38	16.8	16.1	16.7	2.88	2.47	2.78	85.1	83.9	85.0	84	82.8	83.9
39	17.9	17.2	17.8	2.95	2.53	2.85	84.3	83.0	84.2	83	81.7	82.9

40	17.1	16.3	16.9	3.11	2.67	3.01	85	83.7	84.9	83.7	82.4	83.6
41	20.5	19.7	20.3	3.25	2.79	3.14	85.2	83.8	85.1	84.4	83.0	84.3
42	21.1	20.2	20.9	3.43	2.94	3.32	85.7	84.2	85.6	85	83.5	84.9
43	19.3	18.5	19.1	3.37	2.89	3.26	85.4	84.0	85.3	84.7	83.3	84.6
44	19.3	18.5	19.1	3.37	2.89	3.26	85.4	84.0	85.3	84.7	83.3	84.6
45	19.3	18.5	19.1	3.37	2.89	3.26	85.4	84.0	85.3	84.7	83.3	84.6
46	20.5	19.6	20.3	3.48	2.98	3.36	85.8	84.3	85.7	84.2	82.7	84.1
47	15.8	15.2	15.7	2.59	2.22	2.50	83.9	82.8	83.8	83.8	82.7	83.7
48	21.1	20.2	20.9	3.75	3.21	3.63	86.4	84.8	86.3	85.7	84.1	85.6
49	17.8	17.0	17.6	3.08	2.64	2.98	85.4	84.1	85.3	84.6	83.3	84.5
50	20.0	19.1	19.8	3.61	3.09	3.49	85.8	84.3	85.7	84.9	83.4	84.8
51	15.1	14.4	15.0	2.77	2.37	2.68	84.6	83.4	84.5	83.6	82.4	83.5
52	21.1	20.2	20.9	3.72	3.19	3.60	86.1	84.5	86.0	85.4	83.8	85.3
53	16.4	15.7	16.3	2.89	2.48	2.79	85.2	84.0	85.1	84.2	83.0	84.1
54	17.6	16.9	17.5	3.00	2.57	2.90	84.3	83.0	84.2	83.1	81.8	83.0

55	17.0	16.2	16.8	3.12	2.67	3.02	85.2	83.9	85.1	83.8	82.5	83.7
56	20.4	19.6	20.2	3.29	2.82	3.18	85.3	83.9	85.2	84.6	83.2	84.5
57	20.8	19.9	20.6	3.41	2.92	3.30	85.8	84.3	85.7	85.1	83.6	85.0
58	19.2	18.4	19.0	3.38	2.90	3.27	85.6	84.2	85.5	84.8	83.4	84.7
59	19.2	18.4	19.0	3.38	2.90	3.27	85.6	84.2	85.5	84.8	83.4	84.7
60	19.2	18.4	19.0	3.38	2.90	3.27	85.6	84.2	85.5	84.8	83.4	84.7
61	20.4	19.5	20.2	3.51	3.01	3.39	86.0	84.5	85.9	84.3	82.8	84.2
62	15.5	14.9	15.4	2.60	2.23	2.51	84.1	83.0	84.0	84.0	82.9	83.9
63	20.8	19.9	20.6	3.75	3.21	3.63	86.5	84.9	86.4	85.8	84.2	85.7
64	17.6	16.8	17.4	3.09	2.65	2.99	85.5	84.2	85.4	84.8	83.5	84.7
65	19.7	18.8	19.5	3.63	3.11	3.51	85.4	83.8	85.3	85.1	83.5	85.0
66	14.8	14.1	14.7	2.81	2.41	2.72	84.7	83.5	84.6	83.7	82.5	83.6
67	20.9	20.0	20.7	3.74	3.21	3.62	86.3	84.7	86.2	85.6	84.0	85.5
68	16.2	15.5	16.1	2.91	2.49	2.81	85.3	84.1	85.2	84.4	83.2	84.3
69	17.4	16.6	17.2	3.04	2.61	2.94	84.5	83.2	84.4	83.2	81.9	83.1

70	17.0	16.2	16.8	3.15	2.70	3.05	85.3	84.0	85.2	84.0	82.7	83.9
71	20.1	19.3	19.9	3.30	2.83	3.19	85.5	84.1	85.4	84.7	83.3	84.6
72	20.6	19.8	20.4	3.40	2.91	3.29	86.0	84.5	85.9	85.3	83.8	85.2
73	18.8	17.9	18.6	3.41	2.92	3.30	85.7	84.2	85.6	85.0	83.5	84.9
74	18.8	17.9	18.6	3.41	2.92	3.30	85.7	84.2	85.6	85.0	83.5	84.9
75	18.8	17.9	18.6	3.41	2.92	3.30	85.7	84.2	85.6	85.0	83.5	84.9

Table 9.3: Regression coefficient and Root Mean Square Error – Single Screw Extrusion of sprouted quinoa flour

Product Responses	**RSM**		**ANN**	
	R^2	**RMSE**	**R^2**	**RMSE**
Bulk Density (kg/m^3)	0.9769	1.95	0.9843	0.93
Hardness (N)	0.9318	1.13	0.9567	0.73
Water Solubility Index (%)	0.9856	1.27	0.9999	0.77
Water Absorption Index	0.9830	1.08	0.9987	0.57
Total Color Difference	0.9398	1.25	0.9682	0.87
Expansion Ratio	0.9702	1.67	0.9866	1.01
Starch Digestibility (%)	0.9774	1.54	0.9893	0.99
Protein Digestibility (%)	0.9769	1.57	0.9976	0.92

RSM – Response Surface Methodology; ANN – Artificial Neural Network, R^2- Regression Coefficient; RMSE – Root Mean Square Error

Table 9.4: Optimization, validation and comparison of experimental data set (Bulk Density, Hardness, Water Solubility Index, Water Absorption Index) – Single Screw Extrusion of sprouted quinoa flour

No	Moisture Content (%w.b.)	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
					Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
						RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	20	150	115	0	140	138.6	139.3	1.36	1.17	1.31	13.6	10.9	12.24	2.72	1.36	2.04
2	15	80	115	1	136	134.9	135.4	1.13	0.89	1.06	15.4	12.6	14.27	2.83	1.7	2.27
3	20	80	140	0	143	141.6	142.3	1.4	1.24	1.36	12.9	10.4	11.5	2.51	1.11	1.81
4	20	220	140	1	141	139.6	140.3	1.45	1.26	1.4	15.1	12.3	13.65	2.76	1.31	2.04
5	25	150	140	2	148	146.3	147.1	1.73	1.62	1.7	12.9	10.4	11.17	2.51	0.78	1.65
6	20	80	90	2	142	140.8	141.4	1.22	1.04	1.17	12.7	10.2	11.48	2.5	1.28	1.89
7	20	150	115	3	135	133.7	134.4	1.28	1.07	1.22	14	11.2	12.72	2.76	1.48	2.12

8	15	80	115	4	129	127.9	128.5	1.07	0.8	1	15.8	12.9	14.73	2.94	1.87	2.41
9	25	80	115	3	144	142.5	143.2	1.55	1.43	1.52	11.9	9.5	10.35	2.39	0.84	1.62
10	20	80	140	4	138	136.7	137.4	1.27	1.09	1.22	13.4	10.9	12.13	2.54	1.27	1.91

Table 9.5: Optimization, validation and comparison of experimental data set (Total Color Difference, Expansion Ratio, Protein Digestibility, Starch Digestibility) – Single Screw Extrusion of sprouted quinoa flour

No	Moisture Content (%w.b.	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
					Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
						RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	20	150	115	0	19.8	19.0	19.6	3.32	2.85	3.21	83	81.6	82.9	81.6	80.2	81.5
2	15	80	115	1	20.8	19.9	20.6	3.44	2.95	3.33	85.5	84.0	85.4	83.8	82.3	83.7
3	20	80	140	0	20.9	20.1	20.7	3.18	2.73	3.07	82.5	81.1	82.4	81	79.6	80.9
4	20	220	140	1	21.3	20.4	21.1	3.47	2.97	3.35	85.7	84.2	85.6	84.8	83.3	84.7
5	25	150	140	2	16.8	16.1	16.7	2.88	2.47	2.78	85.1	83.9	85.0	84	82.8	83.9
6	20	80	90	2	17.9	17.2	17.8	2.95	2.53	2.85	84.3	83.0	84.2	83	81.7	82.9
7	20	150	115	3	19.2	18.4	19.0	3.38	2.90	3.27	85.6	84.2	85.5	84.8	83.4	84.7
8	15	80	115	4	20.4	19.5	20.2	3.51	3.01	3.39	86	84.5	85.9	84.3	82.8	84.2

9	25	80	115	3	15.8	15.2	15.7	2.59	2.22	2.50	83.9	82.8	83.8	83.8	82.7	83.7
10	20	80	140	4	20.1	19.3	19.9	3.3	2.83	3.19	85.5	84.1	85.4	84.7	83.3	84.6

Table 9.6: Actual and predicted values of Bulk Density, Hardness, Water Solubility Index and Water Absorption Index of sprouted quinoa – corn meal blend by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	119	118.0	118.4	1.13	1.0	1.0	16.5	16.0	16.3	3.46	3.3	3.4
2	145	143.5	144.2	1.6	1.4	1.5	13.3	12.6	13.0	2.68	2.5	2.6
3	115	114.1	114.5	0.95	0.8	0.9	17.3	16.9	17.1	3.67	3.6	3.6
4	132	130.9	131.4	1.25	1.1	1.1	13.7	13.2	13.4	2.46	2.3	2.4
5	137	135.9	136.4	1.24	1.1	1.1	13.3	12.8	13.0	2.7	2.6	2.6
6	130	128.8	129.3	1.35	1.2	1.2	13.6	13.0	13.3	2.74	2.6	2.7
7	125	124.1	124.5	1.04	0.9	0.9	12.4	11.9	12.2	3.38	3.3	3.3
8	116	115.1	115.5	0.97	0.8	0.9	13.4	13.0	13.2	3.67	3.6	3.6
9	128	126.9	127.4	1.16	1.0	1.1	15.8	15.3	15.5	3.3	3.2	3.2

10	145	143.7	144.3	1.46	1.3	1.3	12.7	12.1	12.4	2.44	2.3	2.4
11	107	106.2	106.5	0.91	0.8	0.8	19.5	19.1	19.3	3.75	3.7	3.7
12	128	127.0	127.4	1.14	1.0	1.0	14.4	13.9	14.2	3.67	3.6	3.6
13	129	127.9	128.4	1.23	1.1	1.1	12.8	12.3	12.5	3.3	3.2	3.2
14	127	125.9	126.4	1.22	1.1	1.1	14.1	13.6	13.8	3.5	3.4	3.4
15	130	128.9	129.4	1.23	1.1	1.1	12.4	11.9	12.1	3.47	3.3	3.4
16	120	119.0	119.4	1.13	1.0	1.0	13.7	13.2	13.5	3.55	3.4	3.5
17	120	119.0	119.4	1.14	1.0	1.0	16.4	15.9	16.2	3.44	3.3	3.4
18	130	128.5	129.2	1.62	1.4	1.5	12.8	12.1	12.4	2.68	2.5	2.6
19	112	111.0	111.5	1.05	0.9	1.0	17.9	17.4	17.7	3.63	3.5	3.6
20	128	126.9	127.4	1.23	1.1	1.1	13.7	13.2	13.4	2.46	2.3	2.4
21	145	143.8	144.4	1.28	1.1	1.2	12.3	11.7	12.0	2.46	2.3	2.4
22	132	130.9	131.4	1.25	1.1	1.1	14.4	13.9	14.1	2.68	2.6	2.6
23	124	123.0	123.5	1.1	1.0	1.0	12.6	12.1	12.4	3.47	3.4	3.4
24	116	115.1	115.5	1.01	0.9	0.9	12.9	12.5	12.7	3.55	3.4	3.5

25	124	122.9	123.4	1.17	1.0	1.1	13.4	12.9	13.1	3.5	3.4	3.4
26	124	122.9	123.4	1.17	1.0	1.1	13.4	12.9	13.1	3.5	3.4	3.4
27	124	122.9	123.4	1.17	1.0	1.1	13.4	12.9	13.1	3.5	3.4	3.4
28	118	117.0	117.4	1.12	1.0	1.0	16.6	16.1	16.4	3.47	3.4	3.4
29	143	141.6	142.2	1.59	1.4	1.4	13.5	12.8	13.2	2.7	2.5	2.6
30	114	113.1	113.5	0.94	0.8	0.9	17.4	17.0	17.2	3.68	3.6	3.6
31	130	128.9	129.4	1.23	1.1	1.1	13.8	13.3	13.5	2.48	2.4	2.4
32	136	134.9	135.4	1.22	1.1	1.1	13.5	13.0	13.2	2.72	2.6	2.6
33	128	126.8	127.3	1.32	1.2	1.2	13.8	13.2	13.5	2.76	2.6	2.7
34	124	123.1	123.5	1.03	0.9	0.9	12.5	12.0	12.3	3.39	3.3	3.3
35	115	114.1	114.5	0.96	0.8	0.9	13.4	13.0	13.2	3.68	3.6	3.6
36	126	125.0	125.4	1.14	1.0	1.0	16.1	15.6	15.9	3.32	3.2	3.3
37	143	141.7	142.3	1.43	1.3	1.3	12.9	12.3	12.6	2.46	2.3	2.4
38	106	105.2	105.6	0.9	0.8	0.8	19.6	19.2	19.4	3.76	3.7	3.7
39	127	126.0	126.4	1.14	1.0	1.0	14.6	14.1	14.4	3.68	3.6	3.6

40	128	126.9	127.4	1.22	1.1	1.1	12.9	12.4	12.6	3.33	3.2	3.3
41	126	124.9	125.4	1.2	1.1	1.1	14.2	13.7	13.9	3.53	3.4	3.5
42	129	127.9	128.4	1.22	1.1	1.1	12.5	12.0	12.2	3.5	3.4	3.4
43	119	118.0	118.4	1.12	1.0	1.0	13.8	13.3	13.6	3.57	3.5	3.5
44	118	117.0	117.4	1.13	1.0	1.0	16.5	16.0	16.3	3.45	3.3	3.4
45	128	126.5	127.2	1.61	1.4	1.5	12.9	12.2	12.5	2.7	2.5	2.6
46	111	110.1	110.5	1.04	0.9	0.9	18	17.5	17.8	3.64	3.5	3.6
47	127	125.9	126.4	1.22	1.1	1.1	13.8	13.3	13.5	2.48	2.4	2.4
48	143	141.9	142.4	1.25	1.1	1.1	12.5	12.0	12.2	2.48	2.4	2.4
49	130	128.9	129.4	1.23	1.1	1.1	14.6	14.1	14.3	2.7	2.6	2.6
50	123	122.0	122.5	1.09	1.0	1.0	12.7	12.2	12.5	3.5	3.4	3.4
51	115	114.1	114.5	1	0.9	0.9	13	12.6	12.8	3.57	3.5	3.5
52	123	121.9	122.4	1.16	1.0	1.1	13.5	13.0	13.2	3.54	3.4	3.5
53	123	121.9	122.4	1.16	1.0	1.1	13.5	13.0	13.2	3.54	3.4	3.5
54	123	121.9	122.4	1.16	1.0	1.1	13.5	13.0	13.2	3.54	3.4	3.5

55	117	116.0	116.4	1.11	1.0	1.0	16.7	16.2	16.5	3.48	3.4	3.4
56	142	140.6	141.2	1.58	1.4	1.4	13.6	12.9	13.3	2.71	2.6	2.6
57	113	113.0	113.0	0.03	0.0	0.0	17.5	17.5	17.5	3.69	3.7	3.7
58	129	127.9	128.4	1.22	1.1	1.1	13.9	13.4	13.6	2.49	2.4	2.4
59	134	132.9	133.4	1.21	1.1	1.1	13.6	13.1	13.3	2.73	2.6	2.7
60	127	125.8	126.4	1.3	1.1	1.2	13.9	13.3	13.6	2.77	2.6	2.7
61	123	122.1	122.5	1.02	0.9	0.9	12.6	12.2	12.4	3.4	3.3	3.3
62	114	113.1	113.5	0.95	0.8	0.9	13.5	13.1	13.3	3.69	3.6	3.6
63	124	123.0	123.4	1.13	1.0	1.0	16.2	15.7	16.0	3.33	3.2	3.3
64	142	140.7	141.3	1.41	1.2	1.3	13	12.4	12.7	2.47	2.3	2.4
65	105	104.2	104.6	0.89	0.8	0.8	19.7	19.3	19.5	3.77	3.7	3.7
66	128	127.0	127.4	1.14	1.0	1.0	14.7	14.2	14.5	3.69	3.6	3.6
67	127	125.9	126.4	1.21	1.1	1.1	13	12.5	12.7	3.34	3.2	3.3
68	125	123.9	124.4	1.19	1.0	1.1	14.3	13.8	14.0	3.54	3.4	3.5
69	128	126.9	127.4	1.21	1.1	1.1	12.6	12.1	12.3	3.52	3.4	3.4

70	118	117.0	117.4	1.11	1.0	1.0	13.9	13.4	13.7	3.58	3.5	3.5
71	117	116.0	116.4	1.12	1.0	1.0	16.6	16.1	16.4	3.46	3.3	3.4
72	127	125.5	126.2	1.6	1.4	1.5	13	12.3	12.7	2.71	2.5	2.6
73	110	109.1	109.5	1.03	0.9	0.9	18.1	17.6	17.9	3.65	3.5	3.6
74	126	124.9	125.4	1.21	1.1	1.1	13.9	13.4	13.6	2.49	2.4	2.4
75	142	140.9	141.4	1.24	1.1	1.1	12.6	12.1	12.3	2.49	2.4	2.4
76	129	127.9	128.4	1.22	1.1	1.1	14.7	14.2	14.4	2.71	2.6	2.6
77	122	121.0	121.5	1.08	0.9	1.0	12.8	12.3	12.6	3.52	3.4	3.5
78	114	113.1	113.5	0.99	0.9	0.9	13.1	12.7	12.9	3.58	3.5	3.5
79	122	121.0	121.4	1.15	1.0	1.0	13.6	13.1	13.3	3.55	3.4	3.5
80	122	121.0	121.4	1.15	1.0	1.0	13.6	13.1	13.3	3.55	3.4	3.5
81	122	121.0	121.4	1.15	1.0	1.0	13.6	13.1	13.3	3.55	3.4	3.5
82	116	115.0	115.5	1.1	1.0	1.0	16.8	16.3	16.6	3.49	3.4	3.4
83	141	139.6	140.2	1.57	1.4	1.4	13.7	13.0	13.4	2.72	2.6	2.6
84	112	111.2	111.5	0.92	0.8	0.8	17.6	17.2	17.4	3.7	3.6	3.6

85	128	126.9	127.4	1.21	1.1	1.1	14	13.5	13.7	2.5	2.4	2.4
86	133	131.9	132.4	1.2	1.1	1.1	13.7	13.2	13.4	2.74	2.6	2.7
87	126	124.8	125.4	1.29	1.1	1.2	14	13.4	13.7	2.78	2.6	2.7
88	122	121.1	121.5	1.01	0.9	0.9	12.7	12.3	12.5	3.41	3.3	3.3
89	113	112.1	112.5	0.94	0.8	0.9	13.5	13.1	13.3	3.7	3.6	3.6
90	123	122.0	122.5	1.1	1.0	1.0	16.3	15.8	16.1	3.34	3.2	3.3
91	141	139.7	140.3	1.39	1.2	1.3	13.1	12.5	12.8	2.48	2.3	2.4
92	104	103.2	103.6	0.88	0.8	0.8	19.8	19.4	19.6	3.78	3.7	3.7
93	127	126.0	126.4	1.13	1.0	1.0	14.8	14.3	14.6	3.7	3.6	3.6
94	126	124.9	125.4	1.2	1.1	1.1	13.1	12.6	12.8	3.35	3.2	3.3
95	124	122.9	123.4	1.18	1.0	1.1	14.4	13.9	14.1	3.55	3.4	3.5
96	127	125.9	126.4	1.2	1.1	1.1	12.7	12.2	12.4	3.53	3.4	3.5
97	117	116.0	116.5	1.1	1.0	1.0	14	13.5	13.8	3.59	3.5	3.5
98	116	115.0	115.4	1.11	1.0	1.0	16.7	16.2	16.5	3.47	3.4	3.4
99	126	124.6	125.2	1.59	1.4	1.4	13.1	12.4	12.8	2.72	2.6	2.6

100	109	108.1	108.5	1.02	0.9	0.9	18.2	17.8	18.0	3.66	3.6	3.6
101	125	123.9	124.4	1.2	1.1	1.1	14	13.5	13.7	2.5	2.4	2.4
102	141	139.9	140.4	1.23	1.1	1.1	12.7	12.2	12.4	2.5	2.4	2.4
103	128	126.9	127.4	1.21	1.1	1.1	14.8	14.3	14.5	2.72	2.6	2.6
104	121	120.0	120.5	1.07	0.9	1.0	12.9	12.4	12.7	3.53	3.4	3.5
105	113	112.1	112.5	0.98	0.9	0.9	13.2	12.8	13.0	3.59	3.5	3.5
106	121	120.0	120.4	1.14	1.0	1.0	13.7	13.2	13.5	3.56	3.4	3.5
107	121	120.0	120.4	1.14	1.0	1.0	13.7	13.2	13.5	3.56	3.4	3.5
108	121	120.0	120.4	1.14	1.0	1.0	13.7	13.2	13.5	3.56	3.4	3.5
109	115	114.0	114.5	1.09	1.0	1.0	16.9	16.4	16.7	3.5	3.4	3.4
110	140	138.6	139.2	1.56	1.4	1.4	13.8	13.1	13.5	2.73	2.6	2.6
111	111	110.2	110.5	0.91	0.8	0.8	17.7	17.3	17.5	3.71	3.6	3.7
112	127	125.9	126.4	1.2	1.1	1.1	14.1	13.6	13.8	2.51	2.4	2.4
113	132	130.9	131.4	1.19	1.0	1.1	13.8	13.3	13.5	2.75	2.6	2.7
114	125	123.8	124.4	1.28	1.1	1.2	14.2	13.6	13.9	2.79	2.7	2.7

115	121	120.1	120.5	1	0.9	0.9	12.8	12.4	12.6	3.42	3.3	3.4
116	112	111.2	111.5	0.93	0.8	0.8	13.5	13.1	13.3	3.71	3.6	3.7
117	122	121.0	121.4	1.11	1.0	1.0	16.4	15.9	16.2	3.35	3.2	3.3
118	140	138.8	139.3	1.37	1.2	1.2	13.2	12.6	12.9	2.49	2.4	2.4
119	102	101.2	101.6	0.87	0.8	0.8	19.9	19.5	19.7	3.79	3.7	3.7
120	126	125.0	125.4	1.13	1.0	1.0	14.9	14.4	14.7	3.71	3.6	3.6
121	125	123.9	124.4	1.19	1.0	1.1	13.2	12.7	12.9	3.36	3.2	3.3
122	123	121.9	122.4	1.17	1.0	1.1	14.5	14.0	14.2	3.56	3.4	3.5
123	126	124.9	125.4	1.19	1.0	1.1	12.8	12.3	12.5	3.54	3.4	3.5
124	116	115.0	115.5	1.09	1.0	1.0	14.1	13.6	13.9	3.6	3.5	3.5
125	115	114.0	114.5	1.1	1.0	1.0	16.8	16.3	16.6	3.48	3.4	3.4
126	125	123.6	124.2	1.58	1.4	1.4	13.2	12.5	12.9	2.73	2.6	2.6
127	107	106.1	106.5	1.01	0.9	0.9	18.3	17.9	18.1	3.67	3.6	3.6
128	124	122.9	123.4	1.19	1.0	1.1	14.1	13.6	13.8	2.51	2.4	2.4
129	140	138.9	139.4	1.22	1.1	1.1	12.8	12.3	12.5	2.51	2.4	2.4

130	127	125.9	126.4	1.2	1.1	1.1	14.9	14.4	14.6	2.73	2.6	2.7
131	120	119.0	119.5	1.06	0.9	1.0	13	12.5	12.8	3.54	3.4	3.5
132	112	111.1	111.5	0.97	0.8	0.9	13.3	12.9	13.1	3.6	3.5	3.5
133	120	119.0	119.4	1.13	1.0	1.0	13.8	13.3	13.6	3.57	3.5	3.5
134	120	119.0	119.4	1.13	1.0	1.0	13.8	13.3	13.6	3.57	3.5	3.5
135	120	119.0	119.4	1.13	1.0	1.0	13.8	13.3	13.6	3.57	3.5	3.5

Table 9.7: Actual and predicted values of Total Color Difference, Expansion Ratio, Starch Digestibility and Protein Digestibility of sprouted quinoa – corn meal blend by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	18.5	18.4	18.5	3.59	3.31	3.45	81.1	80.9	81.0	81.1	80.9	81.0
2	16	15.8	16.0	3.11	2.71	2.91	80.8	80.5	80.7	79.8	79.5	79.7
3	16.9	16.8	16.9	3.71	3.47	3.59	83.1	82.9	83.0	83.3	83.1	83.2
4	17.6	17.5	17.6	3.15	2.84	2.99	82.3	82.1	82.2	81.6	81.4	81.5
5	20.6	20.5	20.6	3.2	2.89	3.05	83.6	83.4	83.5	83.2	83.0	83.1
6	17.6	17.5	17.6	3.35	3.01	3.18	83.8	83.6	83.7	83.8	83.6	83.7
7	14.8	14.7	14.8	3.55	3.29	3.42	78.5	78.3	78.4	78.4	78.2	78.3
8	16.7	16.6	16.7	3.67	3.43	3.55	79.4	79.2	79.3	80.3	80.1	80.2
9	21.4	21.3	21.4	3.53	3.24	3.39	83.7	83.5	83.6	84.5	84.3	84.4

10	16.7	16.5	16.7	2.75	2.39	2.57	84.1	83.8	84.0	83	82.7	82.9
11	14.5	14.4	14.5	3.88	3.65	3.77	78.8	78.6	78.7	77.6	77.4	77.5
12	14.8	14.7	14.8	3.3	3.02	3.16	78.4	78.2	78.3	78.5	78.3	78.4
13	16	15.9	16.0	3.3	2.99	3.15	79.2	79.0	79.1	78.5	78.3	78.4
14	16.7	16.6	16.7	3.39	3.09	3.24	79.6	79.4	79.5	80	79.8	79.9
15	17.3	17.2	17.3	3.24	2.93	3.09	80.8	80.6	80.7	80.3	80.1	80.2
16	17.8	17.7	17.8	3.52	3.24	3.38	79	78.8	78.9	81.5	81.3	81.4
17	18.2	18.1	18.2	3.57	3.29	3.43	80.4	80.2	80.3	80.2	80.0	80.1
18	17.6	17.4	17.6	3.11	2.71	2.91	79	78.7	78.9	77.5	77.2	77.4
19	17.4	17.3	17.4	3.77	3.51	3.64	81.3	81.1	81.2	82.6	82.4	82.5
20	17.8	17.7	17.8	3.24	2.93	3.09	82.6	82.4	82.5	82	81.8	81.9
21	17.9	17.8	17.9	2.98	2.66	2.82	83.2	83.0	83.1	81.4	81.2	81.3
22	18	17.9	18.0	3.15	2.84	2.99	84.1	83.9	84.0	84.3	84.1	84.2
23	15.6	15.5	15.6	3.58	3.31	3.44	78.4	78.2	78.3	79.2	79.0	79.1
24	16.4	16.3	16.4	3.63	3.38	3.50	79.7	79.5	79.6	79.4	79.2	79.3

25	16.7	16.6	16.7	3.5	3.21	3.35	79.3	79.1	79.2	81	80.8	80.9
26	16.7	16.6	16.7	3.5	3.21	3.35	79.3	79.1	79.2	81	80.8	80.9
27	16.7	16.6	16.7	3.5	3.21	3.35	79.3	79.1	79.2	81	80.8	80.9
28	18.4	18.3	18.4	3.6	3.32	3.46	82.7	82.5	82.6	82	81.8	81.9
29	16.4	16.2	16.4	3.12	2.72	2.92	78.4	78.1	78.3	79.8	79.5	79.7
30	16.8	16.7	16.8	3.73	3.50	3.61	84	83.8	83.9	84.2	84.0	84.1
31	17.4	17.3	17.4	3.17	2.86	3.02	82.4	82.2	82.3	81.8	81.6	81.7
32	20.4	20.3	20.4	3.23	2.93	3.08	85	84.8	84.9	84.2	84.0	84.1
33	17.4	17.3	17.4	3.38	3.05	3.22	85.2	85.0	85.1	84.7	84.5	84.6
34	14.9	14.8	14.9	3.56	3.30	3.43	78.8	78.6	78.7	78.5	78.3	78.4
35	17	16.9	17.0	3.68	3.44	3.56	79.5	79.3	79.4	80.4	80.2	80.3
36	21.2	21.1	21.2	3.55	3.27	3.41	85.5	85.3	85.4	85.3	85.1	85.2
37	16.5	16.3	16.5	2.78	2.42	2.60	85.1	84.8	85.0	84.1	83.8	84.0
38	14.4	14.3	14.4	3.9	3.68	3.79	80.1	79.9	80.0	79.8	79.6	79.7
39	14.9	14.8	14.9	3.33	3.05	3.19	78.5	78.3	78.4	78.8	78.6	78.7

40	16.4	16.3	16.4	3.33	3.03	3.18	79.3	79.1	79.2	78.8	78.6	78.7
41	17	16.9	17.0	3.41	3.11	3.26	79.7	79.5	79.6	80.2	80.0	80.1
42	17.5	17.4	17.5	3.27	2.97	3.12	78.4	78.2	78.3	80.6	80.4	80.5
43	18.1	18.0	18.1	3.56	3.28	3.42	79.2	79.0	79.1	81.8	81.6	81.7
44	18.1	18.0	18.1	3.58	3.30	3.44	81.7	81.5	81.6	81.5	81.3	81.4
45	17.4	17.2	17.4	3.12	2.72	2.92	79.2	78.9	79.1	77.6	77.3	77.5
46	17.3	17.2	17.3	3.78	3.52	3.65	82.9	82.7	82.8	83.9	83.7	83.8
47	18.1	18.0	18.1	3.27	2.97	3.12	82.8	82.6	82.7	82.2	82.0	82.1
48	17.7	17.6	17.7	3.02	2.71	2.86	84.2	84.0	84.1	82.9	82.7	82.8
49	17.8	17.7	17.8	3.17	2.86	3.02	85.4	85.2	85.3	85	84.8	84.9
50	15.7	15.6	15.7	3.59	3.32	3.45	78.5	78.3	78.4	79.3	79.1	79.2
51	16.5	16.4	16.5	3.64	3.39	3.52	79.8	79.6	79.7	79.5	79.3	79.4
52	17	16.9	17.0	3.53	3.24	3.39	79.5	79.3	79.4	81.1	80.9	81.0
53	17	16.9	17.0	3.53	3.24	3.39	79.5	79.3	79.4	81.1	80.9	81.0
54	17	16.9	17.0	3.53	3.24	3.39	79.5	79.3	79.4	81.1	80.9	81.0

55	18.3	18.2	18.3	3.61	3.33	3.47	82.8	82.6	82.7	82.1	81.9	82.0
56	16.5	16.3	16.5	3.13	2.74	2.93	78.5	78.2	78.4	80	79.7	79.9
57	16.7	16.7	16.7	3.75	3.74	3.75	84.1	84.1	84.1	84.3	84.3	84.3
58	17.3	17.2	17.3	3.18	2.88	3.03	82.5	82.3	82.4	81.9	81.7	81.8
59	20.3	20.2	20.3	3.24	2.94	3.09	85.1	84.9	85.0	84.3	84.1	84.2
60	17.3	17.2	17.3	3.39	3.07	3.23	85.3	85.1	85.2	84.8	84.6	84.7
61	15	14.9	15.0	3.57	3.32	3.44	78.9	78.7	78.8	78.6	78.4	78.5
62	17.1	17.0	17.1	3.69	3.45	3.57	79.6	79.4	79.5	80.5	80.3	80.4
63	21.1	21.0	21.1	3.56	3.28	3.42	85.6	85.4	85.5	85.4	85.2	85.3
64	16.4	16.2	16.4	2.79	2.44	2.61	85.2	84.9	85.1	84.2	84.0	84.1
65	14.3	14.2	14.3	3.92	3.70	3.81	80.2	80.0	80.1	80	79.8	79.9
66	15	14.9	15.0	3.34	3.06	3.20	78.6	78.4	78.5	78.9	78.7	78.8
67	16.5	16.4	16.5	3.34	3.04	3.19	79.4	79.2	79.3	78.9	78.7	78.8
68	17.1	17.0	17.1	3.42	3.12	3.27	79.8	79.6	79.7	80.3	80.1	80.2
69	17.6	17.5	17.6	3.28	2.98	3.13	78.5	78.3	78.4	80.7	80.5	80.6

70	18.2	18.1	18.2	3.57	3.29	3.43	79.3	79.1	79.2	81.9	81.7	81.8
71	18	17.9	18.0	3.59	3.31	3.45	81.8	81.6	81.7	81.6	81.4	81.5
72	17.3	17.1	17.3	3.13	2.73	2.93	79.3	79.0	79.2	77.7	77.4	77.6
73	17.2	17.1	17.2	3.8	3.54	3.67	83	82.8	82.9	84	83.8	83.9
74	18.2	18.1	18.2	3.28	2.98	3.13	82	81.8	81.9	82.3	82.1	82.2
75	17.6	17.5	17.6	3.03	2.72	2.88	84.3	84.1	84.2	83	82.8	82.9
76	17.7	17.6	17.7	3.18	2.88	3.03	85.5	85.3	85.4	85.1	84.9	85.0
77	15.8	15.7	15.8	3.6	3.33	3.47	78.6	78.4	78.5	79.4	79.2	79.3
78	16.6	16.5	16.6	3.65	3.40	3.53	80.3	80.1	80.2	79.6	79.4	79.5
79	17.1	17.0	17.1	3.54	3.25	3.40	79.6	79.4	79.5	81.2	81.0	81.1
80	17.1	17.0	17.1	3.54	3.25	3.40	79.6	79.4	79.5	81.2	81.0	81.1
81	17.1	17.0	17.1	3.54	3.25	3.40	79.6	79.4	79.5	81.2	81.0	81.1
82	18.2	18.1	18.2	3.62	3.35	3.48	82.9	82.7	82.8	82.2	82.0	82.1
83	16.6	16.4	16.6	3.14	2.75	2.94	78.6	78.3	78.5	80.1	79.8	80.0
84	16.6	16.5	16.6	3.76	3.53	3.65	84.2	84.0	84.1	84.4	84.2	84.3

85	17.2	17.1	17.2	3.19	2.89	3.04	82.6	82.4	82.5	82	81.8	81.9
86	20.2	20.1	20.2	3.25	2.95	3.10	85.2	85.0	85.1	84.4	84.2	84.3
87	17.2	17.1	17.2	3.4	3.08	3.24	85.4	85.2	85.3	84.9	84.7	84.8
88	15.1	15.0	15.1	3.58	3.33	3.45	79	78.8	78.9	78.7	78.5	78.6
89	17.2	17.1	17.2	3.7	3.47	3.58	79.7	79.5	79.6	80.6	80.4	80.5
90	21	20.9	21.0	3.57	3.30	3.43	85.7	85.5	85.6	85.5	85.3	85.4
91	16.3	16.1	16.3	2.8	2.45	2.63	85.2	84.9	85.1	84.3	84.1	84.2
92	14.2	14.1	14.2	3.94	3.72	3.83	80.3	80.1	80.2	80.1	79.9	80.0
93	15.1	15.0	15.1	3.35	3.07	3.21	78.7	78.5	78.6	79	78.8	78.9
94	16.6	16.5	16.6	3.35	3.05	3.20	79.5	79.3	79.4	79	78.8	78.9
95	17.2	17.1	17.2	3.43	3.14	3.28	80	79.8	79.9	80.4	80.2	80.3
96	17.7	17.6	17.7	3.29	2.99	3.14	78.6	78.4	78.5	80.8	80.6	80.7
97	18.3	18.2	18.3	3.58	3.31	3.44	79.4	79.2	79.3	82	81.8	81.9
98	17.9	17.8	17.9	3.6	3.32	3.46	81.9	81.7	81.8	81.7	81.5	81.6
99	17.2	17.0	17.2	3.14	2.74	2.94	79.4	79.1	79.3	77.8	77.5	77.7

100	17.1	17.0	17.1	3.81	3.56	3.68	83.1	82.9	83.0	84.1	83.9	84.0
101	18.3	18.2	18.3	3.29	2.99	3.14	83	82.8	82.9	82.4	82.2	82.3
102	17.5	17.4	17.5	3.04	2.73	2.89	84.4	84.2	84.3	83.1	82.9	83.0
103	17.6	17.5	17.6	3.19	2.89	3.04	85.6	85.4	85.5	85.2	85.0	85.1
104	15.9	15.8	15.9	3.61	3.34	3.48	78.7	78.5	78.6	79.5	79.3	79.4
105	16.7	16.6	16.7	3.66	3.42	3.54	80.6	80.4	80.5	79.7	79.5	79.6
106	17.2	17.1	17.2	3.55	3.27	3.41	79.7	79.5	79.6	81.3	81.1	81.2
107	17.2	17.1	17.2	3.55	3.27	3.41	79.7	79.5	79.6	81.3	81.1	81.2
108	17.2	17.1	17.2	3.55	3.27	3.41	79.7	79.5	79.6	81.3	81.1	81.2
109	18.1	18.0	18.1	3.63	3.36	3.49	83	82.8	82.9	82.3	82.1	82.2
110	16.7	16.5	16.7	3.15	2.76	2.96	78.7	78.4	78.6	80.2	79.9	80.1
111	16.5	16.4	16.5	3.78	3.55	3.67	84.3	84.1	84.2	84.5	84.3	84.4
112	17.1	17.0	17.1	3.2	2.90	3.05	82.7	82.5	82.6	82.1	81.9	82.0
113	20.1	20.0	20.1	3.26	2.96	3.11	85.3	85.1	85.2	84.5	84.3	84.4
114	17.1	17.0	17.1	3.41	3.09	3.25	85.5	85.3	85.4	85	84.8	84.9

115	15.2	15.1	15.2	3.59	3.34	3.47	79.1	78.9	79.0	78.8	78.6	78.7
116	17.3	17.2	17.3	3.71	3.48	3.59	79.8	79.6	79.7	80.7	80.5	80.6
117	20.9	20.8	20.9	3.58	3.30	3.44	85.8	85.6	85.7	85.6	85.4	85.5
118	16.2	16.1	16.2	2.81	2.47	2.64	85.3	85.1	85.2	84.4	84.2	84.3
119	14.1	14.0	14.1	3.97	3.75	3.86	80.4	80.2	80.3	80.2	80.0	80.1
120	15.2	15.1	15.2	3.36	3.08	3.22	78.8	78.6	78.7	79.1	78.9	79.0
121	16.7	16.6	16.7	3.36	3.06	3.21	79.6	79.4	79.5	79.1	78.9	79.0
122	17.3	17.2	17.3	3.44	3.15	3.29	80.1	79.9	80.0	80.5	80.3	80.4
123	17.8	17.7	17.8	3.3	3.00	3.15	78.7	78.5	78.6	80.2	80.0	80.1
124	18.4	18.3	18.4	3.59	3.32	3.45	79.5	79.3	79.4	82.1	81.9	82.0
125	17.8	17.7	17.8	3.62	3.35	3.48	82	81.8	81.9	81.8	81.6	81.7
126	17.1	16.9	17.1	3.15	2.76	2.95	79.5	79.2	79.4	77.9	77.6	77.8
127	17	16.9	17.0	3.83	3.58	3.70	83.2	83.0	83.1	84.2	84.0	84.1
128	18.4	18.3	18.4	3.3	3.00	3.15	83.1	82.9	83.0	82.5	82.3	82.4
129	17.4	17.3	17.4	3.05	2.75	2.90	84.5	84.3	84.4	83.2	83.0	83.1

130	17.5	17.4	17.5	3.2	2.90	3.05	85.7	85.5	85.6	85.3	85.1	85.2
131	16	15.9	16.0	3.62	3.36	3.49	78.8	78.6	78.7	79.6	79.4	79.5
132	16.8	16.7	16.8	3.67	3.43	3.55	80.7	80.5	80.6	79.8	79.6	79.7
133	17.3	17.2	17.3	3.56	3.28	3.42	79.8	79.6	79.7	81.4	81.2	81.3
134	17.3	17.2	17.3	3.56	3.28	3.42	79.8	79.6	79.7	81.4	81.2	81.3
135	17.3	17.2	17.3	3.56	3.28	3.42	79.8	79.6	79.7	81.4	81.2	81.3

Table 9.8: Regression coefficient and Root Mean Square Error – Single Screw Extrusion of sprouted quinoa – corn meal blend

Product Responses	**RSM**		**ANN**	
	R^2	**RMSE**	**R^2**	**RMSE**
Bulk Density (kg/m^3)	0.9613	0.91	0.9878	0.43
Hardness (N)	0.7637	2.64	0.9213	1.21
Water Solubility Index (%)	0.8925	2.02	0.9673	0.98
Water Absorption Index	0.8713	1.95	0.9121	0.89
Total Color Difference	0.8351	2.11	0.9651	0.87
Expansion Ratio	0.9629	1.67	0.9923	0.32
Starch Digestibility (%)	0.8918	1.54	0.9336	0.56
Protein Digestibility (%)	0.9269	1.37	0.9875	0.45

RSM – Response Surface Methodology; ANN – Artificial Neural Network, R^2- Regression Coefficient; RMSE – Root Mean Square Error

Table 9.9: Optimization, validation and comparison of experimental data set (Bulk Density, Hardness, Water Solubility Index, Water Absorption Index) – Single Screw Extrusion of sprouted quinoa - corn meal blend

No	Moisture Content	Screw Speed	Die Temperature	Corn Ratio	Germination time	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
						Actual	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**	
	%w.b	**rpm**	**°C**	**%**	**Days**		**RSM**	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**
1	20	220	115	30	2	114	113	114	0.99	0.87	0.90	13.1	12.7	12.9	3.58	3.48	3.52
2	20	150	115	15	2	122	121	121	1.15	1.01	1.05	13.6	13.1	13.3	3.55	3.43	3.48
3	20	150	115	15	0	124	123	123	1.17	1.02	1.06	13.4	12.89	13.14	3.50	3.38	3.43
4	20	220	115	0	3	128	127	127	1.21	1.06	1.10	14.8	14.27	14.54	2.72	2.60	2.65
5	15	220	115	15	4	111	110	111	0.91	0.80	0.83	17.7	17.30	17.50	3.71	3.62	3.65
6	20	80	115	30	4	120	119	119	1.06	0.93	0.96	13.0	12.54	12.77	3.54	3.43	3.48
7	15	150	90	15	3	116	115	115	1.11	0.97	1.01	16.7	16.21	16.46	3.47	3.36	3.40
8	20	80	115	30	1	123	122	122	1.09	0.95	0.99	12.7	12.22	12.46	3.50	3.39	3.43

9	25	150	115	0	1	143	142	142	1.43	1.25	1.30	12.9	12.27	12.59	2.46	2.32	2.37
10	20	150	90	0	2	134	133	133	1.21	1.06	1.10	13.6	13.1	13.3	2.73	2.61	2.66

Table 9.10: Optimization, validation and comparison of experimental data set (Total Color Difference, Expansion Ratio, Protein Digestibility, Starch Digestibility)– Single Screw Extrusion of sprouted quinoa - corn meal blend

No	Moisture Content	Screw Speed	Die Temperature	Corn Ratio	Germination time	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
						Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
	%w.b.	rpm	°C	%	Days		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	20	220	115	30	2	16.6	16.5	16.6	3.65	3.40	3.53	80.3	80.1	80.2	79.6	79.4	79.5
2	20	150	115	15	2	17.1	17.0	17.1	3.54	3.25	3.40	79.6	79.4	79.5	81.2	81.0	81.1
3	20	150	115	15	0	16.7	16.6	16.7	3.50	3.21	3.35	79.3	79.1	79.2	81.0	80.8	80.9
4	20	220	115	0	3	17.6	17.5	17.6	3.19	2.89	3.04	85.6	85.4	85.5	85.2	85.0	85.1
5	15	220	115	15	4	16.5	16.4	16.5	3.78	3.55	3.67	84.3	84.1	84.2	84.5	84.3	84.4
6	20	80	115	30	4	16.0	15.9	16.0	3.62	3.36	3.49	78.8	78.6	78.7	79.6	79.4	79.5
7	15	150	90	15	3	17.9	17.8	17.9	3.60	3.32	3.46	81.9	81.7	81.8	81.7	81.5	81.6
8	20	80	115	30	1	15.7	15.6	15.7	3.59	3.32	3.45	78.5	78.3	78.4	79.3	79.1	79.2

9	25	150	115	0	1	16.5	16.3	16.5	2.78	2.42	2.60	85.1	84.8	85.0	84.1	83.8	84.0
10	20	150	90	0	2	20.3	20.2	20.3	3.24	2.94	3.09	85.1	84.9	85.0	84.3	84.1	84.2

Table 9.11: Actual and predicted values of Bulk Density, Hardness, Water Solubility Index and Water Absorption Index of sprouted quinoa flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by twin screw extrusion

No	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	148	147	148	1.81	1.55	1.70	16.2	16.1	16.1	3.19	3.00	3.09
2	142	141	142	1.68	1.44	1.58	14.9	14.8	14.8	3.3	3.12	3.21
3	171	170	171	2.1	1.80	1.98	18.2	18.1	18.1	2.97	2.75	2.85
4	142	141	142	1.73	1.48	1.63	15.4	15.3	15.3	3.27	3.09	3.17
5	137	136	137	1.61	1.38	1.52	15.5	15.4	15.4	3.36	3.19	3.27
6	150	149	150	1.83	1.57	1.72	16.3	16.2	16.2	3.18	2.99	3.08
7	172	171	172	2.11	1.81	1.99	18.3	18.2	18.2	2.96	2.74	2.84
8	164	163	164	2.05	1.76	1.93	17.6	17.5	17.5	3.03	2.82	2.92
9	144	143	144	1.74	1.49	1.64	15.5	15.4	15.4	3.26	3.08	3.16

10	161	160	161	2.02	1.73	1.90	17.4	17.3	17.3	3.05	2.84	2.94
11	140	139	140	1.66	1.42	1.56	15.1	15.0	15.0	3.32	3.15	3.23
12	141	140	141	1.71	1.47	1.61	15.2	15.1	15.1	3.29	3.11	3.20
13	142	141	142	1.73	1.48	1.63	15.4	15.3	15.3	3.27	3.09	3.17
14	146	145	146	1.76	1.51	1.66	15.7	15.6	15.6	3.24	3.06	3.14
15	143	142	143	1.7	1.46	1.60	14.7	14.6	14.6	3.28	3.10	3.19
16	156	155	156	1.95	1.67	1.84	17.1	17.0	17.0	3.09	2.89	2.98
17	145	144	145	1.75	1.50	1.65	15.6	15.5	15.5	3.25	3.07	3.15
18	165	164	165	2.06	1.77	1.94	17.7	17.6	17.6	3.02	2.80	2.91
19	167	166	167	2.07	1.77	1.95	17.8	17.7	17.7	3.01	2.79	2.90
20	153	152	153	1.88	1.61	1.77	16.5	16.4	16.4	3.15	2.95	3.05
21	155	154	155	1.9	1.63	1.79	16.8	16.7	16.7	3.12	2.92	3.01
22	144	143	144	1.74	1.49	1.64	15.5	15.4	15.4	3.26	3.08	3.16
23	155	154	155	1.94	1.66	1.83	17	16.9	16.9	3.1	2.90	2.99
24	152	151	152	1.87	1.60	1.76	16.5	16.4	16.4	3.16	2.96	3.06

25	149	148	149	1.8	1.54	1.69	16.1	16.0	16.0	3.2	3.01	3.10
26	146	145	146	1.76	1.51	1.66	15.7	15.6	15.6	3.24	3.06	3.14
27	148	147	148	1.77	1.52	1.67	15.8	15.7	15.7	3.22	3.03	3.12
28	133	132	133	1.57	1.35	1.48	15.8	15.7	15.8	3.4	3.24	3.31
29	146	145	146	1.76	1.51	1.66	15.7	15.6	15.6	3.24	3.06	3.14
30	142	141	142	1.73	1.48	1.63	15.4	15.3	15.3	3.27	3.09	3.17
31	156	155	156	1.96	1.68	1.84	17.1	17.0	17.0	3.09	2.89	2.98
32	169	168	169	2.08	1.78	1.96	18	17.9	17.9	2.99	2.77	2.87
33	142	141	142	1.72	1.47	1.62	15.3	15.2	15.2	3.28	3.10	3.18
34	148	147	148	1.77	1.52	1.67	15.8	15.7	15.7	3.22	3.03	3.12
35	153	152	153	1.92	1.65	1.81	16.8	16.7	16.7	3.12	2.92	3.01
36	145	144	145	1.72	1.47	1.62	14.6	14.5	14.5	3.26	3.08	3.16
37	136	135	136	1.6	1.37	1.51	15.5	15.4	15.4	3.37	3.20	3.28
38	135	134	135	1.59	1.36	1.50	15.6	15.5	15.6	3.38	3.21	3.29
39	136	135	136	1.6	1.37	1.51	15.6	15.5	15.5	3.37	3.20	3.28

40	160	159	160	1.95	1.67	1.84	17.2	17.1	17.1	3.08	2.88	2.97
41	140	139	140	1.7	1.46	1.60	15.1	15.0	15.0	3.3	3.12	3.21
42	145	144	145	1.75	1.50	1.65	15.6	15.5	15.5	3.25	3.07	3.15
43	159	158	159	1.99	1.71	1.87	17.3	17.2	17.2	3.07	2.86	2.96
44	146	145	146	1.76	1.51	1.66	15.7	15.6	15.6	3.24	3.06	3.14
45	148	147	148	1.79	1.53	1.68	16	15.9	15.9	3.21	3.02	3.11
46	145	144	145	1.75	1.50	1.65	15.6	15.5	15.5	3.25	3.07	3.15
47	144	143	144	1.74	1.49	1.64	15.5	15.4	15.4	3.26	3.08	3.16
48	147	146	147	1.75	1.50	1.65	14.4	14.3	14.3	3.23	3.05	3.13
49	141	140	141	1.65	1.41	1.55	15.2	15.1	15.1	3.33	3.16	3.24
50	144	143	144	1.74	1.49	1.64	15.5	15.4	15.4	3.26	3.08	3.16
51	147	146	147	1.8	1.54	1.69	16.1	16.0	16.0	3.2	3.01	3.10
52	158	157	158	1.97	1.69	1.85	17.2	17.1	17.1	3.08	2.87	2.97
53	167	166	167	2.08	1.78	1.96	17.9	17.8	17.8	3	2.78	2.88
54	148	147	148	1.77	1.52	1.67	15.8	15.7	15.7	3.22	3.03	3.12

55	145	144	145	1.75	1.50	1.65	15.6	15.5	15.5	3.25	3.07	3.15
56	142	141	142	1.73	1.48	1.63	15.4	15.3	15.3	3.27	3.09	3.17
57	146	145	146	1.76	1.51	1.66	15.7	15.6	15.6	3.24	3.06	3.14
58	162	161	162	2.03	1.74	1.91	17.5	17.4	17.4	3.04	2.83	2.93
59	154	153	154	1.89	1.62	1.78	16.7	16.6	16.6	3.14	2.94	3.04
60	143	142	143	1.67	1.43	1.57	15	14.9	14.9	3.31	3.14	3.22
61	134	133	134	1.58	1.35	1.49	15.7	15.6	15.7	3.39	3.22	3.30
62	158	157	158	1.98	1.70	1.86	17.2	17.1	17.1	3.08	2.87	2.97
63	163	162	163	2.04	1.75	1.92	17.5	17.4	17.4	3.04	2.83	2.93
64	148	147	148	1.77	1.52	1.67	15.8	15.7	15.7	3.22	3.03	3.12
65	147	146	147	1.78	1.53	1.68	15.9	15.8	15.8	3.22	3.03	3.12
66	157	156	157	1.92	1.65	1.81	17	16.9	16.9	3.11	2.91	3.00
67	140	139	140	1.64	1.41	1.54	15.3	15.2	15.2	3.34	3.17	3.25
68	144	143	144	1.74	1.49	1.64	15.5	15.4	15.4	3.26	3.08	3.16
69	160	159	160	2.01	1.72	1.89	17.4	17.3	17.3	3.06	2.85	2.95

70	139	138	139	1.62	1.39	1.52	15.4	15.3	15.3	3.35	3.18	3.26
71	143	142	143	1.73	1.48	1.63	15.4	15.3	15.3	3.27	3.09	3.17
72	139	138	139	1.63	1.40	1.53	15.4	15.3	15.3	3.35	3.18	3.26
73	144	143	144	1.74	1.49	1.64	15.5	15.4	15.4	3.26	3.08	3.16
74	132	131	132	1.56	1.34	1.47	15.9	15.8	15.9	3.41	3.25	3.32
75	154	153	154	1.93	1.65	1.82	16.9	16.8	16.8	3.11	2.91	3.00

Table 9.12: Actual and predicted values of Total Color Difference, Expansion Ratio, Starch Digestibility and Protein Digestibility of sprouted quinoa flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by twin screw extrusion

No	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	17.8	17.5	17.69	2.57	2.35	2.46	82.1	81.9	82.0	82	81.6	81.8
2	17.4	17.2	17.30	2.71	2.50	2.61	83	82.8	82.9	82.6	82.2	82.4
3	19	18.7	18.87	2.33	2.07	2.20	80.6	80.4	80.5	80.1	79.7	79.9
4	17.6	17.4	17.49	2.67	2.46	2.56	82.7	82.5	82.6	82.4	82.0	82.2
5	17.1	16.9	17.00	2.78	2.58	2.68	83.5	83.3	83.4	82.9	82.6	82.7
6	17.9	17.6	17.79	2.56	2.34	2.45	82	81.8	81.9	81.9	81.5	81.7
7	19.1	18.8	18.97	2.31	2.05	2.18	80.5	80.3	80.4	80	79.5	79.8
8	18.7	18.4	18.57	2.39	2.14	2.26	81.1	80.9	81.0	80.6	80.2	80.4
9	18.4	18.2	18.29	2.65	2.44	2.54	81.1	80.9	81.0	80.5	80.1	80.3

10	18.4	18.1	18.28	2.41	2.16	2.29	81.3	81.1	81.2	80.7	80.3	80.5
11	17.2	17.0	17.10	2.73	2.53	2.63	83.1	82.9	83.0	82.8	82.4	82.6
12	17.5	17.3	17.40	2.69	2.48	2.59	82.9	82.7	82.8	82.7	82.3	82.5
13	17.6	17.4	17.49	2.67	2.46	2.56	82.7	82.5	82.6	82.4	82.0	82.2
14	17.3	17.0	17.19	2.64	2.42	2.53	82.4	82.2	82.3	82.1	81.7	81.9
15	17.5	17.3	17.40	2.69	2.48	2.59	82.9	82.7	82.8	82.4	82.0	82.2
16	18.3	18.0	18.18	2.47	2.23	2.35	81.5	81.3	81.4	81.2	80.8	81.0
17	17.4	17.2	17.29	2.65	2.44	2.54	82.5	82.3	82.4	82.2	81.8	82.0
18	18.8	18.5	18.67	2.38	2.13	2.25	81	80.8	80.9	80.3	79.9	80.1
19	18.7	18.4	18.57	2.37	2.12	2.24	81	80.8	80.9	80.4	80.0	80.2
20	19.4	19.1	19.28	2.52	2.29	2.40	79.6	79.4	79.5	81.9	81.5	81.7
21	18.4	18.1	18.28	2.51	2.28	2.39	81.6	81.4	81.5	81.6	81.2	81.4
22	17.5	17.3	17.39	2.66	2.45	2.55	82.6	82.4	82.5	82.3	81.9	82.1
23	18.2	17.9	18.08	2.48	2.24	2.36	81.6	81.4	81.5	81.3	80.9	81.1
24	18	17.7	17.89	2.53	2.30	2.42	82	81.8	81.9	81.8	81.4	81.6

25	17.8	17.5	17.69	2.59	2.37	2.48	82.1	81.9	82.0	82.3	81.9	82.1
26	17.3	17.0	17.19	2.64	2.42	2.53	82.4	82.2	82.3	82.1	81.7	81.9
27	18.7	18.4	18.59	2.63	2.41	2.52	80.7	80.5	80.6	80.7	80.3	80.5
28	16.8	16.6	16.70	2.82	2.63	2.72	84	83.8	83.9	83.3	83.0	83.1
29	17.3	17.0	17.19	2.64	2.42	2.53	82.4	82.2	82.3	82.1	81.7	81.9
30	17.6	17.4	17.49	2.67	2.46	2.56	82.7	82.5	82.6	82.4	82.0	82.2
31	18.2	17.9	18.08	2.46	2.22	2.34	81.6	81.4	81.5	81.2	80.8	81.0
32	18.9	18.6	18.77	2.35	2.10	2.22	80.8	80.6	80.7	80.2	79.8	80.0
33	17.6	17.4	17.49	2.68	2.47	2.57	82.8	82.6	82.7	82.6	82.2	82.4
34	18.7	18.4	18.59	2.63	2.41	2.52	80.7	80.5	80.6	80.7	80.3	80.5
35	18	17.7	17.88	2.5	2.26	2.38	81.8	81.6	81.7	81.5	81.1	81.3
36	17.6	17.4	17.49	2.67	2.46	2.56	82.7	82.5	82.6	82.2	81.8	82.0
37	17.6	17.4	17.50	2.78	2.58	2.68	81.5	81.3	81.4	81.8	81.5	81.6
38	16.9	16.7	16.80	2.8	2.61	2.70	83.7	83.5	83.6	83.1	82.8	82.9
39	17	16.8	16.90	2.79	2.59	2.69	83.6	83.4	83.5	83	82.7	82.8

40	19.9	19.6	19.78	2.48	2.24	2.36	79.1	78.9	79.0	78.2	77.8	78.0
41	17.4	17.2	17.30	2.7	2.49	2.60	83	82.8	82.9	82.8	82.4	82.6
42	17.4	17.2	17.29	2.65	2.44	2.54	82.5	82.3	82.4	82.2	81.8	82.0
43	18.4	18.1	18.28	2.44	2.20	2.32	81.4	81.2	81.3	81	80.6	80.8
44	17.3	17.0	17.19	2.64	2.42	2.53	82.4	82.2	82.3	82.1	81.7	81.9
45	17.6	17.3	17.49	2.6	2.38	2.49	82.2	82.0	82.1	82.4	82.0	82.2
46	17.4	17.2	17.29	2.65	2.44	2.54	82.5	82.3	82.4	82.2	81.8	82.0
47	17.5	17.3	17.39	2.66	2.45	2.55	82.6	82.4	82.5	82.3	81.9	82.1
48	19.3	19.1	19.19	2.64	2.43	2.53	80.1	79.9	80.0	80.2	79.8	80.0
49	17.3	17.1	17.20	2.74	2.54	2.64	83	82.8	82.9	82.7	82.3	82.5
50	17.5	17.3	17.39	2.66	2.45	2.55	82.6	82.4	82.5	82.3	81.9	82.1
51	17.7	17.4	17.59	2.58	2.36	2.47	82.2	82.0	82.1	82.1	81.7	81.9
52	19.2	18.9	19.08	2.45	2.21	2.33	80.1	79.9	80.0	78.6	78.2	78.4
53	20.2	19.9	20.07	2.36	2.11	2.23	79.1	78.9	79.0	77.7	77.3	77.5
54	18.7	18.4	18.59	2.63	2.41	2.52	80.7	80.5	80.6	80.7	80.3	80.5

55	17.4	17.2	17.29	2.65	2.44	2.54	82.5	82.3	82.4	82.2	81.8	82.0
56	17.6	17.4	17.49	2.67	2.46	2.56	82.7	82.5	82.6	82.4	82.0	82.2
57	17.3	17.0	17.19	2.64	2.42	2.53	82.4	82.2	82.3	82.1	81.7	81.9
58	19.8	19.5	19.68	2.41	2.16	2.29	79.6	79.4	79.5	78.2	77.8	78.0
59	18.2	17.9	18.08	2.52	2.29	2.40	81.8	81.6	81.7	81.7	81.3	81.5
60	18.8	18.6	18.70	2.72	2.52	2.62	80.7	80.5	80.6	80.6	80.2	80.4
61	16.8	16.6	16.70	2.81	2.62	2.71	83.8	83.6	83.7	83.2	82.9	83.0
62	18.3	18.0	18.18	2.45	2.21	2.33	81.5	81.3	81.4	81.1	80.7	80.9
63	18.6	18.3	18.48	2.4	2.15	2.28	81.2	81.0	81.1	80.5	80.1	80.3
64	18.7	18.4	18.59	2.63	2.41	2.52	80.7	80.5	80.6	80.7	80.3	80.5
65	17.5	17.2	17.39	2.62	2.40	2.51	82.3	82.1	82.2	82.5	82.1	82.3
66	18.5	18.2	18.38	2.5	2.26	2.38	81.5	81.3	81.4	81.4	81.0	81.2
67	17.1	16.9	17.00	2.75	2.55	2.65	83.1	82.9	83.0	82.8	82.4	82.6
68	17.5	17.3	17.39	2.66	2.45	2.55	82.6	82.4	82.5	82.3	81.9	82.1
69	18.5	18.2	18.38	2.43	2.18	2.31	81.3	81.1	81.2	80.8	80.4	80.6

70	18.4	18.2	18.30	2.76	2.56	2.66	81.1	80.9	81.0	81.1	80.8	80.9
71	17.7	17.5	17.59	2.67	2.46	2.56	82.7	82.5	82.6	82.5	82.1	82.3
72	17	16.8	16.90	2.76	2.56	2.66	83.2	83.0	83.1	82.9	82.6	82.7
73	17.5	17.3	17.39	2.66	2.45	2.55	82.6	82.4	82.5	82.3	81.9	82.1
74	16.7	16.5	16.60	2.83	2.64	2.73	84.1	83.9	84.0	83.4	83.1	83.2
75	18.1	17.8	17.98	2.49	2.25	2.37	81.7	81.5	81.6	81.4	81.0	81.2

Table 9.13: Regression coefficient and Root Mean Square Error – Twin Screw Extrusion of sprouted quinoa flour

Product Responses	**RSM**		**ANN**	
	R^2	**RMSE**	**R^2**	**RMSE**
Bulk Density (kg/m^3)	0.9913	2.04	0.9999	0.89
Hardness (N)	0.9969	1.22	0.9999	0.69
Water Solubility Index (%)	0.9534	1.36	0.9889	0.73
Water Absorption Index	0.9962	1.17	0.9992	0.53
Total Color Difference	0.9745	1.34	0.9959	0.83
Expansion Ratio	0.9969	1.76	0.9995	0.97
Starch Digestibility (%)	0.9933	1.63	0.9989	0.95
Protein Digestibility (%)	0.9202	1.66	0.9798	0.88

RSM – Response Surface Methodology; ANN – Artificial Neural Network, R^2- Regression Coefficient; RMSE – Root Mean Square Error

Table 9.14: Optimization, validation and comparison of experimental data set (Bulk Density, Hardness, Water Solubility Index, Water Absorption Index) – Twin Screw Extrusion of sprouted quinoa flour

No	Moisture Content (%w.b.)	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
					Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
						RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	25	110	80	2	164	163	164	2.05	1.76	1.93	17.6	17.5	17.5	3.03	2.82	2.92
2	15	80	150	4	140	139	140	1.66	1.42	1.56	15.1	15.0	15.0	3.32	3.15	3.23
3	15	80	150	2	143	142	143	1.70	1.46	1.60	14.7	14.6	14.6	3.28	3.10	3.19
4	20	80	220	0	153	152	153	1.88	1.61	1.77	16.5	16.4	16.4	3.15	2.95	3.05
5	25	80	150	3	169	168	169	2.08	1.78	1.96	18.0	17.9	17.9	2.99	2.77	2.87
6	15	110	220	3	135	134	135	1.59	1.36	1.50	15.6	15.5	15.6	3.38	3.21	3.29
7	20	80	80	0	160	159	160	1.95	1.67	1.84	17.2	17.1	17.1	3.08	2.88	2.97

8	20	110	150	2	145	144	145	1.75	1.50	1.65	15.6	15.5	15.5	3.25	3.07	3.15
9	25	80	150	0	175	174	175	2.14	1.83	2.01	18.5	18.4	18.4	2.93	2.71	2.81
10	25	110	220	0	162	161	162	2.03	1.74	1.91	17.5	17.4	17.4	3.04	2.83	2.93

Table 9.15: Optimization, validation and comparison of experimental data set (Total Color Difference, Expansion Ratio, Protein Digestibility, Starch Digestibility) – Twin Screw Extrusion of sprouted quinoa flour

No	Moisture Content (%w.b.)	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
					Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
						RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	25	110	80	2	18.7	18.4	18.6	2.39	2.14	2.26	81.1	80.9	81.0	80.6	80.2	80.4
2	15	80	150	4	17.2	17.0	17.1	2.73	2.53	2.63	83.1	82.9	83.0	82.8	82.4	82.6
3	15	80	150	2	17.5	17.3	17.4	2.69	2.48	2.59	82.9	82.7	82.8	82.4	82.0	82.2
4	20	80	220	0	19.4	19.1	19.3	2.52	2.29	2.40	79.6	79.4	79.5	81.9	81.5	81.7
5	25	80	150	3	18.9	18.6	18.8	2.35	2.10	2.22	80.8	80.6	80.7	80.2	79.8	80.0
6	15	110	220	3	16.9	16.7	16.8	2.80	2.61	2.70	83.7	83.5	83.6	83.1	82.8	82.9

7	20	80	80	0	19.9	19.6	19.8	2.48	2.24	2.36	79.1	78.9	79.0	78.2	77.8	78.0
8	20	110	150	2	17.4	17.2	17.3	2.65	2.44	2.54	82.5	82.3	82.4	82.2	81.8	82.0
9	25	80	150	0	20.8	20.5	20.7	2.28	2.02	2.15	78.4	78.2	78.3	77.1	76.6	76.9
10	25	110	220	0	19.8	19.5	19.7	2.41	2.16	2.29	79.6	79.4	79.5	78.2	77.8	78.0

Table 9.16: Actual and predicted values of Bulk Density, Hardness, Water Solubility Index and Water Absorption Index of sprouted proso millet flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	120	119	120	1.08	0.90	1.02	17.7	17.5	17.63	4.28	4.13	4.22
2	137	136	137	1.3	1.08	1.22	14.8	14.5	14.72	4.02	3.85	3.95
3	105	104	105	1.05	0.88	0.99	18.3	18.1	18.23	4.37	4.23	4.31
4	126	125	126	1.16	0.97	1.09	16	15.8	15.93	4.1	3.94	4.04
5	112	111	112	1.13	0.94	1.06	17	16.8	16.93	4.25	4.10	4.19
6	131	130	131	1.24	1.03	1.17	15.3	15.0	15.22	4	3.83	3.93
7	107	106	107	1.06	0.88	1.00	18	17.8	17.93	4.31	4.17	4.25
8	129	128	129	1.19	0.99	1.12	15.8	15.6	15.72	4.07	3.91	4.00
9	131	130	131	1.27	1.06	1.20	16.2	15.9	16.12	4.1	3.93	4.03

10	123	122	123	1.19	0.99	1.12	17.2	17.0	17.12	4.17	4.01	4.10
11	124	123	124	1.18	0.98	1.11	16.6	16.4	16.52	4.14	3.98	4.07
12	115	114	115	1.09	0.91	1.03	17.8	17.6	17.73	4.25	4.10	4.19
13	121	120	121	1.12	0.93	1.05	17.5	17.3	17.43	4.22	4.07	4.16
14	121	120	121	1.12	0.93	1.05	17.5	17.3	17.43	4.22	4.07	4.16
15	121	120	121	1.12	0.93	1.05	17.5	17.3	17.43	4.22	4.07	4.16
16	121	120	121	1.12	0.93	1.05	17.5	17.3	17.43	4.22	4.07	4.16
17	121	120	121	1.12	0.93	1.05	17.5	17.3	17.43	4.22	4.07	4.16
18	119	118	119	1.07	0.89	1.01	17.8	17.6	17.73	4.29	4.15	4.23
19	135	134	135	1.28	1.07	1.20	15	14.7	14.92	4.03	3.86	3.96
20	104	103	104	1.04	0.87	0.98	18.4	18.2	18.33	4.38	4.24	4.32
21	125	124	125	1.15	0.96	1.08	16.1	15.9	16.03	4.11	3.96	4.05
22	111	110	111	1.12	0.93	1.05	17.1	16.9	17.03	4.26	4.11	4.20
23	130	129	130	1.23	1.03	1.16	15.5	15.2	15.42	4.01	3.84	3.94
24	105	104	105	1.05	0.88	0.99	18.1	17.9	18.03	4.32	4.18	4.26

25	127	126	127	1.18	0.98	1.11	15.9	15.7	15.82	4.08	3.92	4.01
26	130	129	130	1.25	1.04	1.18	16.4	16.1	16.32	4.11	3.94	4.04
27	122	121	122	1.18	0.98	1.11	17.3	17.1	17.22	4.18	4.02	4.11
28	123	122	123	1.16	0.97	1.09	16.8	16.6	16.73	4.15	3.99	4.09
29	114	113	114	1.08	0.90	1.02	17.9	17.7	17.83	4.26	4.11	4.20
30	119	118	119	1.11	0.93	1.04	17.6	17.4	17.53	4.23	4.08	4.17
31	119	118	119	1.11	0.93	1.04	17.6	17.4	17.53	4.23	4.08	4.17
32	119	118	119	1.11	0.93	1.04	17.6	17.4	17.53	4.23	4.08	4.17
33	119	118	119	1.11	0.93	1.04	17.6	17.4	17.53	4.23	4.08	4.17
34	119	118	119	1.11	0.93	1.04	17.6	17.4	17.53	4.23	4.08	4.17
35	118	117	118	1.06	0.88	1.00	18	17.8	17.93	4.3	4.16	4.24
36	133	132	133	1.27	1.06	1.20	15.2	14.9	15.12	4.04	3.87	3.97
37	108	107	108	1.03	0.86	0.97	18.5	18.3	18.43	4.39	4.25	4.33
38	124	123	124	1.14	0.95	1.07	16.2	16.0	16.13	4.12	3.97	4.06
39	110	109	110	1.11	0.93	1.04	17.3	17.1	17.23	4.27	4.12	4.21

40	129	128	129	1.21	1.01	1.14	15.7	15.4	15.62	4.01	3.85	3.94
41	104	103	104	1.04	0.87	0.98	18.2	18.0	18.13	4.34	4.20	4.28
42	126	125	126	1.17	0.98	1.10	16	15.8	15.93	4.08	3.92	4.02
43	129	128	129	1.23	1.03	1.16	16.5	16.2	16.42	4.12	3.95	4.05
44	121	120	121	1.17	0.98	1.10	17.4	17.2	17.33	4.19	4.03	4.13
45	122	121	122	1.14	0.95	1.07	17.1	16.9	17.03	4.16	4.01	4.10
46	112	111	112	1.07	0.89	1.01	18	17.8	17.93	4.27	4.13	4.21
47	117	116	117	1.1	0.92	1.04	17.7	17.5	17.63	4.24	4.09	4.18
48	117	116	117	1.1	0.92	1.04	17.7	17.5	17.63	4.24	4.09	4.18
49	117	116	117	1.1	0.92	1.04	17.7	17.5	17.63	4.24	4.09	4.18
50	117	116	117	1.1	0.92	1.04	17.7	17.5	17.63	4.24	4.09	4.18
51	117	116	117	1.1	0.92	1.04	17.7	17.5	17.63	4.24	4.09	4.18
52	116	115	116	1.05	0.88	0.99	18.1	17.9	18.03	4.31	4.17	4.25
53	131	130	131	1.25	1.04	1.18	15.4	15.1	15.32	4.05	3.88	3.98
54	102	101	102	1.02	0.85	0.96	18.6	18.4	18.53	4.4	4.26	4.34

55	122	121	122	1.13	0.94	1.06	16.3	16.1	16.23	4.13	3.98	4.07
56	109	108	109	1.09	0.91	1.03	17.5	17.3	17.43	4.28	4.13	4.22
57	128	127	128	1.2	1.00	1.13	15.8	15.6	15.72	4.02	3.86	3.95
58	103	102	103	1.03	0.86	0.97	18.3	18.1	18.23	4.35	4.21	4.29
59	125	124	125	1.16	0.97	1.09	16.1	15.9	16.03	4.09	3.93	4.03
60	127	126	127	1.21	1.01	1.14	16.6	16.3	16.52	4.13	3.97	4.06
61	120	119	120	1.16	0.97	1.09	17.5	17.3	17.43	4.2	4.04	4.14
62	121	120	121	1.12	0.93	1.05	17.2	17.0	17.13	4.17	4.02	4.11
63	110	109	110	1.05	0.88	0.99	18.1	17.9	18.03	4.28	4.14	4.22
64	116	115	116	1.09	0.91	1.03	17.8	17.6	17.73	4.25	4.10	4.19
65	116	115	116	1.09	0.91	1.03	17.8	17.6	17.73	4.25	4.10	4.19
66	116	115	116	1.09	0.91	1.03	17.8	17.6	17.73	4.25	4.10	4.19
67	116	115	116	1.09	0.91	1.03	17.8	17.6	17.73	4.25	4.10	4.19
68	116	115	116	1.09	0.91	1.03	17.8	17.6	17.73	4.25	4.10	4.19
69	114	113	114	1.04	0.87	0.98	18.2	18.0	18.13	4.32	4.18	4.26

70	129	128	129	1.23	1.03	1.16	15.5	15.2	15.42	4.06	3.89	3.99
71	101	100	101	1.01	0.84	0.95	18.7	18.5	18.64	4.41	4.27	4.35
72	121	120	121	1.12	0.93	1.05	16.4	16.2	16.33	4.14	3.99	4.08
73	107	106	107	1.07	0.89	1.01	17.7	17.5	17.63	4.29	4.15	4.23
74	127	126	127	1.18	0.98	1.11	15.9	15.7	15.82	4.03	3.87	3.96
75	102	101	102	1.03	0.86	0.97	18.5	18.3	18.43	4.36	4.22	4.30

Table 9.17: Actual and predicted values of Total Color Difference, Expansion Ratio, Starch Digestibility and Protein Digestibility of sprouted proso millet flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	17.1	17.0	17.0	3.65	3.52	3.62	84.8	84.6	84.7	84.7	84.5	84.6
2	15.9	15.8	15.8	3.35	3.20	3.31	79.1	78.9	79.0	81.4	81.2	81.3
3	13.5	13.4	13.4	3.71	3.59	3.68	85.8	85.6	85.7	85.6	85.4	85.5
4	14.5	14.4	14.4	3.45	3.31	3.41	80.1	79.9	80.0	82.5	82.3	82.4
5	14.7	14.6	14.6	3.58	3.45	3.54	84.4	84.2	84.3	84.1	83.9	84.0
6	15.8	15.7	15.7	3.28	3.13	3.24	78.2	78.0	78.1	80.9	80.7	80.8
7	15.3	15.2	15.2	3.69	3.57	3.66	85.3	85.1	85.2	85.1	84.9	85.0
8	16.2	16.1	16.1	3.4	3.26	3.36	79.8	79.6	79.7	82.1	81.9	82.0
9	16.5	16.4	16.4	3.45	3.30	3.41	81.4	81.2	81.3	82.1	81.9	82.0

10	13	12.9	12.9	3.56	3.42	3.52	82.7	82.5	82.6	83.6	83.4	83.5
11	16	15.9	15.9	3.52	3.38	3.48	82.2	82.0	82.1	82.9	82.7	82.8
12	14.1	14.0	14.0	3.63	3.50	3.60	83.5	83.3	83.4	84.8	84.6	84.7
13	16	15.9	15.9	3.61	3.48	3.57	83.1	82.9	83.0	84.1	83.9	84.0
14	16	15.9	15.9	3.61	3.48	3.57	83.1	82.9	83.0	84.1	83.9	84.0
15	16	15.9	15.9	3.61	3.48	3.57	83.1	82.9	83.0	84.1	83.9	84.0
16	16	15.9	15.9	3.61	3.48	3.57	83.1	82.9	83.0	84.1	83.9	84.0
17	16	15.9	15.9	3.61	3.48	3.57	83.1	82.9	83.0	84.1	83.9	84.0
18	16.9	16.8	16.8	3.66	3.53	3.63	85.7	85.5	85.6	87	86.8	86.9
19	15.8	15.7	15.7	3.36	3.21	3.32	81.8	81.6	81.7	84	83.8	83.9
20	13.7	13.6	13.6	3.72	3.60	3.69	86.3	86.1	86.2	87.4	87.2	87.3
21	14.6	14.5	14.5	3.46	3.32	3.42	82.4	82.2	82.3	84.8	84.6	84.7
22	14.9	14.8	14.8	3.6	3.47	3.56	85.3	85.1	85.2	86.6	86.4	86.5
23	15.9	15.8	15.8	3.3	3.16	3.26	81.3	81.1	81.2	83.6	83.4	83.5
24	15.4	15.3	15.3	3.7	3.58	3.67	86.1	85.9	86.0	87.2	87.0	87.1

25	16.3	16.2	16.2	3.42	3.28	3.38	82	81.8	81.9	84.4	84.2	84.3
26	16.3	16.2	16.2	3.47	3.32	3.43	83.5	83.3	83.4	85.3	85.1	85.2
27	13.1	13.0	13.0	3.58	3.44	3.54	83.9	83.7	83.8	86.4	86.2	86.3
28	15.9	15.8	15.8	3.53	3.39	3.49	84	83.8	83.9	85.9	85.7	85.8
29	14.2	14.1	14.1	3.65	3.52	3.62	84.7	84.5	84.6	87.1	86.9	87.0
30	16.1	16.0	16.0	3.62	3.49	3.59	84.4	84.2	84.3	86.9	86.7	86.8
31	16.1	16.0	16.0	3.62	3.49	3.59	84.4	84.2	84.3	86.9	86.7	86.8
32	16.1	16.0	16.0	3.62	3.49	3.59	84.4	84.2	84.3	86.9	86.7	86.8
33	16.1	16.0	16.0	3.62	3.49	3.59	84.4	84.2	84.3	86.9	86.7	86.8
34	16.1	16.0	16.0	3.62	3.49	3.59	84.4	84.2	84.3	86.9	86.7	86.8
35	16.8	16.7	16.7	3.67	3.55	3.64	85.8	85.6	85.7	87.1	86.9	87.0
36	15.7	15.6	15.6	3.38	3.23	3.34	82	81.8	81.9	84.1	83.9	84.0
37	13.9	13.8	13.8	3.73	3.61	3.70	86.4	86.2	86.3	87.5	87.3	87.4
38	14.7	14.6	14.6	3.48	3.35	3.44	82.5	82.3	82.4	85.1	84.9	85.0
39	15	14.9	14.9	3.62	3.49	3.59	85.5	85.3	85.4	86.8	86.6	86.7

40	16	15.9	15.9	3.31	3.17	3.27	81.4	81.2	81.3	83.7	83.5	83.6
41	15.6	15.5	15.5	3.71	3.59	3.68	86.2	86.0	86.1	87.4	87.2	87.3
42	16.4	16.3	16.3	3.43	3.29	3.39	82.1	81.9	82.0	84.5	84.3	84.4
43	16.2	16.1	16.1	3.49	3.35	3.45	83.6	83.4	83.5	85.5	85.3	85.4
44	13.3	13.2	13.2	3.59	3.45	3.55	84.1	83.9	84.0	86.6	86.4	86.5
45	15.8	15.7	15.7	3.55	3.42	3.51	84.1	83.9	84.0	86	85.8	85.9
46	14.4	14.3	14.3	3.67	3.54	3.64	84.8	84.6	84.7	87.2	87.0	87.1
47	16.2	16.1	16.1	3.63	3.50	3.60	84.5	84.3	84.4	87	86.8	86.9
48	16.2	16.1	16.1	3.63	3.50	3.60	84.5	84.3	84.4	87	86.8	86.9
49	16.2	16.1	16.1	3.63	3.50	3.60	84.5	84.3	84.4	87	86.8	86.9
50	16.2	16.1	16.1	3.63	3.50	3.60	84.5	84.3	84.4	87	86.8	86.9
51	16.2	16.1	16.1	3.63	3.50	3.60	84.5	84.3	84.4	87	86.8	86.9
52	16.6	16.5	16.5	3.69	3.57	3.66	86	85.8	85.9	87.3	87.1	87.2
53	15.6	15.5	15.5	3.4	3.25	3.36	82.1	81.9	82.0	84.3	84.1	84.2
54	14.1	14.0	14.0	3.74	3.62	3.71	86.5	86.3	86.4	87.6	87.4	87.5

55	14.8	14.7	14.7	3.49	3.36	3.45	82.7	82.5	82.6	85.2	85.0	85.1
56	15.2	15.1	15.1	3.64	3.51	3.61	85.6	85.4	85.5	86.9	86.7	86.8
57	16.1	16.0	16.0	3.33	3.19	3.29	81.6	81.4	81.5	83.9	83.7	83.8
58	15.7	15.6	15.6	3.72	3.60	3.69	86.3	86.1	86.2	87.5	87.3	87.4
59	16.5	16.4	16.4	3.45	3.31	3.41	82.3	82.1	82.2	84.6	84.4	84.5
60	16	15.9	15.9	3.51	3.37	3.47	83.8	83.6	83.7	85.7	85.5	85.6
61	13.4	13.3	13.3	3.6	3.46	3.56	84.3	84.1	84.2	86.8	86.6	86.7
62	15.7	15.6	15.6	3.56	3.43	3.52	84.2	84.0	84.1	86.1	85.9	86.0
63	14.5	14.4	14.4	3.68	3.56	3.65	85	84.8	84.9	87.3	87.1	87.2
64	16.3	16.2	16.2	3.64	3.51	3.61	84.6	84.4	84.5	87.1	86.9	87.0
65	16.3	16.2	16.2	3.64	3.51	3.61	84.6	84.4	84.5	87.1	86.9	87.0
66	16.3	16.2	16.2	3.64	3.51	3.61	84.6	84.4	84.5	87.1	86.9	87.0
67	16.3	16.2	16.2	3.64	3.51	3.61	84.6	84.4	84.5	87.1	86.9	87.0
68	16.3	16.2	16.2	3.64	3.51	3.61	84.6	84.4	84.5	87.1	86.9	87.0
69	16.4	16.3	16.3	3.71	3.59	3.68	86.2	86.0	86.1	87.4	87.2	87.3

70	15.5	15.4	15.4	3.41	3.27	3.37	82.2	82.0	82.1	84.5	84.3	84.4
71	14.2	14.1	14.1	3.75	3.63	3.72	86.6	86.4	86.5	87.7	87.5	87.6
72	14.9	14.8	14.8	3.51	3.38	3.47	82.8	82.6	82.7	85.3	85.1	85.2
73	15.4	15.3	15.3	3.66	3.53	3.63	85.8	85.6	85.7	87.1	86.9	87.0
74	16.3	16.2	16.2	3.35	3.21	3.31	81.8	81.6	81.7	84.1	83.9	84.0
75	15.9	15.8	15.8	3.73	3.61	3.70	86.4	86.2	86.3	87.6	87.4	87.5

Table 9.18: Regression coefficient and Root Mean Square Error – Single Screw Extrusion of sprouted proso millet flour

Product Responses	**RSM**		**ANN**	
	R^2	**RMSE**	**R^2**	**RMSE**
Bulk Density (kg/m^3)	0.9817	2.03	0.9912	0.94
Hardness (N)	0.9731	1.21	0.9964	0.74
Water Solubility Index (%)	0.9859	1.35	0.9978	0.78
Water Absorption Index	0.9992	1.16	0.9999	0.58
Total Color Difference	0.9575	1.33	0.9879	0.88
Expansion Ratio	0.9924	1.75	0.9979	1.02
Starch Digestibility (%)	0.9961	1.62	0.9999	1
Protein Digestibility (%)	0.9922	1.65	0.9999	0.93

RSM – Response Surface Methodology; ANN – Artificial Neural Network, R^2- Regression Coefficient; RMSE – Root Mean Square Error

Table 9.19: Optimization, validation and comparison of experimental data set (Bulk Density, Hardness, Water Solubility Index, Water Absorption Index) – Single Screw Extrusion of sprouted proso millet flour

No	Moisture Content (%w.b.)	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Bulk Density kg/m³			Hardness N			Water Solubility Index %			Water Absorption Index		
					Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
						RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	25	80	115	0	137	136	137	1.3	1.1	1.2	14.8	14.5	14.7	4.02	3.85	3.95
2	25	150	140	0	129	128	129	1.19	1.0	1.1	15.8	15.6	15.7	4.07	3.91	4.00
3	15	220	115	1	104	103	104	1.04	0.9	1.0	18.4	18.2	18.3	4.38	4.24	4.32
4	25	150	140	1	127	126	127	1.18	1.0	1.1	15.9	15.7	15.8	4.08	3.92	4.01
5	20	220	90	2	121	120	121	1.17	1.0	1.1	17.4	17.2	17.3	4.19	4.03	4.13
6	15	220	115	3	102	101	102	1.02	0.9	1.0	18.6	18.4	18.5	4.4	4.26	4.34
7	20	80	90	3	127	126	127	1.21	1.0	1.1	16.6	16.3	16.5	4.13	3.97	4.06
8	20	150	115	3	116	115	116	1.09	0.9	1.0	17.8	17.6	17.7	4.25	4.10	4.19

9	25	150	90	4	127	126	127	1.18	1.0	1.1	15.9	15.7	15.8	4.03	3.87	3.96
10	25	80	115	0	137	136	137	1.3	1.1	1.2	14.8	14.5	14.7	4.02	3.85	3.95

Table 9.20: Optimization, validation and comparison of experimental data set (Total Color Difference, Expansion Ratio, Protein Digestibility, Starch Digestibility) – Single Screw Extrusion of sprouted proso millet flour

No	Moisture Content (%w.b.)	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
					Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
						RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	25	80	115	0	15.9	15.8	15.8	3.35	3.20	3.31	79.1	78.9	79.0	81.4	81.2	81.3
2	25	150	140	0	16.2	16.1	16.1	3.4	3.26	3.36	79.8	79.6	79.7	82.1	81.9	82.0
3	15	220	115	1	13.7	13.6	13.6	3.72	3.60	3.69	86.3	86.1	86.2	87.4	87.2	87.3
4	25	150	140	1	16.3	16.2	16.2	3.42	3.28	3.38	82	81.8	81.9	84.4	84.2	84.3
5	20	220	90	2	13.3	13.2	13.2	3.59	3.45	3.55	84.1	83.9	84.0	86.6	86.4	86.5
6	15	220	115	3	14.1	14.0	14.0	3.74	3.62	3.71	86.5	86.3	86.4	87.6	87.4	87.5
7	20	80	90	3	16	15.9	15.9	3.51	3.37	3.47	83.8	83.6	83.7	85.7	85.5	85.6
8	20	150	115	3	16.3	16.2	16.2	3.64	3.51	3.61	84.6	84.4	84.5	87.1	86.9	87.0

9	25	150	90	4	16.3	16.2	16.2	3.35	3.21	3.31	81.8	81.6	81.7	84.1	83.9	84.0
10	25	80	115	0	15.9	15.8	15.8	3.35	3.20	3.31	79.1	78.9	79.0	81.4	81.2	81.3

Table 9.21: Actual and predicted values of Bulk Density, Hardness, Water Solubility Index and Water Absorption Index of sprouted proso millet – corn meal blend by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	117	116	117	3.58	3.31	3.51	1.1	0.83	0.96	16.7	16.4	16.6
2	143	142	143	3.1	2.71	3.00	1.57	1.18	1.37	13.5	13.1	13.4
3	113	113	113	3.7	3.47	3.64	0.92	0.69	0.81	17.5	17.3	17.4
4	130	129	130	3.14	2.84	3.06	1.22	0.92	1.07	13.9	13.6	13.8
5	135	134	135	3.19	2.89	3.11	1.21	0.91	1.06	13.5	13.2	13.4
6	128	127	128	3.34	3.01	3.25	1.32	0.99	1.16	13.8	13.5	13.7
7	123	122	123	3.54	3.29	3.47	1.01	0.76	0.88	12.6	12.3	12.5
8	114	114	114	3.66	3.43	3.60	0.94	0.71	0.82	13.6	13.4	13.5
9	126	125	126	3.52	3.24	3.44	1.13	0.85	0.99	16	15.7	15.9

10	143	142	143	2.74	2.38	2.64	1.43	1.07	1.25	12.9	12.5	12.8
11	105	105	105	3.87	3.65	3.81	0.88	0.66	0.77	19.7	19.5	19.6
12	126	125	126	3.29	3.01	3.22	1.11	0.83	0.97	14.6	14.3	14.5
13	127	126	127	3.29	2.99	3.21	1.2	0.90	1.05	13	12.7	12.9
14	125	124	125	3.38	3.08	3.30	1.19	0.89	1.04	14.3	14.0	14.2
15	128	127	128	3.23	2.93	3.15	1.2	0.90	1.05	12.6	12.3	12.5
16	118	117	118	3.51	3.24	3.44	1.1	0.83	0.96	13.9	13.6	13.8
17	118	117	118	3.56	3.28	3.49	1.11	0.83	0.97	16.6	16.3	16.5
18	128	127	128	3.1	2.70	2.99	1.59	1.19	1.39	13	12.6	12.9
19	110	109	110	3.76	3.51	3.69	1.02	0.77	0.89	18.1	17.8	18.0
20	126	125	126	3.23	2.93	3.15	1.2	0.90	1.05	13.9	13.6	13.8
21	143	142	143	2.97	2.66	2.89	1.25	0.94	1.09	12.5	12.2	12.4
22	130	129	130	3.14	2.84	3.06	1.22	0.92	1.07	14.6	14.3	14.5
23	122	121	122	3.57	3.30	3.50	1.07	0.80	0.94	12.8	12.5	12.7
24	114	114	114	3.62	3.38	3.55	0.98	0.74	0.86	13.1	12.9	13.0

25	122	121	122	3.49	3.21	3.41	1.14	0.86	1.00	13.6	13.3	13.5
26	122	121	122	3.49	3.21	3.41	1.14	0.86	1.00	13.6	13.3	13.5
27	122	121	122	3.49	3.21	3.41	1.14	0.86	1.00	13.6	13.3	13.5
28	116	115	116	3.59	3.32	3.52	1.09	0.82	0.95	16.8	16.5	16.7
29	141	140	141	3.11	2.72	3.01	1.56	1.17	1.37	13.7	13.3	13.6
30	112	112	112	3.72	3.49	3.66	0.91	0.68	0.80	17.6	17.4	17.5
31	128	127	128	3.16	2.86	3.08	1.2	0.90	1.05	14	13.7	13.9
32	134	133	134	3.22	2.92	3.14	1.19	0.89	1.04	13.7	13.4	13.6
33	126	125	126	3.37	3.05	3.28	1.29	0.97	1.13	14	13.7	13.9
34	122	122	122	3.55	3.30	3.48	1	0.75	0.88	12.7	12.5	12.6
35	113	113	113	3.67	3.44	3.61	0.93	0.70	0.81	13.6	13.4	13.5
36	124	123	124	3.54	3.26	3.47	1.11	0.83	0.97	16.3	16.0	16.2
37	141	140	141	2.77	2.42	2.68	1.4	1.05	1.23	13.1	12.8	13.0
38	104	104	104	3.89	3.67	3.83	0.87	0.65	0.76	19.8	19.6	19.7
39	125	124	125	3.32	3.04	3.25	1.11	0.83	0.97	14.8	14.5	14.7

40	126	125	126	3.32	3.02	3.24	1.19	0.89	1.04	13.1	12.8	13.0
41	124	123	124	3.4	3.11	3.32	1.17	0.88	1.02	14.4	14.1	14.3
42	127	126	127	3.26	2.96	3.18	1.19	0.89	1.04	12.7	12.4	12.6
43	117	116	117	3.55	3.28	3.48	1.09	0.82	0.95	14	13.7	13.9
44	116	115	116	3.57	3.30	3.50	1.1	0.83	0.96	16.7	16.4	16.6
45	126	125	126	3.11	2.72	3.00	1.58	1.19	1.38	13.1	12.7	13.0
46	109	108	109	3.77	3.52	3.70	1.01	0.76	0.88	18.2	17.9	18.1
47	125	124	125	3.26	2.96	3.18	1.19	0.89	1.04	14	13.7	13.9
48	141	140	141	3.01	2.71	2.93	1.22	0.92	1.07	12.7	12.4	12.6
49	128	127	128	3.16	2.86	3.08	1.2	0.90	1.05	14.8	14.5	14.7
50	121	120	121	3.58	3.32	3.51	1.06	0.80	0.93	12.9	12.6	12.8
51	113	113	113	3.63	3.39	3.57	0.97	0.73	0.85	13.2	13.0	13.1
52	121	120	121	3.52	3.24	3.44	1.13	0.85	0.99	13.7	13.4	13.6
53	121	120	121	3.52	3.24	3.44	1.13	0.85	0.99	13.7	13.4	13.6
54	121	120	121	3.52	3.24	3.44	1.13	0.85	0.99	13.7	13.4	13.6

55	115	114	115	3.6	3.33	3.53	1.08	0.81	0.95	16.9	16.6	16.8
56	140	139	140	3.12	2.73	3.02	1.55	1.16	1.36	13.8	13.4	13.7
57	111	111	111	3.74	3.49	3.67	1	0.75	0.88	17.7	17.5	17.6
58	127	126	127	3.17	2.87	3.09	1.19	0.89	1.04	14.1	13.8	14.0
59	132	131	132	3.23	2.94	3.15	1.18	0.89	1.03	13.8	13.5	13.7
60	125	124	125	3.38	3.06	3.30	1.27	0.95	1.11	14.1	13.8	14.0
61	121	121	121	3.56	3.31	3.49	0.99	0.74	0.87	12.8	12.6	12.7
62	112	112	112	3.68	3.45	3.62	0.92	0.69	0.81	13.7	13.5	13.6
63	122	121	122	3.55	3.28	3.48	1.1	0.83	0.96	16.4	16.1	16.3
64	140	139	140	2.78	2.44	2.69	1.38	1.04	1.21	13.2	12.9	13.1
65	103	103	103	3.91	3.70	3.85	0.86	0.65	0.75	19.9	19.7	19.8
66	126	125	126	3.33	3.05	3.26	1.11	0.83	0.97	14.9	14.6	14.8
67	125	124	125	3.33	3.04	3.25	1.18	0.89	1.03	13.2	12.9	13.1
68	123	122	123	3.41	3.12	3.33	1.16	0.87	1.02	14.5	14.2	14.4
69	126	125	126	3.27	2.98	3.19	1.18	0.89	1.03	12.8	12.5	12.7

70	116	115	116	3.56	3.29	3.49	1.08	0.81	0.95	14.1	13.8	14.0
71	115	114	115	3.58	3.31	3.51	1.09	0.82	0.95	16.8	16.5	16.7
72	125	124	125	3.12	2.73	3.02	1.57	1.18	1.37	13.2	12.8	13.1
73	108	108	108	3.79	3.54	3.72	1	0.75	0.88	18.3	18.1	18.2
74	124	123	124	3.27	2.98	3.19	1.18	0.89	1.03	14.1	13.8	14.0
75	140	139	140	3.02	2.72	2.94	1.21	0.91	1.06	12.8	12.5	12.7
76	127	126	127	3.17	2.87	3.09	1.19	0.89	1.04	14.9	14.6	14.8
77	120	119	120	3.59	3.33	3.52	1.05	0.79	0.92	13	12.7	12.9
78	112	112	112	3.64	3.40	3.58	0.96	0.72	0.84	13.3	13.1	13.2
79	120	119	120	3.53	3.25	3.46	1.12	0.84	0.98	13.8	13.5	13.7
80	120	119	120	3.53	3.25	3.46	1.12	0.84	0.98	13.8	13.5	13.7
81	120	119	120	3.53	3.25	3.46	1.12	0.84	0.98	13.8	13.5	13.7
82	114	113	114	3.61	3.34	3.54	1.07	0.80	0.94	17	16.7	16.9
83	139	138	139	3.13	2.75	3.03	1.54	1.16	1.35	13.9	13.5	13.8
84	110	110	110	3.75	3.53	3.69	0.89	0.67	0.78	17.8	17.6	17.7

85	126	125	126	3.18	2.89	3.10	1.18	0.89	1.03	14.2	13.9	14.1
86	131	130	131	3.24	2.95	3.16	1.17	0.88	1.02	13.9	13.6	13.8
87	124	123	124	3.39	3.08	3.31	1.26	0.95	1.10	14.2	13.9	14.1
88	120	120	120	3.57	3.33	3.50	0.98	0.74	0.86	12.9	12.7	12.8
89	111	111	111	3.69	3.46	3.63	0.91	0.68	0.80	13.7	13.5	13.6
90	121	120	121	3.56	3.29	3.49	1.07	0.80	0.94	16.5	16.2	16.4
91	139	138	139	2.79	2.45	2.70	1.36	1.02	1.19	13.3	13.0	13.2
92	102	102	102	3.93	3.72	3.87	0.85	0.64	0.74	20	19.8	19.9
93	125	124	125	3.34	3.07	3.27	1.1	0.83	0.96	15	14.7	14.9
94	124	123	124	3.34	3.05	3.26	1.17	0.88	1.02	13.3	13.0	13.2
95	122	121	122	3.42	3.13	3.34	1.15	0.86	1.01	14.6	14.3	14.5
96	125	124	125	3.28	2.99	3.20	1.17	0.88	1.02	12.9	12.6	12.8
97	115	114	115	3.57	3.30	3.50	1.07	0.80	0.94	14.2	13.9	14.1
98	114	113	114	3.59	3.32	3.52	1.08	0.81	0.95	16.9	16.6	16.8
99	124	123	124	3.13	2.74	3.03	1.56	1.17	1.37	13.3	12.9	13.2

100	107	107	107	3.8	3.55	3.73	0.99	0.74	0.87	18.4	18.2	18.3
101	123	122	123	3.28	2.99	3.20	1.17	0.88	1.02	14.2	13.9	14.1
102	139	138	139	3.03	2.73	2.95	1.2	0.90	1.05	12.9	12.6	12.8
103	126	125	126	3.18	2.89	3.10	1.18	0.89	1.03	15	14.7	14.9
104	119	118	119	3.6	3.34	3.53	1.04	0.78	0.91	13.1	12.8	13.0
105	111	111	111	3.65	3.41	3.59	0.95	0.71	0.83	13.4	13.2	13.3
106	119	118	119	3.54	3.26	3.47	1.11	0.83	0.97	13.9	13.6	13.8
107	119	118	119	3.54	3.26	3.47	1.11	0.83	0.97	13.9	13.6	13.8
108	119	118	119	3.54	3.26	3.47	1.11	0.83	0.97	13.9	13.6	13.8
109	113	112	113	3.62	3.36	3.55	1.06	0.80	0.93	17.1	16.8	17.0
110	138	137	138	3.14	2.76	3.04	1.53	1.15	1.34	14	13.6	13.9
111	109	109	109	3.77	3.55	3.71	0.88	0.66	0.77	17.9	17.7	17.8
112	125	124	125	3.19	2.90	3.11	1.17	0.88	1.02	14.3	14.0	14.2
113	130	129	130	3.25	2.96	3.17	1.16	0.87	1.02	14	13.7	13.9
114	123	122	123	3.4	3.09	3.32	1.25	0.94	1.09	14.4	14.1	14.3

115	119	119	119	3.58	3.34	3.52	0.97	0.73	0.85	13	12.8	12.9
116	110	110	110	3.7	3.48	3.64	0.9	0.68	0.79	13.7	13.5	13.6
117	120	119	120	3.57	3.30	3.50	1.08	0.81	0.95	16.6	16.3	16.5
118	138	137	138	2.8	2.47	2.71	1.34	1.01	1.17	13.4	13.1	13.3
119	100	100	100	3.96	3.75	3.90	0.84	0.63	0.74	20.1	19.9	20.0
120	124	123	124	3.35	3.08	3.28	1.1	0.83	0.96	15.1	14.8	15.0
121	123	122	123	3.35	3.06	3.27	1.16	0.87	1.02	13.4	13.1	13.3
122	121	120	121	3.43	3.15	3.35	1.14	0.86	1.00	14.7	14.4	14.6
123	124	123	124	3.29	3.00	3.21	1.16	0.87	1.02	13	12.7	12.9
124	114	113	114	3.58	3.32	3.51	1.06	0.80	0.93	14.3	14.0	14.2
125	113	112	113	3.61	3.34	3.54	1.07	0.80	0.94	17	16.7	16.9
126	123	122	123	3.14	2.75	3.04	1.55	1.16	1.36	13.4	13.0	13.3
127	105	105	105	3.82	3.58	3.75	0.98	0.74	0.86	18.5	18.3	18.4
128	122	121	122	3.29	3.00	3.21	1.16	0.87	1.02	14.3	14.0	14.2
129	138	137	138	3.04	2.74	2.96	1.19	0.89	1.04	13	12.7	12.9

130	125	124	125	3.19	2.90	3.11	1.17	0.88	1.02	15.1	14.8	15.0
131	118	117	118	3.61	3.35	3.54	1.03	0.77	0.90	13.2	12.9	13.1
132	110	110	110	3.66	3.43	3.60	0.94	0.71	0.82	13.5	13.3	13.4
133	118	117	118	3.55	3.28	3.48	1.1	0.83	0.96	14	13.7	13.9
134	118	117	118	3.55	3.28	3.48	1.1	0.83	0.96	14	13.7	13.9
135	118	117	118	3.55	3.28	3.48	1.1	0.83	0.96	14	13.7	13.9

Table 9.22: Actual and predicted values of Total Color Difference, Expansion Ratio, Starch Digestibility and Protein Digestibility of sprouted proso millet – corn meal blend by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by single screw extrusion

No	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	3.57	3.30	3.51	17.47	17.33	17.37	81.2	80.9	81.1	81.3	81.2	81.2
2	2.79	2.40	2.70	14.97	14.86	14.89	80.9	80.5	80.8	80	79.9	80.0
3	3.78	3.55	3.73	15.87	15.72	15.77	83.2	83.0	83.1	83.5	83.3	83.4
4	2.57	2.27	2.50	16.57	16.47	16.50	82.4	82.1	82.3	81.8	81.7	81.8
5	2.81	2.51	2.74	19.57	19.46	19.49	83.7	83.4	83.6	83.4	83.3	83.4
6	2.85	2.52	2.78	16.57	16.46	16.49	83.9	83.6	83.8	84	83.9	84.0
7	3.49	3.24	3.43	13.77	13.63	13.68	78.6	78.3	78.5	78.6	78.5	78.5
8	3.78	3.55	3.73	15.67	15.52	15.57	79.5	79.3	79.4	80.5	80.3	80.4
9	3.41	3.13	3.35	20.37	20.23	20.28	83.8	83.5	83.7	84.7	84.6	84.6

10	2.55	2.19	2.47	15.67	15.57	15.60	84.2	83.8	84.1	83.2	83.1	83.2
11	3.86	3.64	3.81	13.47	13.32	13.37	78.9	78.7	78.8	77.8	77.6	77.7
12	3.78	3.50	3.72	13.77	13.62	13.67	78.5	78.2	78.4	78.7	78.5	78.6
13	3.41	3.11	3.34	14.97	14.83	14.88	79.3	79.0	79.2	78.7	78.6	78.6
14	3.61	3.31	3.54	15.67	15.53	15.57	79.7	79.4	79.6	80.2	80.1	80.1
15	3.58	3.28	3.51	16.27	16.13	16.17	80.9	80.6	80.8	80.5	80.4	80.4
16	3.66	3.39	3.60	16.77	16.62	16.67	79.1	78.8	79.0	81.7	81.6	81.6
17	3.55	3.27	3.49	17.17	17.03	17.07	80.5	80.2	80.4	80.4	80.3	80.3
18	2.79	2.39	2.70	16.57	16.46	16.49	79.1	78.7	79.0	77.7	77.6	77.7
19	3.74	3.49	3.68	16.37	16.22	16.27	81.4	81.1	81.3	82.8	82.7	82.7
20	2.57	2.27	2.50	16.77	16.67	16.70	82.7	82.4	82.6	82.2	82.1	82.2
21	2.57	2.26	2.50	16.87	16.77	16.80	83.3	83.0	83.2	81.6	81.5	81.6
22	2.79	2.49	2.72	16.97	16.86	16.89	84.2	83.9	84.1	84.5	84.4	84.5
23	3.58	3.31	3.52	14.57	14.43	14.47	78.5	78.2	78.4	79.4	79.3	79.3
24	3.66	3.42	3.61	15.37	15.22	15.27	79.8	79.6	79.7	79.6	79.5	79.5

25	3.61	3.33	3.55	15.67	15.53	15.57	79.4	79.1	79.3	81.2	81.1	81.1
26	3.61	3.33	3.55	15.67	15.53	15.57	79.4	79.1	79.3	81.2	81.1	81.1
27	3.61	3.33	3.55	15.67	15.53	15.57	79.4	79.1	79.3	81.2	81.1	81.1
28	3.58	3.31	3.52	17.37	17.23	17.27	82.8	82.5	82.7	82.2	82.1	82.1
29	2.81	2.42	2.72	15.37	15.26	15.29	78.5	78.1	78.4	80	79.9	80.0
30	3.79	3.56	3.74	15.77	15.62	15.67	84.1	83.9	84.0	84.4	84.2	84.3
31	2.59	2.29	2.52	16.37	16.27	16.30	82.5	82.2	82.4	82	81.9	82.0
32	2.83	2.53	2.76	19.37	19.26	19.29	85.1	84.8	85.0	84.4	84.3	84.4
33	2.87	2.55	2.80	16.37	16.26	16.29	85.3	85.0	85.2	84.9	84.8	84.9
34	3.5	3.25	3.44	13.87	13.73	13.78	78.9	78.7	78.8	78.7	78.6	78.6
35	3.79	3.56	3.74	15.97	15.82	15.87	79.6	79.4	79.5	80.6	80.4	80.5
36	3.43	3.15	3.37	20.17	20.03	20.08	85.6	85.3	85.5	85.5	85.4	85.4
37	2.57	2.22	2.49	15.47	15.37	15.40	85.2	84.9	85.1	84.3	84.2	84.3
38	3.87	3.65	3.82	13.37	13.22	13.27	80.2	80.0	80.1	80	79.8	79.9
39	3.79	3.51	3.73	13.87	13.72	13.77	78.6	78.3	78.5	79	78.8	78.9

40	3.44	3.14	3.37	15.37	15.23	15.28	79.4	79.1	79.3	79	78.9	78.9
41	3.64	3.35	3.58	15.97	15.82	15.87	79.8	79.5	79.7	80.4	80.3	80.3
42	3.61	3.31	3.54	16.47	16.33	16.37	78.5	78.2	78.4	80.8	80.7	80.7
43	3.68	3.41	3.62	17.07	16.92	16.97	79.3	79.0	79.2	82	81.9	81.9
44	3.56	3.29	3.50	17.07	16.93	16.97	81.8	81.5	81.7	81.7	81.6	81.6
45	2.81	2.42	2.72	16.37	16.26	16.29	79.3	78.9	79.2	77.8	77.7	77.8
46	3.75	3.50	3.69	16.27	16.12	16.17	83	82.7	82.9	84.1	84.0	84.0
47	2.59	2.29	2.52	17.07	16.97	17.00	82.9	82.6	82.8	82.4	82.3	82.4
48	2.59	2.29	2.52	16.67	16.57	16.60	84.3	84.0	84.2	83.1	83.0	83.1
49	2.81	2.51	2.74	16.77	16.66	16.69	85.5	85.2	85.4	85.2	85.1	85.2
50	3.61	3.35	3.55	14.67	14.53	14.57	78.6	78.3	78.5	79.5	79.4	79.4
51	3.68	3.44	3.63	15.47	15.32	15.37	79.9	79.7	79.8	79.7	79.6	79.6
52	3.65	3.37	3.59	15.97	15.82	15.87	79.6	79.3	79.5	81.3	81.2	81.2
53	3.65	3.37	3.59	15.97	15.82	15.87	79.6	79.3	79.5	81.3	81.2	81.2
54	3.65	3.37	3.59	15.97	15.82	15.87	79.6	79.3	79.5	81.3	81.2	81.2

55	3.59	3.32	3.53	17.27	17.13	17.17	82.9	82.6	82.8	82.3	82.2	82.2
56	2.82	2.43	2.73	15.47	15.36	15.39	78.6	78.2	78.5	80.2	80.1	80.2
57	3.8	3.55	3.74	15.67	15.52	15.57	84.2	84.0	84.1	84.5	84.3	84.4
58	2.6	2.30	2.53	16.27	16.17	16.20	82.6	82.3	82.5	82.1	82.0	82.1
59	2.84	2.55	2.77	19.27	19.16	19.19	85.2	84.9	85.1	84.5	84.4	84.5
60	2.88	2.56	2.81	16.27	16.15	16.19	85.4	85.1	85.3	85	84.9	85.0
61	3.51	3.26	3.46	13.97	13.83	13.88	79	78.8	78.9	78.8	78.7	78.7
62	3.8	3.57	3.75	16.07	15.92	15.97	79.7	79.5	79.6	80.7	80.5	80.6
63	3.44	3.17	3.38	20.07	19.93	19.98	85.7	85.4	85.6	85.6	85.5	85.5
64	2.58	2.24	2.50	15.37	15.27	15.30	85.3	85.0	85.2	84.4	84.3	84.4
65	3.88	3.67	3.83	13.27	13.11	13.17	80.3	80.1	80.2	80.2	80.0	80.1
66	3.8	3.52	3.74	13.97	13.82	13.87	78.7	78.4	78.6	79.1	78.9	79.0
67	3.45	3.16	3.38	15.47	15.33	15.38	79.5	79.2	79.4	79.1	79.0	79.0
68	3.65	3.36	3.59	16.07	15.92	15.97	79.9	79.6	79.8	80.5	80.4	80.4
69	3.63	3.34	3.56	16.57	16.42	16.47	78.6	78.3	78.5	80.9	80.8	80.8

70	3.69	3.42	3.63	17.17	17.02	17.07	79.4	79.1	79.3	82.1	82.0	82.0
71	3.57	3.30	3.51	16.97	16.83	16.87	81.9	81.6	81.8	81.8	81.7	81.7
72	2.82	2.43	2.73	16.27	16.16	16.19	79.4	79.0	79.3	77.9	77.8	77.9
73	3.76	3.51	3.70	16.17	16.02	16.07	83.1	82.9	83.0	84.2	84.0	84.1
74	2.6	2.31	2.53	17.17	17.07	17.10	82.1	81.8	82.0	82.5	82.4	82.5
75	2.6	2.30	2.53	16.57	16.47	16.50	84.4	84.1	84.3	83.2	83.1	83.2
76	2.82	2.52	2.75	16.67	16.56	16.59	85.6	85.3	85.5	85.3	85.2	85.3
77	3.63	3.37	3.57	14.77	14.62	14.67	78.7	78.4	78.6	79.6	79.5	79.5
78	3.69	3.45	3.64	15.57	15.42	15.47	80.4	80.2	80.3	79.8	79.7	79.7
79	3.66	3.38	3.60	16.07	15.92	15.97	79.7	79.4	79.6	81.4	81.3	81.3
80	3.66	3.38	3.60	16.07	15.92	15.97	79.7	79.4	79.6	81.4	81.3	81.3
81	3.66	3.38	3.60	16.07	15.92	15.97	79.7	79.4	79.6	81.4	81.3	81.3
82	3.6	3.33	3.54	17.17	17.03	17.07	83	82.7	82.9	82.4	82.3	82.3
83	2.83	2.45	2.74	15.57	15.46	15.49	78.7	78.3	78.6	80.3	80.2	80.3
84	3.81	3.59	3.76	15.57	15.42	15.47	84.3	84.1	84.2	84.6	84.4	84.5

85	2.61	2.32	2.54	16.17	16.07	16.10	82.7	82.4	82.6	82.2	82.1	82.2
86	2.85	2.56	2.79	19.17	19.06	19.09	85.3	85.0	85.2	84.6	84.5	84.6
87	2.89	2.58	2.82	16.17	16.05	16.09	85.5	85.2	85.4	85.1	85.0	85.1
88	3.52	3.28	3.47	14.07	13.93	13.97	79.1	78.9	79.0	78.9	78.8	78.8
89	3.81	3.58	3.76	16.17	16.02	16.07	79.8	79.6	79.7	80.8	80.6	80.7
90	3.45	3.18	3.39	19.97	19.83	19.88	85.8	85.5	85.7	85.7	85.6	85.6
91	2.59	2.25	2.51	15.27	15.17	15.20	85.3	85.0	85.2	84.5	84.4	84.5
92	3.89	3.68	3.84	13.17	13.01	13.06	80.4	80.2	80.3	80.3	80.1	80.2
93	3.81	3.54	3.75	14.07	13.92	13.97	78.8	78.5	78.7	79.2	79.0	79.1
94	3.46	3.17	3.40	15.57	15.43	15.48	79.6	79.3	79.5	79.2	79.1	79.1
95	3.66	3.37	3.60	16.17	16.02	16.07	80.1	79.8	80.0	80.6	80.5	80.5
96	3.64	3.35	3.58	16.67	16.52	16.57	78.7	78.4	78.6	81	80.9	80.9
97	3.7	3.43	3.64	17.27	17.12	17.17	79.5	79.2	79.4	82.2	82.1	82.1
98	3.58	3.31	3.52	16.87	16.73	16.77	82	81.7	81.9	81.9	81.8	81.8
99	2.83	2.44	2.74	16.17	16.06	16.09	79.5	79.1	79.4	78	77.9	78.0

100	3.77	3.52	3.72	16.07	15.92	15.97	83.2	83.0	83.1	84.3	84.1	84.2
101	2.61	2.32	2.55	17.27	17.17	17.20	83.1	82.8	83.0	82.6	82.5	82.6
102	2.61	2.31	2.54	16.47	16.37	16.40	84.5	84.2	84.4	83.3	83.2	83.3
103	2.83	2.54	2.76	16.57	16.46	16.49	85.7	85.4	85.6	85.4	85.3	85.4
104	3.64	3.38	3.58	14.87	14.72	14.77	78.8	78.5	78.7	79.7	79.6	79.6
105	3.7	3.46	3.65	15.67	15.52	15.57	80.7	80.5	80.6	79.9	79.8	79.8
106	3.67	3.39	3.61	16.17	16.02	16.07	79.8	79.5	79.7	81.5	81.4	81.4
107	3.67	3.39	3.61	16.17	16.02	16.07	79.8	79.5	79.7	81.5	81.4	81.4
108	3.67	3.39	3.61	16.17	16.02	16.07	79.8	79.5	79.7	81.5	81.4	81.4
109	3.61	3.35	3.55	17.07	16.93	16.97	83.1	82.8	83.0	82.5	82.4	82.4
110	2.84	2.46	2.76	15.67	15.56	15.59	78.8	78.4	78.7	80.4	80.3	80.4
111	3.82	3.60	3.77	15.47	15.32	15.37	84.4	84.2	84.3	84.7	84.5	84.6
112	2.62	2.33	2.56	16.07	15.97	16.00	82.8	82.5	82.7	82.3	82.2	82.3
113	2.86	2.57	2.80	19.07	18.96	18.99	85.4	85.1	85.3	84.7	84.6	84.7
114	2.9	2.59	2.83	16.07	15.95	15.99	85.6	85.3	85.5	85.2	85.1	85.2

115	3.53	3.29	3.48	14.17	14.03	14.07	79.2	79.0	79.1	79	78.9	78.9
116	3.82	3.60	3.77	16.27	16.12	16.17	79.9	79.7	79.8	80.9	80.7	80.8
117	3.46	3.19	3.40	19.87	19.73	19.78	85.9	85.6	85.8	85.8	85.7	85.7
118	2.6	2.27	2.53	15.17	15.07	15.10	85.4	85.1	85.3	84.6	84.5	84.6
119	3.9	3.69	3.85	13.07	12.91	12.96	80.5	80.3	80.4	80.4	80.2	80.3
120	3.82	3.55	3.76	14.17	14.02	14.07	78.9	78.6	78.8	79.3	79.1	79.2
121	3.47	3.18	3.41	15.67	15.53	15.58	79.7	79.4	79.6	79.3	79.2	79.2
122	3.67	3.39	3.61	16.27	16.12	16.17	80.2	79.9	80.1	80.7	80.6	80.6
123	3.65	3.36	3.59	16.77	16.62	16.67	78.8	78.5	78.7	80.4	80.3	80.3
124	3.71	3.45	3.65	17.37	17.22	17.27	79.6	79.3	79.5	82.3	82.2	82.2
125	3.59	3.32	3.53	16.77	16.63	16.67	82.1	81.8	82.0	82	81.9	81.9
126	2.84	2.45	2.75	16.07	15.96	15.99	79.6	79.2	79.5	78.1	78.0	78.1
127	3.78	3.54	3.73	15.97	15.82	15.87	83.3	83.1	83.2	84.4	84.2	84.3
128	2.62	2.33	2.56	17.37	17.27	17.30	83.2	82.9	83.1	82.7	82.6	82.7
129	2.62	2.32	2.55	16.37	16.27	16.30	84.6	84.3	84.5	83.4	83.3	83.4

130	2.84	2.55	2.78	16.47	16.36	16.39	85.8	85.5	85.7	85.5	85.4	85.5
131	3.65	3.39	3.59	14.97	14.82	14.87	78.9	78.6	78.8	79.8	79.7	79.7
132	3.71	3.48	3.66	15.77	15.62	15.67	80.8	80.6	80.7	80	79.9	79.9
133	3.68	3.41	3.62	16.27	16.12	16.17	79.9	79.6	79.8	81.6	81.5	81.5
134	3.68	3.41	3.62	16.27	16.12	16.17	79.9	79.6	79.8	81.6	81.5	81.5
135	3.68	3.41	3.62	16.27	16.12	16.17	79.9	79.6	79.8	81.6	81.5	81.5

Table 9.23: Regression coefficient and Root Mean Square Error – Single Screw Extrusion of sprouted proso millet – corn meal blend

Product Responses	RSM		ANN	
	R^2	RMSE	R^2	RMSE
Bulk Density (kg/m^3)	0.9613	1.00	0.9811	0.37
Hardness (N)	0.9731	2.73	0.9932	1.15
Water Solubility Index (%)	0.8925	2.11	0.9429	0.92
Water Absorption Index	0.8713	2.04	0.9368	0.83
Total Color Difference	0.9992	2.20	0.9999	0.81
Expansion Ratio	0.9629	1.76	0.9873	0.26
Starch Digestibility (%)	0.8918	1.63	0.9451	0.50
Protein Digestibility (%)	0.9924	1.46	0.9992	0.39

RSM – Response Surface Methodology; ANN – Artificial Neural Network, R^2- Regression Coefficient; RMSE – Root Mean Square Error

Table 9.24: Optimization, validation and comparison of experimental data set (Bulk Density, Hardness, Water Solubility Index, Water Absorption Index) – Single Screw Extrusion of sprouted proso millet - corn meal blend

No	Moisture Content	Screw Speed	Die Temperature	Corn Ratio	Germination time	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
						Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
	%w.b.	rpm	°C	%	Days		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	20	150	90	0	0	135	134	135	3.19	2.89	3.11	1.21	0.91	1.06	13.5	13.2	13.4
2	20	220	140	15	0	118	117	118	3.51	3.24	3.44	1.1	0.83	0.96	13.9	13.6	13.8
3	15	80	115	15	1	116	115	116	3.59	3.32	3.52	1.09	0.82	0.95	16.8	16.5	16.7
4	20	220	115	30	1	113	113	113	3.63	3.39	3.57	0.97	0.73	0.85	13.2	13.0	13.1
5	20	150	90	0	2	132	131	132	3.23	2.94	3.15	1.18	0.89	1.03	13.8	13.5	13.7
6	25	150	90	15	2	125	124	125	3.12	2.73	3.02	1.57	1.18	1.37	13.2	12.8	13.1
7	25	150	115	0	3	139	138	139	2.79	2.45	2.70	1.36	1.02	1.19	13.3	13.0	13.2

8	20	220	115	0	3	126	125	126	3.18	2.89	3.10	1.18	0.89	1.03	15	14.7	14.9
9	20	80	90	15	4	123	122	123	3.35	3.06	3.27	1.16	0.87	1.02	13.4	13.1	13.3
10	20	220	115	30	4	110	110	110	3.66	3.43	3.60	0.94	0.71	0.82	13.5	13.3	13.4

Table 9.25: Optimization, validation and comparison of experimental data set (Total Color Difference, Expansion Ratio, Protein Digestibility, Starch Digestibility)– Single Screw Extrusion of sprouted proso millet - corn meal blend

No	**Moisture Content**	**Screw Speed**	**Die Temperature**	**Corn Ratio**	**Germination time**	**Total Color Difference**			**Expansion ratio**			**Protein Digestibility %**			**Starch Digestibility %**		
						Actual	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**	
	%w.b.	**rpm**	**°C**	**%**	**Days**		**RSM**	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**
1	2.81	2.51	2.74	19.57	19.46	19.49	83.7	83.4	83.6	83.4	83.3	83.4	2.81	2.51	2.74	19.57	19.46
2	3.66	3.39	3.60	16.77	16.62	16.67	79.1	78.8	79.0	81.7	81.6	81.6	3.66	3.39	3.60	16.77	16.62
3	3.58	3.31	3.52	17.37	17.23	17.27	82.8	82.5	82.7	82.2	82.1	82.1	3.58	3.31	3.52	17.37	17.23
4	3.68	3.44	3.63	15.47	15.32	15.37	79.9	79.7	79.8	79.7	79.6	79.6	3.68	3.44	3.63	15.47	15.32
5	2.84	2.55	2.77	19.27	19.16	19.19	85.2	84.9	85.1	84.5	84.4	84.5	2.84	2.55	2.77	19.27	19.16
6	2.82	2.43	2.73	16.27	16.16	16.19	79.4	79.0	79.3	77.9	77.8	77.9	2.82	2.43	2.73	16.27	16.16
7	2.59	2.25	2.51	15.27	15.17	15.20	85.3	85.0	85.2	84.5	84.4	84.5	2.59	2.25	2.51	15.27	15.17
8	2.83	2.54	2.76	16.57	16.46	16.49	85.7	85.4	85.6	85.4	85.3	85.4	2.83	2.54	2.76	16.57	16.46

9	3.47	3.18	3.41	15.67	15.53	15.58	79.7	79.4	79.6	79.3	79.2	79.2	3.47	3.18	3.41	15.67	15.53
10	3.71	3.48	3.66	15.77	15.62	15.67	80.8	80.6	80.7	80	79.9	79.9	3.71	3.48	3.66	15.77	15.62

Table 9.26: Actual and predicted values of Bulk Density, Hardness, Water Solubility Index and Water Absorption Index of sprouted proso millet flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by twin screw extrusion

No	Bulk Density kg/m³			Hardness N			Water Solubility Index %			Water Absorption Index		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	139	138	139	1.33	1.11	1.26	19	18.6	18.9	3.59	3.41	3.51
2	131	130	131	1.24	1.03	1.17	20	19.7	19.9	3.69	3.52	3.62
3	158	157	158	1.51	1.26	1.43	16.8	16.4	16.7	3.41	3.21	3.32
4	131	130	131	1.26	1.05	1.19	19.7	19.4	19.6	3.67	3.50	3.60
5	125	124	125	1.18	0.98	1.12	20.6	20.3	20.5	3.74	3.58	3.67
6	140	139	140	1.35	1.13	1.28	18.9	18.5	18.8	3.58	3.40	3.50
7	159	158	159	1.52	1.27	1.44	16.6	16.2	16.5	3.39	3.18	3.30
8	152	151	152	1.45	1.21	1.37	17.6	17.2	17.5	3.48	3.28	3.39
9	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.67	3.50	3.59

10	150	149	150	1.43	1.19	1.35	17.8	17.4	17.7	3.5	3.31	3.42
11	129	128	129	1.22	1.02	1.16	20.1	19.8	20.0	3.71	3.54	3.64
12	129	128	129	1.24	1.03	1.17	19.9	19.6	19.8	3.7	3.53	3.63
13	131	130	131	1.26	1.05	1.19	19.7	19.4	19.6	3.67	3.50	3.60
14	134	133	134	1.29	1.08	1.22	19.4	19.0	19.3	3.64	3.47	3.56
15	133	132	133	1.26	1.05	1.19	19.8	19.5	19.7	3.67	3.50	3.60
16	146	145	146	1.4	1.17	1.33	18.2	17.8	18.1	3.53	3.34	3.45
17	133	132	133	1.28	1.07	1.21	19.5	19.1	19.4	3.65	3.48	3.57
18	153	152	153	1.46	1.22	1.38	17.5	17.1	17.4	3.46	3.26	3.37
19	154	153	154	1.47	1.23	1.39	17.2	16.8	17.1	3.45	3.25	3.36
20	142	141	142	1.38	1.15	1.31	18.6	18.2	18.5	3.55	3.36	3.47
21	145	144	145	1.41	1.18	1.34	18.4	18.0	18.3	3.52	3.33	3.44
22	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.66	3.49	3.58
23	145	144	145	1.39	1.16	1.32	18.3	17.9	18.2	3.54	3.35	3.46
24	141	140	141	1.37	1.14	1.30	18.7	18.3	18.6	3.56	3.37	3.48

25	137	136	137	1.32	1.10	1.25	19.1	18.7	19.0	3.61	3.43	3.53
26	134	133	134	1.29	1.08	1.22	19.4	19.0	19.3	3.64	3.47	3.56
27	135	134	135	1.3	1.08	1.23	19.3	18.9	19.2	3.63	3.45	3.55
28	120	119	120	1.14	0.95	1.08	21	20.7	20.9	3.79	3.64	3.72
29	134	133	134	1.29	1.08	1.22	19.4	19.0	19.3	3.64	3.47	3.56
30	131	130	131	1.26	1.05	1.19	19.7	19.4	19.6	3.67	3.50	3.60
31	146	145	146	1.4	1.17	1.33	18.2	17.8	18.1	3.53	3.34	3.45
32	156	155	156	1.49	1.24	1.41	17	16.6	16.9	3.43	3.23	3.34
33	130	129	130	1.25	1.04	1.18	19.8	19.5	19.7	3.69	3.52	3.62
34	135	134	135	1.3	1.08	1.23	19.3	18.9	19.2	3.63	3.45	3.55
35	143	142	143	1.37	1.14	1.30	18.5	18.1	18.4	3.56	3.37	3.48
36	134	133	134	1.27	1.06	1.20	19.6	19.2	19.5	3.65	3.48	3.57
37	123	122	123	1.17	0.98	1.11	20.7	20.4	20.6	3.76	3.60	3.69
38	123	122	123	1.16	0.97	1.10	20.8	20.5	20.7	3.76	3.60	3.69
39	124	123	124	1.17	0.98	1.11	20.7	20.4	20.6	3.75	3.59	3.68

40	148	147	148	1.45	1.21	1.37	18	17.6	17.9	3.48	3.28	3.39
41	128	127	128	1.23	1.03	1.17	20	19.7	19.9	3.71	3.54	3.64
42	133	132	133	1.28	1.07	1.21	19.5	19.1	19.4	3.65	3.48	3.57
43	148	147	148	1.42	1.18	1.35	18	17.6	17.9	3.51	3.32	3.43
44	134	133	134	1.29	1.08	1.22	19.4	19.0	19.3	3.64	3.47	3.56
45	136	135	136	1.31	1.09	1.24	19.2	18.8	19.1	3.62	3.44	3.54
46	133	132	133	1.28	1.07	1.21	19.5	19.1	19.4	3.65	3.48	3.57
47	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.66	3.49	3.58
48	136	135	136	1.3	1.08	1.23	19.4	19.0	19.3	3.62	3.44	3.54
49	128	127	128	1.21	1.01	1.15	20.2	19.9	20.1	3.71	3.55	3.64
50	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.66	3.49	3.58
51	138	137	138	1.32	1.10	1.25	19.1	18.7	19.0	3.6	3.42	3.52
52	147	146	147	1.41	1.18	1.34	18.1	17.7	18.0	3.52	3.33	3.44
53	155	154	155	1.48	1.23	1.40	17.3	16.9	17.2	3.44	3.24	3.35
54	135	134	135	1.3	1.08	1.23	19.3	18.9	19.2	3.63	3.45	3.55

55	133	132	133	1.28	1.07	1.21	19.5	19.1	19.4	3.65	3.48	3.57
56	131	130	131	1.26	1.05	1.19	19.7	19.4	19.6	3.67	3.50	3.60
57	134	133	134	1.29	1.08	1.22	19.4	19.0	19.3	3.64	3.47	3.56
58	150	149	150	1.45	1.21	1.37	17.8	17.4	17.7	3.49	3.29	3.40
59	143	142	143	1.39	1.16	1.32	18.5	18.1	18.4	3.54	3.35	3.46
60	129	128	129	1.23	1.03	1.17	20.1	19.8	20.0	3.7	3.53	3.63
61	122	121	122	1.15	0.96	1.09	20.9	20.6	20.8	3.77	3.61	3.70
62	147	146	147	1.41	1.18	1.34	18.1	17.7	18.0	3.52	3.33	3.44
63	151	150	151	1.44	1.20	1.36	17.7	17.3	17.6	3.49	3.30	3.40
64	135	134	135	1.3	1.08	1.23	19.3	18.9	19.2	3.63	3.45	3.55
65	135	134	135	1.3	1.08	1.23	19.3	18.9	19.2	3.63	3.45	3.55
66	146	145	146	1.42	1.18	1.35	18.2	17.8	18.1	3.51	3.32	3.43
67	127	126	127	1.2	1.00	1.14	20.3	20.0	20.2	3.72	3.56	3.65
68	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.66	3.49	3.58
69	149	148	149	1.43	1.19	1.35	17.9	17.5	17.8	3.5	3.31	3.42

70	127	126	127	1.19	0.99	1.13	20.5	20.2	20.4	3.73	3.57	3.66
71	131	130	131	1.26	1.05	1.19	19.7	19.4	19.6	3.68	3.51	3.61
72	126	125	126	1.19	0.99	1.13	20.4	20.1	20.3	3.73	3.57	3.66
73	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.66	3.49	3.58
74	119	118	119	1.13	0.94	1.07	21.1	20.8	21.0	3.8	3.65	3.73
75	144	143	144	1.38	1.15	1.31	18.4	18.0	18.3	3.55	3.36	3.47

Table 9.27: Actual and predicted values of Total Color Difference, Expansion Ratio, Starch Digestibility and Protein Digestibility of sprouted proso millet flour by Response Surface Methodology (RSM) and Artificial Neural Network (ANN) by twin screw extrusion

No	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
	Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
		RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	20.4	20.2	20.3	3.34	3.19	3.27	83.5	83.3	83.4	83.7	83.5	83.6
2	19.3	19.1	19.2	3.48	3.34	3.41	84.4	84.3	84.3	84	83.8	83.9
3	22.4	22.1	22.3	3.07	2.90	2.99	80.3	80.1	80.2	82.5	82.3	82.4
4	19.6	19.4	19.5	3.43	3.29	3.36	84.1	84.0	84.0	84	83.8	83.9
5	18.7	18.5	18.6	3.57	3.44	3.51	84.8	84.7	84.7	84.6	84.5	84.5
6	20.5	20.3	20.4	3.32	3.17	3.25	83.4	83.2	83.3	83.6	83.4	83.5
7	22.6	22.3	22.5	3.05	2.88	2.97	80.2	80.0	80.1	82.4	82.2	82.3
8	21.8	21.6	21.7	3.16	3.00	3.08	81	80.8	80.9	82.8	82.6	82.7
9	19.7	19.5	19.6	3.43	3.29	3.36	82.1	81.9	82.0	82.5	82.3	82.4

10	21.6	21.4	21.5	3.18	3.02	3.10	82.2	82.0	82.1	83	82.8	82.9
11	19.1	18.9	19.0	3.5	3.36	3.44	84.5	84.4	84.4	84.2	84.0	84.1
12	19.4	19.2	19.3	3.46	3.32	3.39	84.2	84.1	84.1	84.1	83.9	84.0
13	19.6	19.4	19.5	3.43	3.29	3.36	84.1	84.0	84.0	84	83.8	83.9
14	19.9	19.7	19.8	3.4	3.26	3.33	83.8	83.6	83.7	83.7	83.5	83.6
15	19.5	19.3	19.4	3.46	3.32	3.39	84.2	84.1	84.1	83.8	83.6	83.7
16	21.2	21.0	21.1	3.22	3.06	3.15	82.5	82.3	82.4	83.1	82.9	83.0
17	19.8	19.6	19.7	3.41	3.27	3.34	83.9	83.7	83.8	83.8	83.6	83.7
18	21.9	21.7	21.8	3.15	2.99	3.07	80.9	80.7	80.8	82.6	82.4	82.5
19	22	21.8	21.9	3.11	2.95	3.03	80.7	80.5	80.6	82.7	82.5	82.6
20	20.8	20.6	20.7	3.29	3.14	3.22	79.6	79.4	79.5	81.3	81.1	81.2
21	21.1	20.9	21.0	3.26	3.10	3.19	83	82.8	82.9	83.1	82.9	83.0
22	19.7	19.5	19.6	3.42	3.28	3.35	84	83.8	83.9	83.9	83.7	83.8
23	21.1	20.9	21.0	3.23	3.08	3.16	82.6	82.4	82.5	83.2	83.0	83.1
24	20.7	20.5	20.6	3.3	3.15	3.23	83.3	83.1	83.2	83.5	83.3	83.4

25	20.2	20.0	20.1	3.36	3.21	3.29	83.6	83.4	83.5	83.4	83.2	83.3
26	19.9	19.7	19.8	3.4	3.26	3.33	83.8	83.6	83.7	83.7	83.5	83.6
27	20	19.8	19.9	3.39	3.25	3.32	81.6	81.4	81.5	82.1	81.9	82.0
28	18.2	18.0	18.1	3.62	3.49	3.56	85.3	85.2	85.2	85	84.9	84.9
29	19.9	19.7	19.8	3.4	3.26	3.33	83.8	83.6	83.7	83.7	83.5	83.6
30	19.6	19.4	19.5	3.43	3.29	3.36	84.1	84.0	84.0	84	83.8	83.9
31	21.2	21.0	21.1	3.21	3.05	3.14	82.5	82.3	82.4	83.2	83.0	83.1
32	22.2	22.0	22.1	3.09	2.92	3.01	80.5	80.3	80.4	82.6	82.4	82.5
33	19.5	19.3	19.4	3.45	3.31	3.38	84.1	84.0	84.0	84	83.8	83.9
34	20	19.8	19.9	3.39	3.25	3.32	81.6	81.4	81.5	82.1	81.9	82.0
35	20.9	20.7	20.8	3.25	3.10	3.18	82.8	82.6	82.7	83.4	83.2	83.3
36	19.7	19.5	19.6	3.45	3.31	3.38	84	83.8	83.9	83.7	83.5	83.6
37	18.5	18.3	18.4	3.59	3.46	3.53	83.6	83.5	83.5	83.3	83.2	83.2
38	18.5	18.3	18.4	3.59	3.46	3.53	85	84.9	84.9	84.8	84.7	84.7
39	18.6	18.4	18.5	3.58	3.45	3.52	84.9	84.8	84.8	84.7	84.6	84.6

40	21.5	21.3	21.4	3.21	3.05	3.13	79.3	79.1	79.2	80.6	80.4	80.5
41	19.3	19.1	19.2	3.47	3.33	3.41	84.3	84.2	84.2	84.2	84.0	84.1
42	19.8	19.6	19.7	3.41	3.27	3.34	83.9	83.7	83.8	83.8	83.6	83.7
43	21.4	21.2	21.3	3.19	3.03	3.12	82.3	82.1	82.2	83	82.8	82.9
44	19.9	19.7	19.8	3.4	3.26	3.33	83.8	83.6	83.7	83.7	83.5	83.6
45	20.1	19.9	20.0	3.37	3.22	3.30	83.7	83.5	83.6	83.5	83.3	83.4
46	19.8	19.6	19.7	3.41	3.27	3.34	83.9	83.7	83.8	83.8	83.6	83.7
47	19.7	19.5	19.6	3.42	3.28	3.35	84	83.8	83.9	83.9	83.7	83.8
48	19.9	19.7	19.8	3.42	3.28	3.35	82.3	82.1	82.2	82.1	81.9	82.0
49	19.1	18.9	19.0	3.53	3.40	3.47	84.6	84.5	84.5	84.1	83.9	84.0
50	19.7	19.5	19.6	3.42	3.28	3.35	84	83.8	83.9	83.9	83.7	83.8
51	20.3	20.1	20.2	3.35	3.20	3.28	83.6	83.4	83.5	83.8	83.6	83.7
52	21.3	21.1	21.2	3.21	3.05	3.14	79.6	79.4	79.5	81.4	81.2	81.3
53	22.1	21.9	22.0	3.13	2.97	3.05	79.1	78.9	79.0	80.4	80.2	80.3
54	20	19.8	19.9	3.39	3.25	3.32	81.6	81.4	81.5	82.1	81.9	82.0

55	19.8	19.6	19.7	3.41	3.27	3.34	83.9	83.7	83.8	83.8	83.6	83.7
56	19.6	19.4	19.5	3.43	3.29	3.36	84.1	84.0	84.0	84	83.8	83.9
57	19.9	19.7	19.8	3.4	3.26	3.33	83.8	83.6	83.7	83.7	83.5	83.6
58	21.7	21.5	21.6	3.17	3.01	3.09	79.4	79.2	79.3	80.9	80.7	80.8
59	20.9	20.7	20.8	3.28	3.13	3.21	83.1	82.9	83.0	83.3	83.1	83.2
60	19.2	19.0	19.1	3.52	3.38	3.46	82.8	82.7	82.7	82.6	82.4	82.5
61	18.4	18.2	18.3	3.6	3.47	3.54	85.1	85.0	85.0	84.9	84.8	84.8
62	21.3	21.1	21.2	3.2	3.04	3.13	82.4	82.2	82.3	83.1	82.9	83.0
63	21.7	21.5	21.6	3.17	3.01	3.09	82.1	81.9	82.0	82.9	82.7	82.8
64	20	19.8	19.9	3.39	3.25	3.32	81.6	81.4	81.5	82.1	81.9	82.0
65	20	19.8	19.9	3.38	3.24	3.31	83.8	83.6	83.7	83.6	83.4	83.5
66	21.3	21.1	21.2	3.24	3.08	3.17	82.8	82.6	82.7	82.9	82.7	82.8
67	19	18.8	18.9	3.54	3.41	3.48	84.7	84.6	84.6	84.3	84.2	84.2
68	19.7	19.5	19.6	3.42	3.28	3.35	84	83.8	83.9	83.9	83.7	83.8
69	21.5	21.3	21.4	3.18	3.02	3.10	82.2	82.0	82.1	82.8	82.6	82.7

70	18.8	18.6	18.7	3.56	3.43	3.50	83.1	83.0	83.0	83	82.9	82.9
71	19.6	19.4	19.5	3.44	3.30	3.37	84	83.9	83.9	83.9	83.7	83.8
72	18.9	18.7	18.8	3.55	3.42	3.49	84.8	84.7	84.7	84.4	84.3	84.3
73	19.7	19.5	19.6	3.42	3.28	3.35	84	83.8	83.9	83.9	83.7	83.8
74	18.1	17.9	18.0	3.63	3.50	3.57	85.4	85.3	85.3	85.1	85.0	85.0
75	21	20.8	20.9	3.24	3.09	3.17	82.7	82.5	82.6	83.3	83.1	83.2

Table 9.28: Regression coefficient and Root Mean Square Error – Twin Screw Extrusion of sprouted proso millet flour

Product Responses	RSM		ANN	
	R^2	RMSE	R^2	RMSE
Bulk Density (kg/m^3)	0.9329	2.11	0.9789	0.85
Hardness (N)	0.9957	1.29	0.9972	0.65
Water Solubility Index (%)	0.9937	1.43	0.9993	0.69
Water Absorption Index	0.9946	1.24	0.9976	0.49
Total Color Difference	0.9964	1.41	0.9994	0.79
Expansion Ratio	0.9959	1.83	0.9990	0.93
Starch Digestibility (%)	0.9672	1.70	0.9934	0.91
Protein Digestibility (%)	0.9892	1.73	0.9947	0.84

RSM – Response Surface Methodology; ANN – Artificial Neural Network, R^2- Regression Coefficient; RMSE – Root Mean Square Error

Table 9.29: Optimization, validation and comparison of experimental data set (Bulk Density, Hardness, Water Solubility Index, Water Absorption Index) – Twin Screw Extrusion of sprouted proso millet flour

No	Moisture Content (%w.b.)	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Bulk Density kg/m^3			Hardness N			Water Solubility Index %			Water Absorption Index		
					Actual	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**		**Actual**	**Predicted**	
						RSM	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**		**RSM**	**ANN**
1	15	110	220	1	125	124	125	1.18	0.98	1.12	20.6	20.3	20.5	3.74	3.58	3.67
2	15	80	150	4	129	128	129	1.22	1.02	1.16	20.1	19.8	20.0	3.71	3.54	3.64
3	20	140	220	0	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.67	3.50	3.59
4	15	80	150	2	133	132	133	1.26	1.05	1.19	19.8	19.5	19.7	3.67	3.50	3.60
5	20	110	150	3	132	131	132	1.27	1.06	1.20	19.6	19.2	19.5	3.66	3.49	3.58
6	20	80	80	4	141	140	141	1.37	1.14	1.30	18.7	18.3	18.6	3.56	3.37	3.48
7	20	110	150	0	135	134	135	1.3	1.08	1.23	19.3	18.9	19.2	3.63	3.45	3.55
8	15	140	150	3	120	119	120	1.14	0.95	1.08	21	20.7	20.9	3.79	3.64	3.72

9	20	110	150	1	134	133	134	1.29	1.08	1.22	19.4	19.0	19.3	3.64	3.47	3.56
10	20	110	150	2	133	132	133	1.28	1.07	1.21	19.5	19.1	19.4	3.65	3.48	3.57

Table 9.30: Optimization, validation and comparison of experimental data set (Total Color Difference, Expansion Ratio, Protein Digestibility, Starch Digestibility) – Twin Screw Extrusion of sprouted proso millet flour

No	Moisture Content (%w.b.)	Screw Speed (rpm)	Die Temperature (°C)	Germination time (Days)	Total Color Difference			Expansion ratio			Protein Digestibility %			Starch Digestibility %		
					Actual	Predicted		Actual	Predicted		Actual	Predicted		Actual	Predicted	
						RSM	ANN		RSM	ANN		RSM	ANN		RSM	ANN
1	15	110	220	1	18.7	18.5	18.6	3.57	3.44	3.51	84.8	84.7	84.7	84.6	84.5	84.5
2	15	80	150	4	19.1	18.9	19.0	3.5	3.36	3.44	84.5	84.4	84.4	84.2	84.0	84.1
3	20	140	220	0	19.7	19.5	19.6	3.43	3.29	3.36	82.1	81.9	82.0	82.5	82.3	82.4
4	15	80	150	2	19.5	19.3	19.4	3.46	3.32	3.39	84.2	84.1	84.1	83.8	83.6	83.7
5	20	110	150	3	19.7	19.5	19.6	3.42	3.28	3.35	84	83.8	83.9	83.9	83.7	83.8
6	20	80	80	4	20.7	20.5	20.6	3.3	3.15	3.23	83.3	83.1	83.2	83.5	83.3	83.4
7	20	110	150	0	20	19.8	19.9	3.39	3.25	3.32	81.6	81.4	81.5	82.1	81.9	82.0
8	15	140	150	3	18.2	18.0	18.1	3.62	3.49	3.56	85.3	85.2	85.2	85	84.9	84.9

9	20	110	150	1	19.9	19.7	19.8	3.4	3.26	3.33	83.8	83.6	83.7	83.7	83.5	83.6
10	20	110	150	2	19.8	19.6	19.7	3.41	3.27	3.34	83.9	83.7	83.8	83.8	83.6	83.7

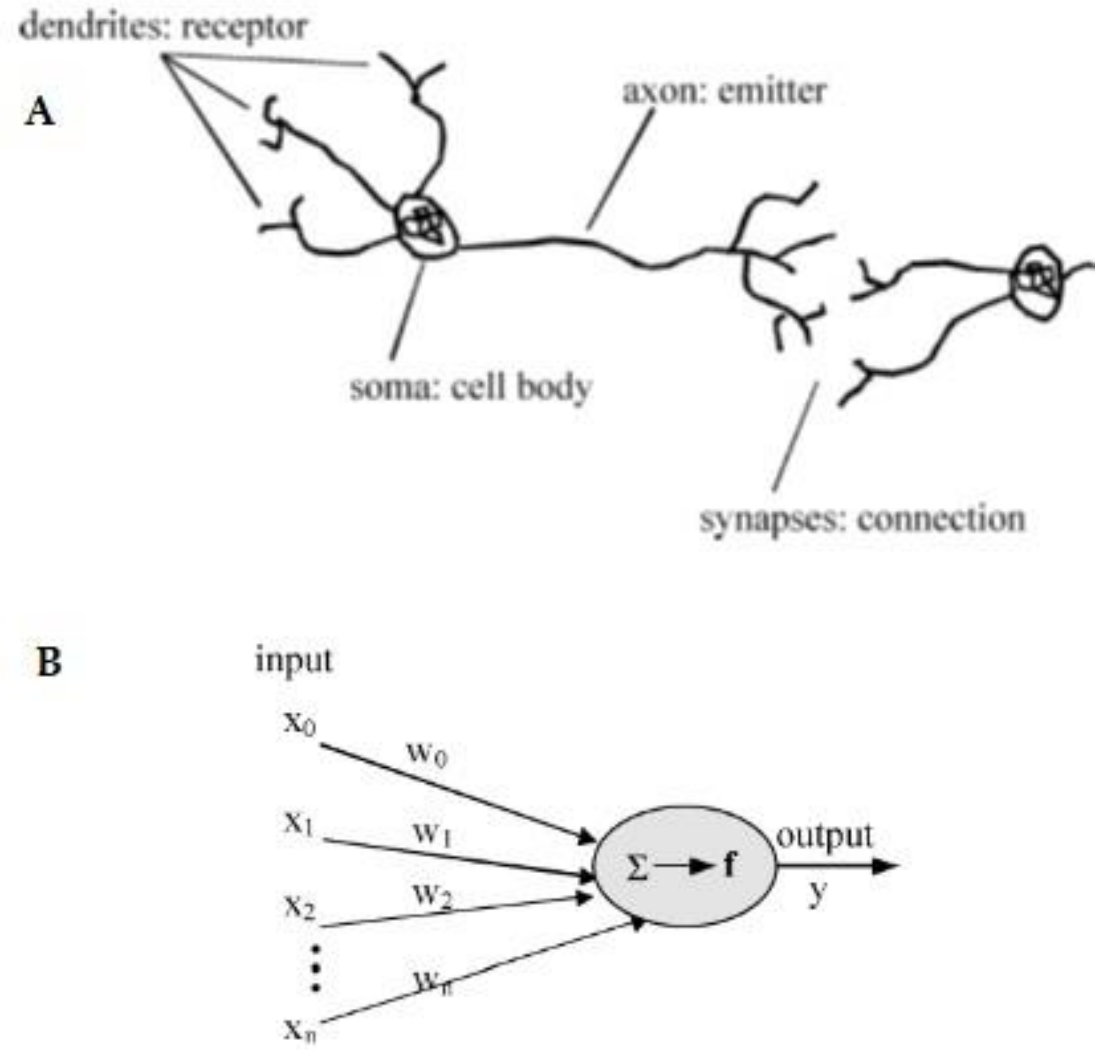


Figure 9.1: (A) Schematic representation of a biological neuron (B) Basic design of an Artificial Neural Network

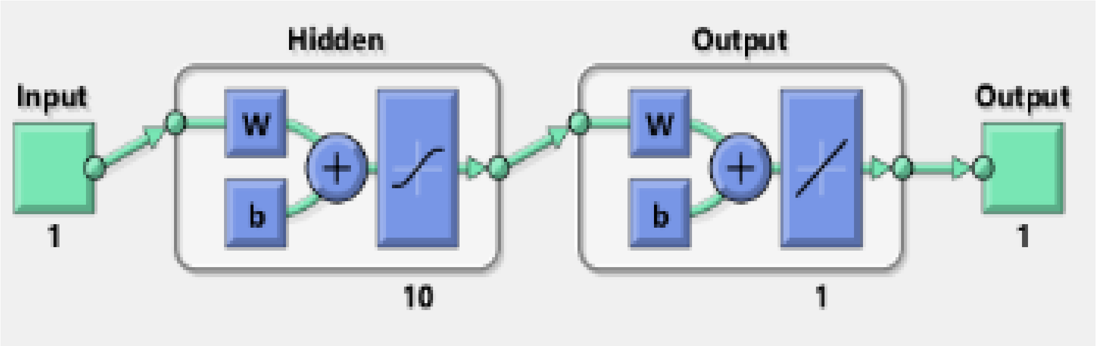


Figure 9.2: Three-layered feedforward networks were used with a backpropagation algorithm

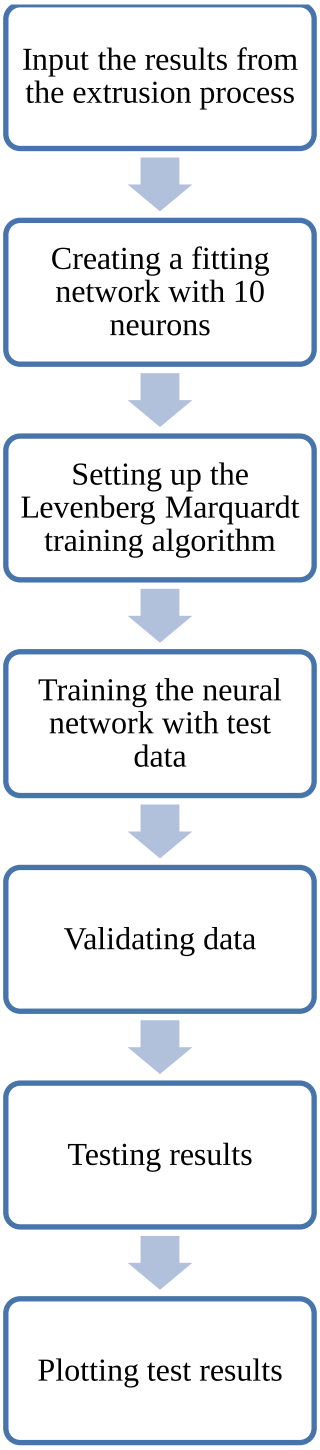


Figure 9.3: Neural Network methodology for training and validating data

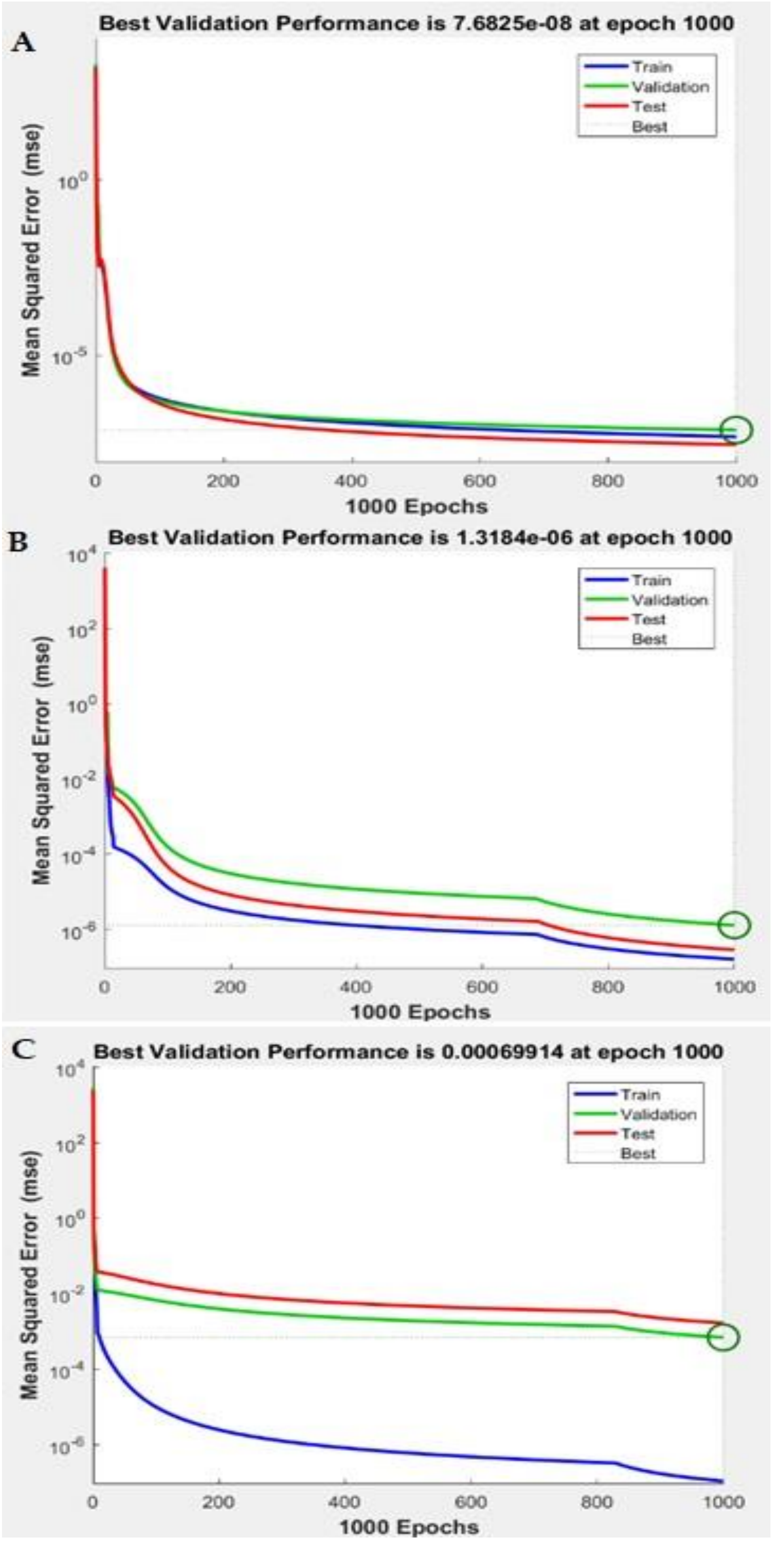


Figure 9.4: Validation of predicted data (A) Single-screw extrusion of sprouted quinoa (B) Single-screw extrusion of sprouted quinoa-corn meal blend (C) Twin-screw extrusion of sprouted quinoa

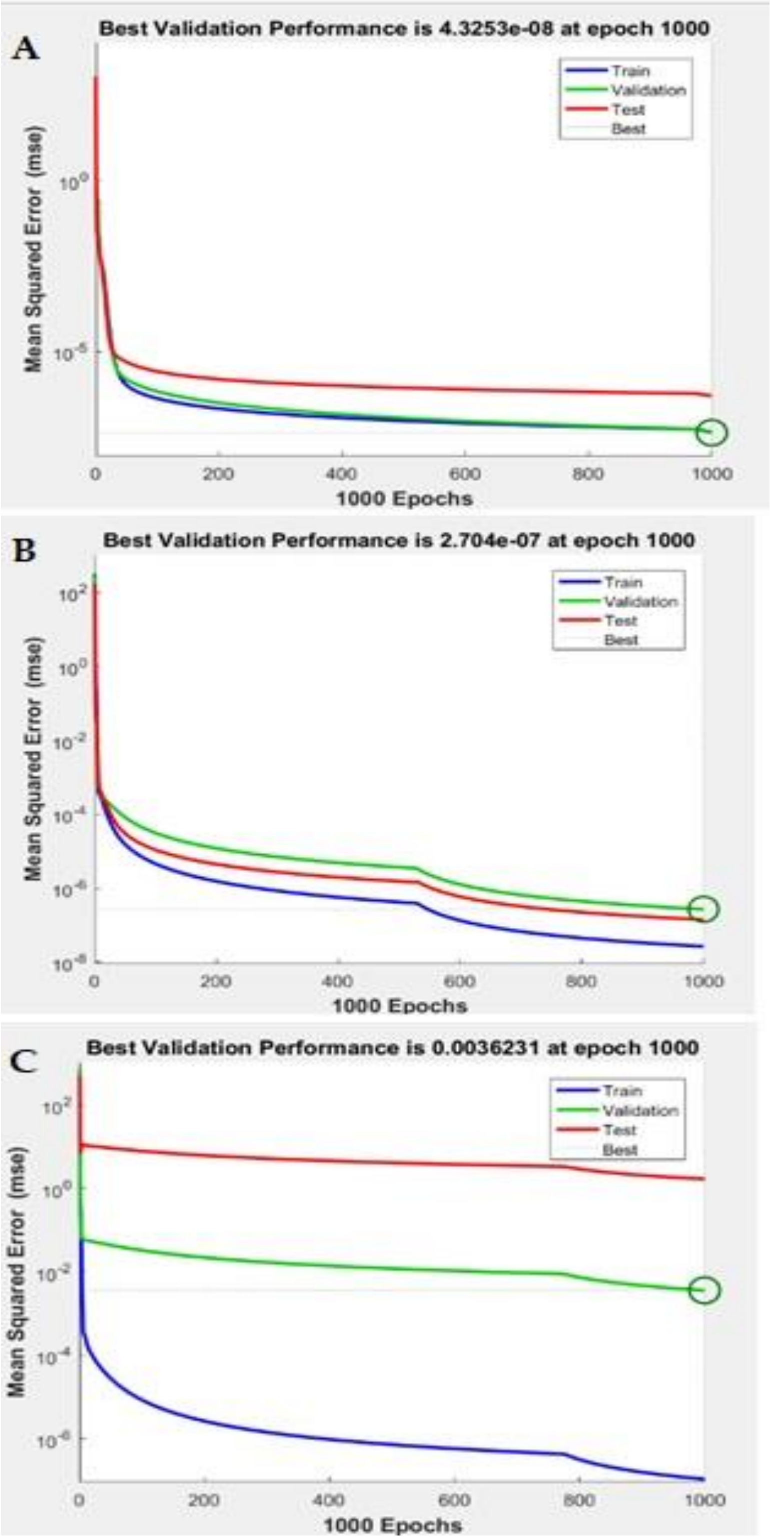


Figure 9.5: Validation of predicted data (A) Single-screw extrusion of sprouted proso millet (B) Single-screw extrusion of sprouted proso millet-corn meal blend (C) Twin-screw extrusion of sprouted proso millet

Program 9.1

```
% Solve an Input-Output Fitting problem with a Neural Network
% Script generated by Neural Fitting app
% This script assumes these variables are defined:
% data - input data.
% data - target data.
 x = data';
t = data';

% Choose a Training Function
% For a list of all training functions type: help nntrain
% 'trainlm' is usually fastest.
% 'trainbr' takes longer but may be better for challenging problems.
% 'trainscg' uses less memory. Suitable in low memory situations.
trainFcn = 'trainlm'; % Levenberg-Marquardt backpropagation.

% Create a Fitting Network
hiddenLayerSize = 10;
net = fitnet(hiddenLayerSize,trainFcn);

% Choose Input and Output Pre/Post-Processing Functions
% For a list of all processing functions type: help nnprocess
net.input.processFcns = {'removeconstantrows','mapminmax'}x;
net.output.processFcns = {'removeconstantrows','mapminmax'};
```

```
% Setup Division of Data for Training, Validation, Testing
% For a list of all data division functions type: help nndivide
net.divideFcn = 'dividerand'; % Divide data randomly
net.divideMode = 'sample'; % Divide up every sample
net.divideParam.trainRatio = 70/100;
net.divideParam.valRatio = 15/100;
net.divideParam.testRatio = 15/100;

% Choose a Performance Function
% For a list of all performance functions type: help nnperformance
net.performFcn = 'mse'; % Mean Squared Error

% Choose Plot Functions
% For a list of all plot functions type: help nnplot
net.plotFcns = {'plotperform','plottrainstate','ploterrhist',...
 'plotregression', 'plotfit'};

% Train the Network
[net,tr] = train(net,x,t);

% Test the Network
y = net(x);
e = gsubtract(t,y);
performance = perform(net,t,y)
```

```
% Recalculate Training, Validation and Test Performance
trainTargets = t.* tr.trainMask{1};
valTargets = t.* tr.valMask{1};
testTargets = t.* tr.testMask{1};
trainPerformance = perform(net,trainTargets,y)
valPerformance = perform(net,valTargets,y)
testPerformance = perform(net,testTargets,y)

% View the Network
view(net)

% Plots
% Uncomment these lines to enable various plots.
%figure, plotperform(tr)
%figure, plottrainstate(tr)
%figure, ploterrhist(e)
%figure, plotregression(t,y)
%figure, plotfit(net,x,t)

% Deployment
% Change the (false) values to (true) to enable the following code blocks.
% See the help for each generation function for more information.
if (false)
 % Generate MATLAB function for neural network for application
 % deployment in MATLAB scripts or with MATLAB Compiler and Builder
```

```
 % tools, or simply to examine the calculations your trained neural
 % network performs.
 genFunction(net,'myNeuralNetworkFunction');
 y = myNeuralNetworkFunction(x);
end
if (false)
 % Generate a matrix-only MATLAB function for neural network code
 % generation with MATLAB Coder tools.
 genFunction(net,'myNeuralNetworkFunction','MatrixOnly','yes');
 y = myNeuralNetworkFunction(x);
end
if (false)
 % Generate a Simulink diagram for simulation or deployment with.
 % Simulink Coder tools.
 gensim(net);
end
```

CHAPTER 10: CONCLUSIONS

The specific conclusions from this dissertation are as follows:

1. Soaking and germination significantly reduced the saponin and phytic acid content of quinoa flour. Saponin content reduced from 0.80g/100g (raw quinoa) to 0.10g/100g (Day 4 germination). The phytic acid content reduced from 1.10g/100g (raw quinoa) to 0.21g/100 g (Day 4 germination).
2. Single screw extrusion of sprouted quinoa-corn meal blend showed higher expansion ratio. The addition of 30% corn meal significantly increased the expansion ratio from 3.11 – 3.56 (sprouted quinoa) to (3.29 – 3.96) (sprouted quinoa and corn meal blend)
3. Increasing the corn meal ratio to 30% decreased the total color difference between the extrudates from 20.9 – 21.3 (sprouted quinoa flour-control) to 13.1 – 16.1.
4. Corn meal addition to sprouted quinoa flour reduced the protein and starch digestibility. The addition of 30% corn meal reduced the starch digestibility from 80.4 – 85.7% to 77.7 – 78.2% and protein digestibility from 80.1– 86.3% to 77.1 – 78.5%.
5. The single screw extrusion processing of sprouted quinoa flour showed that the protein digestibility and starch digestibility are (80.1 – 86.3%) and (80.4 – 85.7%), respectively. On the other hand, the twin screw extrusion processing showed lower protein digestibility (78.4 – 84.1%) and starch digestibility (77.1 – 83.4%).
6. Single screw extrusion processing of sprouted quinoa flour proved to be more promising than twin screw extrusion processing in terms of product quality. The single screw sprouted quinoa extrudates had a higher expansion ratio (3.1 – 3.56) than twin screw extrudates (2.28 – 2.83).

7. The water solubility index (11.5 – 16.5%) and water absorption index (2.36 – 3.51) of single screw extruded sprouted quinoa was significantly lower than the water solubility index (14.4-18.5%) and water absorption index (2.93 – 3.41) of twin screw extruded sprouted quinoa
8. Soaking and germination significantly reduced the phytic acid content from 1.5g/100g (raw quinoa) to 0.2g/100 g (Day 4 germination).
9. The addition of 30% corn meal to sprouted proso millet in the single screw extrusion process significantly increased the expansion ratio from 3.10 – 3.65 to 3.35 – 3.96.
10. The total color difference of the single screw extruded proso millet extrudates was significantly reduced from 20.9 – 21.3 to 13.1 – 16.12 (only sprouted proso millet flour) by 30% corn meal addition.
11. Addition of 30% corn meal reduced the overall starch and protein digestibility of sprouted proso millets. The addition of 30% corn meal reduced the protein digestibility from 85.9% to 78.5%. and starch digestibility from 85.8% to 77.7%.
12. The water absorption index (2.93-3.41) of twin screw extruded sprouted proso millet was significantly lower than the water absorption index (4.0-4.4) of single screw extruded sprouted proso millet.
13. The expansion ratio of sprouted quinoa (3.29 – 3.96) and sprouted proso millet (3.35 – 3.96) were in the same range when 30% corn meal was added.
14. RSM predicted regression coefficients in the range (0.7637-0.9969) and root mean square error in the range (0.98-2.16) for sprouted quinoa extrusion processes and the corresponding ANN predictions were in the range (0.9121-0.9999) and (0.32-0.98) respectively

15. RSM predicted regression coefficients in the range (0.871-0.999) and root mean square error in the range (1.13-2.30) for sprouted proso millet extrusion processes and the corresponding ANN predictions were in the range (0.937-0.999) and (0.36 – 1.03), respectively.
16. The dissertation highlights that it is possible to manufacture puffed snacks using single screw extrusion of sprouted quinoa – corn meal blend and sprouted proso millet – corn meal blend.

CHAPTER 11: RECOMMENDATIONS FOR FUTURE WORK

We discovered several important findings from this timely research work. At the same time, it also showed some new ideas of interest for future research and development, which are summarized as follows:

1. Replacing corn meal with novel gluten free cereals like cahokia rice, buckwheat, amaranth and other millet varieties without changing the main source of protein (quinoa and proso millet).
2. The quinoa and proso millet could also be combined with legumes like fava bean, navy beans and chickpea to present a complete plant protein based extruded food.
3. Studying the effect of other extrusion parameters such as different screw configuration, die nozzles, feed rate and varying the particle size of raw ingredients.
4. Further studies on the internal structure and changes in molecular conformation can be investigated by varying one of the processing conditions such as extrusion die temperature, screw speed and feed moisture content.
5. Developing a viscosity model for the single screw and conical twin screw extrusion of proso millet and quinoa. This will help in analyzing the changes in apparent viscosity in the die during extrusion.
6. Future research in using different post processing methods such as frying might be useful in developing acceptable snacks with addition of flavors.
7. Sensory analysis of the extruded products will be an important study to understand consumer preference and modify ingredients and procedures.
8. It is relevant to investigate possible scale-up of the extrusion studies using pilot-scale extruders which might find potential application in snack food industries.

APPENDIX A - RESULTS OF SINGLE SCREW EXTRUSION OF SPROUTED QUINOA

No	**Run**	**Moisture Content**	**Screw Speed**	**Die Temperature**	**Germination time**	**Bulk Density**	**Hardness**	**Water Solubility Index**	**Water Absorption Index**	**Total Color Difference ΔE**	**Expansion ratio**	**Protein digestibility**	**Starch digestibility**
		%w.b.	**rpm**	**°C**	**Day**	**kg/m^3**	**N**	**%**				**%**	**%**
1	49	15	80	115	0	138	1.16	15.2	2.80	21.1	3.42	83.5	81.1
2	38	25	80	115	0	149	1.63	11.5	2.36	16.5	2.48	80.5	80.9
3	44	15	220	115	0	128	1.10	16.2	3.41	21.7	3.71	83.0	82.0
4	73	25	220	115	0	149	1.69	13.0	2.51	18.4	3.04	81.9	81.4
5	37	15	150	90	0	130	1.13	15.8	2.94	20.5	3.57	83.9	81.9
6	62	25	150	90	0	146	1.50	12.0	2.42	15.8	2.68	81.5	80.5
7	30	15	150	140	0	132	1.27	16.1	3.22	21.4	3.68	83.0	82.1
8	48	25	150	140	0	154	1.80	12.6	2.50	17.1	2.81	81.7	81.0

9	3	20	80	90	0	145	1.30	12.4	2.49	18.4	2.92	81.0	80.7
10	2	20	220	90	0	136	1.29	14.4	2.69	17.4	3.03	82.0	80.9
11	7	20	80	140	0	143	1.40	12.9	2.51	20.9	3.18	82.5	81.0
12	29	20	220	140	0	141	1.47	15.1	2.74	21.4	3.51	82.1	81.9
13	51	20	150	115	0	140	1.36	13.6	2.72	19.8	3.32	83.0	81.6
14	36	20	150	115	0	140	1.36	13.6	2.72	19.8	3.32	83.0	81.6
15	14	20	150	115	0	140	1.36	13.6	2.72	19.8	3.32	83.0	81.6
16	42	15	80	115	1	136	1.13	15.4	2.83	20.8	3.44	85.5	83.8
17	10	25	80	115	1	148	1.60	11.7	2.36	16.2	2.52	83.6	83.5
18	35	15	220	115	1	125	1.70	16.3	3.43	21.6	3.72	86.1	85.3
19	58	25	220	115	1	145	1.65	13.2	2.52	18.1	3.06	85.1	84.3
20	33	15	150	90	1	129	1.10	15.9	2.96	20.3	3.58	85.6	84.6
21	4	25	150	90	1	144	1.46	12.2	2.43	15.5	2.72	84.3	83.3
22	69	15	150	140	1	131	1.22	16.2	3.26	21.2	3.69	85.8	85.1

23	65	25	150	140	1	151	1.77	12.7	2.50	17.0	2.85	85.1	83.9
24	5	20	80	90	1	143	1.25	12.6	2.49	18.2	2.94	84.1	83.5
25	19	20	220	90	1	133	1.25	14.6	2.69	17.3	3.07	84.8	83.5
26	59	20	80	140	1	142	1.36	13.1	2.53	20.7	3.21	85.0	84.2
27	41	20	220	140	1	141	1.45	15.1	2.76	21.3	3.47	85.7	84.8
28	39	20	150	115	1	138	1.35	13.8	2.73	19.6	3.35	85.3	84.5
29	34	20	150	115	1	138	1.35	13.8	2.73	19.6	3.35	85.3	84.5
30	25	20	150	115	1	138	1.35	13.8	2.73	19.6	3.35	85.3	84.5
31	43	15	80	115	2	135	1.10	15.6	2.86	20.7	3.47	85.7	84.0
32	56	25	80	115	2	145	1.56	11.8	2.37	16.1	2.56	83.7	83.6
33	71	15	220	115	2	122	1.15	16.4	3.46	21.4	3.74	86.2	85.5
34	74	25	220	115	2	143	1.63	13.3	2.52	17.9	3.08	85.2	84.5
35	45	15	150	90	2	127	1.08	16.0	2.99	20.2	3.60	85.7	84.8
36	15	25	150	90	2	144	1.43	12.4	2.43	15.4	2.75	84.4	83.5

37	17	15	150	140	2	129	1.19	16.2	3.28	21.1	3.70	86.0	85.3
38	31	25	150	140	2	148	1.73	12.9	2.51	16.8	2.88	85.1	84.0
39	12	20	80	90	2	142	1.22	12.7	2.50	17.9	2.95	84.3	83.0
40	13	20	220	90	2	130	1.23	14.8	2.70	17.1	3.11	85.0	83.7
41	21	20	80	140	2	140	1.33	13.3	2.53	20.5	3.25	85.2	84.4
42	67	20	220	140	2	140	1.43	15.2	2.77	21.1	3.43	85.7	85.0
43	61	20	150	115	2	137	1.31	13.9	2.74	19.3	3.37	85.4	84.7
44	8	20	150	115	2	137	1.31	13.9	2.74	19.3	3.37	85.4	84.7
45	50	20	150	115	2	137	1.31	13.9	2.74	19.3	3.37	85.4	84.7
46	66	15	80	115	3	132	1.09	15.7	2.88	20.5	3.48	85.8	84.2
47	53	25	80	115	3	144	1.55	11.9	2.39	15.8	2.59	83.9	83.8
48	40	15	220	115	3	120	1.15	16.4	3.48	21.1	3.75	86.4	85.7
49	11	25	220	115	3	142	1.60	13.4	2.52	17.8	3.08	85.4	84.6
50	70	15	150	90	3	126	1.07	16.1	3.03	20.0	3.61	85.8	84.9

51	1	25	150	90	3	143	1.41	12.5	2.45	15.1	2.77	84.6	83.6
52	60	15	150	140	3	126	1.17	16.3	3.33	21.1	3.72	86.1	85.4
53	72	25	150	140	3	146	1.69	13.1	2.51	16.4	2.89	85.2	84.2
54	75	20	80	90	3	140	1.21	12.7	2.51	17.6	3.00	84.3	83.1
55	52	20	220	90	3	127	1.22	14.8	2.72	17.0	3.12	85.2	83.8
56	16	20	80	140	3	140	1.29	13.3	2.54	20.4	3.29	85.3	84.6
57	57	20	220	140	3	137	1.41	15.3	2.77	20.8	3.41	85.8	85.1
58	27	20	150	115	3	135	1.28	14.0	2.76	19.2	3.38	85.6	84.8
59	54	20	150	115	3	135	1.28	14.0	2.76	19.2	3.38	85.6	84.8
60	26	20	150	115	3	135	1.28	14.0	2.76	19.2	3.38	85.6	84.8
61	68	15	80	115	4	129	1.07	15.8	2.94	20.4	3.51	86.0	84.3
62	24	25	80	115	4	144	1.53	12.1	2.41	15.5	2.60	84.1	84.0
63	22	15	220	115	4	116	1.13	16.5	3.51	20.8	3.75	86.5	85.8
64	20	25	220	115	4	140	1.58	13.4	2.54	17.6	3.09	85.5	84.8

65	28	15	150	90	4	122	1.05	16.2	3.05	19.7	3.63	85.4	85.1
66	55	25	150	90	4	141	1.37	12.7	2.47	14.8	2.81	84.7	83.7
67	47	15	150	140	4	125	1.15	16.4	3.35	20.9	3.74	86.3	85.6
68	9	25	150	140	4	146	1.64	13.2	2.53	16.2	2.91	85.3	84.4
69	63	20	80	90	4	140	1.19	12.8	2.51	17.4	3.04	84.5	83.2
70	6	20	220	90	4	123	1.20	14.9	2.73	17.0	3.15	85.3	84.0
71	64	20	80	140	4	138	1.27	13.4	2.54	20.1	3.30	85.5	84.7
72	32	20	220	140	4	135	1.37	15.4	2.79	20.6	3.40	86.0	85.3
73	18	20	150	115	4	132	1.25	14.2	2.77	18.8	3.41	85.7	85.0
74	23	20	150	115	4	132	1.25	14.2	2.77	18.8	3.41	85.7	85.0
75	46	20	150	115	4	132	1.25	14.2	2.77	18.8	3.41	85.7	85.0

APPENDIX B: RESULTS OF SINGLE SCREW EXTRUSION OF SPROUTED QUINOA-CORN MEAL BLEND

No	**Run**	**Moisture Content**	**Screw Speed**	**Die Temperature**	**Corn meal Ratio**	**Germination time**	**Bulk Density**	**Hardness**	**Water Solubility Index**	**Water Absorption Index**	**Total Color Difference ΔE**	**Expansion ratio**	**Protein digestibility**	**Starch digestibility**
		%w.b.	**rpm**	**°C**	**%**	**Day**	**kg/m^3**	**N**	**%**				**%**	**%**
1	1	15	80	115	15	0	119	3.59	1.13	16.5	3.46	18.5	81.1	81.1
2	13	25	80	115	15	0	145	3.11	1.60	13.3	2.68	16.0	80.8	79.8
3	34	15	220	115	15	0	115	3.71	0.95	17.3	3.67	16.9	83.1	83.3
4	49	25	220	115	15	0	132	3.15	1.25	13.7	2.46	17.6	82.3	81.6
5	5	20	150	90	0	0	137	3.20	1.24	13.3	2.70	20.6	83.6	83.2
6	77	20	150	140	0	0	130	3.35	1.35	13.6	2.74	17.6	83.8	83.8
7	116	20	150	90	30	0	125	3.55	1.04	12.4	3.38	14.8	78.5	78.4
8	23	20	150	140	30	0	116	3.67	0.97	13.4	3.67	16.7	79.4	80.3

9	37	15	150	115	0	0	128	3.53	1.16	15.8	3.30	21.4	83.7	84.5
10	4	25	150	115	0	0	145	2.75	1.46	12.7	2.44	16.7	84.1	83.0
11	113	15	150	115	30	0	107	3.88	0.91	19.5	3.75	14.5	78.8	77.6
12	108	25	150	115	30	0	128	3.30	1.14	14.4	3.67	14.8	78.4	78.5
13	124	20	80	90	15	0	129	3.30	1.23	12.8	3.30	16.0	79.2	78.5
14	66	20	220	90	15	0	127	3.39	1.22	14.1	3.50	16.7	79.6	80.0
15	79	20	80	140	15	0	130	3.24	1.23	12.4	3.47	17.3	80.8	80.3
16	115	20	220	140	15	0	120	3.52	1.13	13.7	3.55	17.8	79.0	81.5
17	43	15	150	90	15	0	120	3.57	1.14	16.4	3.44	18.2	80.4	80.2
18	53	25	150	90	15	0	130	3.11	1.62	12.8	2.68	17.6	79.0	77.5
19	119	15	150	140	15	0	112	3.77	1.05	17.9	3.63	17.4	81.3	82.6
20	84	25	150	140	15	0	128	3.24	1.23	13.7	2.46	17.8	82.6	82.0
21	132	20	80	115	0	0	145	2.98	1.28	12.3	2.46	17.9	83.2	81.4
22	18	20	220	115	0	0	132	3.15	1.25	14.4	2.68	18.0	84.1	84.3

23	61	20	80	115	30	0	124	3.58	1.10	12.6	3.47	15.6	78.4	79.2
24	105	20	220	115	30	0	116	3.63	1.01	12.9	3.55	16.4	79.7	79.4
25	85	20	150	115	15	0	124	3.50	1.17	13.4	3.50	16.7	79.3	81.0
26	30	20	150	115	15	0	124	3.50	1.17	13.4	3.50	16.7	79.3	81.0
27	35	20	150	115	15	0	124	3.50	1.17	13.4	3.50	16.7	79.3	81.0
28	103	15	80	115	15	1	118	3.60	1.12	16.6	3.47	18.4	82.7	82.0
29	129	25	80	115	15	1	143	3.12	1.59	13.5	2.70	16.4	78.4	79.8
30	74	15	220	115	15	1	114	3.73	0.94	17.4	3.68	16.8	84.0	84.2
31	65	25	220	115	15	1	130	3.17	1.23	13.8	2.48	17.4	82.4	81.8
32	51	20	150	90	0	1	136	3.23	1.22	13.5	2.72	20.4	85.0	84.2
33	52	20	150	140	0	1	128	3.38	1.32	13.8	2.76	17.4	85.2	84.7
34	60	20	150	90	30	1	124	3.56	1.03	12.5	3.39	14.9	78.8	78.5
35	122	20	150	140	30	1	115	3.68	0.96	13.4	3.68	17.0	79.5	80.4
36	71	15	150	115	0	1	126	3.55	1.14	16.1	3.32	21.2	85.5	85.3

37	59	25	150	115	0	1	143	2.78	1.43	12.9	2.46	16.5	85.1	84.1
38	33	15	150	115	30	1	106	3.90	0.90	19.6	3.76	14.4	80.1	79.8
39	111	25	150	115	30	1	127	3.33	1.14	14.6	3.68	14.9	78.5	78.8
40	10	20	80	90	15	1	128	3.33	1.22	12.9	3.33	16.4	79.3	78.8
41	2	20	220	90	15	1	126	3.41	1.20	14.2	3.53	17.0	79.7	80.2
42	91	20	80	140	15	1	129	3.27	1.22	12.5	3.50	17.5	78.4	80.6
43	14	20	220	140	15	1	119	3.56	1.12	13.8	3.57	18.1	79.2	81.8
44	92	15	150	90	15	1	118	3.58	1.13	16.5	3.45	18.1	81.7	81.5
45	121	25	150	90	15	1	128	3.12	1.61	12.9	2.70	17.4	79.2	77.6
46	97	15	150	140	15	1	111	3.78	1.04	18.0	3.64	17.3	82.9	83.9
47	62	25	150	140	15	1	127	3.27	1.22	13.8	2.48	18.1	82.8	82.2
48	89	20	80	115	0	1	143	3.02	1.25	12.5	2.48	17.7	84.2	82.9
49	99	20	220	115	0	1	130	3.17	1.23	14.6	2.70	17.8	85.4	85.0
50	112	20	80	115	30	1	123	3.59	1.09	12.7	3.50	15.7	78.5	79.3

51	80	20	220	115	30	1	115	3.64	1.00	13.0	3.57	16.5	79.8	79.5
52	75	20	150	115	15	1	123	3.53	1.16	13.5	3.54	17.0	79.5	81.1
53	45	20	150	115	15	1	123	3.53	1.16	13.5	3.54	17.0	79.5	81.1
54	46	20	150	115	15	1	123	3.53	1.16	13.5	3.54	17.0	79.5	81.1
55	44	15	80	115	15	2	117	3.61	1.11	16.7	3.48	18.3	82.8	82.1
56	32	25	80	115	15	2	142	3.13	1.58	13.6	2.71	16.5	78.5	80.0
57	98	15	220	115	15	2	113	3.75	0.03	17.5	3.69	16.7	84.1	84.3
58	94	25	220	115	15	2	129	3.18	1.22	13.9	2.49	17.3	82.5	81.9
59	8	20	150	90	0	2	134	3.24	1.21	13.6	2.73	20.3	85.1	84.3
60	12	20	150	140	0	2	127	3.39	1.30	13.9	2.77	17.3	85.3	84.8
61	106	20	150	90	30	2	123	3.57	1.02	12.6	3.40	15.0	78.9	78.6
62	130	20	150	140	30	2	114	3.69	0.95	13.5	3.69	17.1	79.6	80.5
63	78	15	150	115	0	2	124	3.56	1.13	16.2	3.33	21.1	85.6	85.4
64	54	25	150	115	0	2	142	2.79	1.41	13.0	2.47	16.4	85.2	84.2

65	83	15	150	115	30	2	105	3.92	0.89	19.7	3.77	14.3	80.2	80.0
66	28	25	150	115	30	2	128	3.34	1.14	14.7	3.69	15.0	78.6	78.9
67	47	20	80	90	15	2	127	3.34	1.21	13.0	3.34	16.5	79.4	78.9
68	38	20	220	90	15	2	125	3.42	1.19	14.3	3.54	17.1	79.8	80.3
69	39	20	80	140	15	2	128	3.28	1.21	12.6	3.52	17.6	78.5	80.7
70	6	20	220	140	15	2	118	3.57	1.11	13.9	3.58	18.2	79.3	81.9
71	96	15	150	90	15	2	117	3.59	1.12	16.6	3.46	18.0	81.8	81.6
72	110	25	150	90	15	2	127	3.13	1.60	13.0	2.71	17.3	79.3	77.7
73	135	15	150	140	15	2	110	3.80	1.03	18.1	3.65	17.2	83.0	84.0
74	25	25	150	140	15	2	126	3.28	1.21	13.9	2.49	18.2	82.0	82.3
75	50	20	80	115	0	2	142	3.03	1.24	12.6	2.49	17.6	84.3	83.0
76	93	20	220	115	0	2	129	3.18	1.22	14.7	2.71	17.7	85.5	85.1
77	76	20	80	115	30	2	122	3.60	1.08	12.8	3.52	15.8	78.6	79.4
78	127	20	220	115	30	2	114	3.65	0.99	13.1	3.58	16.6	80.3	79.6

79	21	20	150	115	15	2	122	3.54	1.15	13.6	3.55	17.1	79.6	81.2
80	107	20	150	115	15	2	122	3.54	1.15	13.6	3.55	17.1	79.6	81.2
81	9	20	150	115	15	2	122	3.54	1.15	13.6	3.55	17.1	79.6	81.2
82	101	15	80	115	15	3	116	3.62	1.10	16.8	3.49	18.2	82.9	82.2
83	70	25	80	115	15	3	141	3.14	1.57	13.7	2.72	16.6	78.6	80.1
84	128	15	220	115	15	3	112	3.76	0.92	17.6	3.70	16.6	84.2	84.4
85	24	25	220	115	15	3	128	3.19	1.21	14.0	2.50	17.2	82.6	82.0
86	104	20	150	90	0	3	133	3.25	1.20	13.7	2.74	20.2	85.2	84.4
87	67	20	150	140	0	3	126	3.40	1.29	14.0	2.78	17.2	85.4	84.9
88	72	20	150	90	30	3	122	3.58	1.01	12.7	3.41	15.1	79.0	78.7
89	82	20	150	140	30	3	113	3.70	0.94	13.5	3.70	17.2	79.7	80.6
90	69	15	150	115	0	3	123	3.57	1.10	16.3	3.34	21.0	85.7	85.5
91	125	25	150	115	0	3	141	2.80	1.39	13.1	2.48	16.3	85.2	84.3
92	48	15	150	115	30	3	104	3.94	0.88	19.8	3.78	14.2	80.3	80.1

93	56	25	150	115	30	3	127	3.35	1.13	14.8	3.70	15.1	78.7	79.0
94	15	20	80	90	15	3	126	3.35	1.20	13.1	3.35	16.6	79.5	79.0
95	81	20	220	90	15	3	124	3.43	1.18	14.4	3.55	17.2	80.0	80.4
96	64	20	80	140	15	3	127	3.29	1.20	12.7	3.53	17.7	78.6	80.8
97	134	20	220	140	15	3	117	3.58	1.10	14.0	3.59	18.3	79.4	82.0
98	3	15	150	90	15	3	116	3.60	1.11	16.7	3.47	17.9	81.9	81.7
99	117	25	150	90	15	3	126	3.14	1.59	13.1	2.72	17.2	79.4	77.8
100	131	15	150	140	15	3	109	3.81	1.02	18.2	3.66	17.1	83.1	84.1
101	133	25	150	140	15	3	125	3.29	1.20	14.0	2.50	18.3	83.0	82.4
102	42	20	80	115	0	3	141	3.04	1.23	12.7	2.50	17.5	84.4	83.1
103	126	20	220	115	0	3	128	3.19	1.21	14.8	2.72	17.6	85.6	85.2
104	88	20	80	115	30	3	121	3.61	1.07	12.9	3.53	15.9	78.7	79.5
105	86	20	220	115	30	3	113	3.66	0.98	13.2	3.59	16.7	80.6	79.7
106	31	20	150	115	15	3	121	3.55	1.14	13.7	3.56	17.2	79.7	81.3

107	63	20	150	115	15	3	121	3.55	1.14	13.7	3.56	17.2	79.7	81.3
108	40	20	150	115	15	3	121	3.55	1.14	13.7	3.56	17.2	79.7	81.3
109	90	15	80	115	15	4	115	3.63	1.09	16.9	3.50	18.1	83.0	82.3
110	17	25	80	115	15	4	140	3.15	1.56	13.8	2.73	16.7	78.7	80.2
111	102	15	220	115	15	4	111	3.78	0.91	17.7	3.71	16.5	84.3	84.5
112	29	25	220	115	15	4	127	3.20	1.20	14.1	2.51	17.1	82.7	82.1
113	36	20	150	90	0	4	132	3.26	1.19	13.8	2.75	20.1	85.3	84.5
114	41	20	150	140	0	4	125	3.41	1.28	14.2	2.79	17.1	85.5	85.0
115	57	20	150	90	30	4	121	3.59	1.00	12.8	3.42	15.2	79.1	78.8
116	87	20	150	140	30	4	112	3.71	0.93	13.5	3.71	17.3	79.8	80.7
117	123	15	150	115	0	4	122	3.58	1.11	16.4	3.35	20.9	85.8	85.6
118	68	25	150	115	0	4	140	2.81	1.37	13.2	2.49	16.2	85.3	84.4
119	11	15	150	115	30	4	102	3.97	0.87	19.9	3.79	14.1	80.4	80.2
120	120	25	150	115	30	4	126	3.36	1.13	14.9	3.71	15.2	78.8	79.1

121	19	20	80	90	15	4	125	3.36	1.19	13.2	3.36	16.7	79.6	79.1
122	55	20	220	90	15	4	123	3.44	1.17	14.5	3.56	17.3	80.1	80.5
123	95	20	80	140	15	4	126	3.30	1.19	12.8	3.54	17.8	78.7	80.2
124	22	20	220	140	15	4	116	3.59	1.09	14.1	3.60	18.4	79.5	82.1
125	26	15	150	90	15	4	115	3.62	1.10	16.8	3.48	17.8	82.0	81.8
126	58	25	150	90	15	4	125	3.15	1.58	13.2	2.73	17.1	79.5	77.9
127	114	15	150	140	15	4	107	3.83	1.01	18.3	3.67	17.0	83.2	84.2
128	16	25	150	140	15	4	124	3.30	1.19	14.1	2.51	18.4	83.1	82.5
129	27	20	80	115	0	4	140	3.05	1.22	12.8	2.51	17.4	84.5	83.2
130	73	20	220	115	0	4	127	3.20	1.20	14.9	2.73	17.5	85.7	85.3
131	100	20	80	115	30	4	120	3.62	1.06	13.0	3.54	16.0	78.8	79.6
132	7	20	220	115	30	4	112	3.67	0.97	13.3	3.60	16.8	80.7	79.8
133	109	20	150	115	15	4	120	3.56	1.13	13.8	3.57	17.3	79.8	81.4
134	118	20	150	115	15	4	120	3.56	1.13	13.8	3.57	17.3	79.8	81.4

135	20	20	150	115	15	4	120	3.56	1.13	13.8	3.57	17.3	79.8	81.4

APPENDIX C: RESULTS OF TWIN-SCREW EXTRUSION OF SPROUTED QUINOA

No	Run	Moisture Content	Screw Speed	Die Temperature	Germination time	Bulk Density	Hardness	Water Solubility Index	Water Absorption Index	Total Color Difference ΔE	Expansion ratio	Protein digestibility	Starch digestibility
		%w.b.	rpm	°C	Day	kg/m^3	N	%				%	%
1	62	20	80	220	3	148	1.81	16.2	3.19	17.8	2.57	82.1	82.0
2	52	15	80	150	3	142	1.68	14.9	3.30	17.4	2.71	83.0	82.6
3	36	25	80	150	2	171	2.10	18.2	2.97	19.0	2.33	80.6	80.1
4	85	20	110	150	4	142	1.73	15.4	3.27	17.6	2.67	82.7	82.4
5	24	15	110	220	1	137	1.61	15.5	3.36	17.1	2.78	83.5	82.9
6	45	20	80	220	2	150	1.83	16.3	3.18	17.9	2.56	82.0	81.9
7	19	25	80	150	1	172	2.11	18.3	2.96	19.1	2.31	80.5	80.0

8	40	25	110	80	2	164	2.05	17.6	3.03	18.7	2.39	81.1	80.6
9	12	20	140	220	0	144	1.74	15.5	3.26	18.4	2.65	81.1	80.5
10	74	25	110	80	4	161	2.02	17.4	3.05	18.4	2.41	81.3	80.7
11	69	15	80	150	4	140	1.66	15.1	3.32	17.2	2.73	83.1	82.8
12	63	20	140	220	3	141	1.71	15.2	3.29	17.5	2.69	82.9	82.7
13	83	20	110	150	4	142	1.73	15.4	3.27	17.6	2.67	82.7	82.4
14	32	20	110	150	1	146	1.76	15.7	3.24	17.3	2.64	82.4	82.1
15	35	15	80	150	2	143	1.70	14.7	3.28	17.5	2.69	82.9	82.4
16	21	25	140	150	1	156	1.95	17.1	3.09	18.3	2.47	81.5	81.2
17	48	20	110	150	2	145	1.75	15.6	3.25	17.4	2.65	82.5	82.2
18	23	25	110	80	1	165	2.06	17.7	3.02	18.8	2.38	81.0	80.3
19	70	25	80	150	4	167	2.07	17.8	3.01	18.7	2.37	81.0	80.4
20	11	20	80	220	0	153	1.88	16.5	3.15	19.4	2.52	79.6	81.9

21	43	20	80	80	2	155	1.90	16.8	3.12	18.4	2.51	81.6	81.6
22	64	20	110	150	3	144	1.74	15.5	3.26	17.5	2.66	82.6	82.3
23	38	25	140	150	2	155	1.94	17.0	3.10	18.2	2.48	81.6	81.3
24	77	20	80	80	4	152	1.87	16.5	3.16	18.0	2.53	82.0	81.8
25	27	20	140	80	1	149	1.80	16.1	3.20	17.8	2.59	82.1	82.3
26	30	20	110	150	1	146	1.76	15.7	3.24	17.3	2.64	82.4	82.1
27	13	20	110	150	0	148	1.77	15.8	3.22	18.7	2.63	80.7	80.7
28	54	15	140	150	3	133	1.57	15.8	3.40	16.8	2.82	84.0	83.3
29	34	20	110	150	1	146	1.76	15.7	3.24	17.3	2.64	82.4	82.1
30	82	20	110	150	4	142	1.73	15.4	3.27	17.6	2.67	82.7	82.4
31	76	25	110	220	4	156	1.96	17.1	3.09	18.2	2.46	81.6	81.2
32	53	25	80	150	3	169	2.08	18.0	2.99	18.9	2.35	80.8	80.2
33	46	20	140	220	2	142	1.72	15.3	3.28	17.6	2.68	82.8	82.6

34	14	20	110	150	0	148	1.77	15.8	3.22	18.7	2.63	80.7	80.7
35	72	25	140	150	4	153	1.92	16.8	3.12	18.0	2.50	81.8	81.5
36	18	15	80	150	1	145	1.72	14.6	3.26	17.6	2.67	82.7	82.2
37	3	15	140	150	0	136	1.60	15.5	3.37	17.6	2.78	81.5	81.8
38	58	15	110	220	3	135	1.59	15.6	3.38	16.9	2.80	83.7	83.1
39	41	15	110	220	2	136	1.60	15.6	3.37	17.0	2.79	83.6	83.0
40	9	20	80	80	0	160	1.95	17.2	3.08	19.9	2.48	79.1	78.2
41	80	20	140	220	4	140	1.70	15.1	3.3	17.4	2.70	83.0	82.8
42	49	20	110	150	2	145	1.75	15.6	3.25	17.4	2.65	82.5	82.2
43	42	25	110	220	2	159	1.99	17.3	3.07	18.4	2.44	81.4	81.0
44	31	20	110	150	1	146	1.76	15.7	3.24	17.3	2.64	82.4	82.1
45	44	20	140	80	2	148	1.79	16.0	3.21	17.6	2.60	82.2	82.4
46	50	20	110	150	2	145	1.75	15.6	3.25	17.4	2.65	82.5	82.2

47	67	20	110	150	3	144	1.74	15.5	3.26	17.5	2.66	82.6	82.3
48	1	15	80	150	0	147	1.75	14.4	3.23	19.3	2.64	80.1	80.2
49	22	15	110	80	1	141	1.65	15.2	3.33	17.3	2.74	83.0	82.7
50	68	20	110	150	3	144	1.74	15.5	3.26	17.5	2.66	82.6	82.3
51	79	20	80	220	4	147	1.80	16.1	3.20	17.7	2.58	82.2	82.1
52	4	25	140	150	0	158	1.97	17.2	3.08	19.2	2.45	80.1	78.6
53	6	25	110	80	0	167	2.08	17.9	3.00	20.2	2.36	79.1	77.7
54	17	20	110	150	0	148	1.77	15.8	3.22	18.7	2.63	80.7	80.7
55	51	20	110	150	2	145	1.75	15.6	3.25	17.4	2.65	82.5	82.2
56	84	20	110	150	4	142	1.73	15.4	3.27	17.6	2.67	82.7	82.4
57	33	20	110	150	1	146	1.76	15.7	3.24	17.3	2.64	82.4	82.1
58	8	25	110	220	0	162	2.03	17.5	3.04	19.8	2.41	79.6	78.2
59	60	20	80	80	3	154	1.89	16.7	3.14	18.2	2.52	81.8	81.7

60	5	15	110	80	0	143	1.67	15.0	3.31	18.8	2.72	80.7	80.6
61	75	15	110	220	4	134	1.58	15.7	3.39	16.8	2.81	83.8	83.2
62	59	25	110	220	3	158	1.98	17.2	3.08	18.3	2.45	81.5	81.1
63	57	25	110	80	3	163	2.04	17.5	3.04	18.6	2.40	81.2	80.5
64	15	20	110	150	0	148	1.77	15.8	3.22	18.7	2.63	80.7	80.7
65	61	20	140	80	3	147	1.78	15.9	3.22	17.5	2.62	82.3	82.5
66	26	20	80	80	1	157	1.92	17.0	3.11	18.5	2.50	81.5	81.4
67	39	15	110	80	2	140	1.64	15.3	3.34	17.1	2.75	83.1	82.8
68	66	20	110	150	3	144	1.74	15.5	3.26	17.5	2.66	82.6	82.3
69	25	25	110	220	1	160	2.01	17.4	3.06	18.5	2.43	81.3	80.8
70	7	15	110	220	0	139	1.62	15.4	3.35	18.4	2.76	81.1	81.1
71	29	20	140	220	1	143	1.73	15.4	3.27	17.7	2.67	82.7	82.5
72	56	15	110	80	3	139	1.63	15.4	3.35	17.0	2.76	83.2	82.9

73	65	20	110	150	3	144	1.74	15.5	3.26	17.5	2.66	82.6	82.3
74	71	15	140	150	4	132	1.56	15.9	3.41	16.7	2.83	84.1	83.4
75	55	25	140	150	3	154	1.93	16.9	3.11	18.1	2.49	81.7	81.4
76	73	15	110	80	4	137	1.61	15.5	3.36	16.9	2.77	83.4	83.0
77	47	20	110	150	2	145	1.75	15.6	3.25	17.4	2.65	82.5	82.2
78	81	20	110	150	4	142	1.73	15.4	3.27	17.6	2.67	82.7	82.4
79	28	20	80	220	1	151	1.85	16.4	3.17	18.1	2.55	81.9	78.9
80	78	20	140	80	4	146	1.76	15.8	3.23	17.4	2.63	82.4	82.6
81	20	15	140	150	1	135	1.59	15.6	3.38	16.9	2.80	83.8	83.1
82	10	20	140	80	0	150	1.82	16.2	3.19	19.1	2.57	80.3	79.4
83	16	20	110	150	0	148	1.77	15.8	3.22	18.7	2.63	80.7	80.7
84	2	25	80	150	0	175	2.14	18.5	2.93	20.8	2.28	78.4	77.1
85	37	15	140	150	2	134	1.58	15.7	3.39	16.9	2.81	83.9	83.2

APPENDIX D: RESULTS OF SINGLE SCREW EXTRUSION OF SPROUTED PROSO MILLET

No	Run	Moisture Content	Screw Speed	Die Temperature	Germination time	Bulk Density	Hardness	Water Solubility Index	Water Absorption Index	Total Color Difference ΔE	Expansion ratio	Protein digestibility	Starch digestibility
		%w.b.	rpm	°C	Day	kg/m^3	N	%				%	%
1	47	15	80	115	0	120	1.08	17.7	4.28	17.1	3.65	84.8	84.7
2	9	25	80	115	0	137	1.30	14.8	4.02	15.9	3.35	79.1	81.4
3	70	15	220	115	0	105	1.05	18.3	4.37	13.5	3.71	85.8	85.6
4	14	25	220	115	0	126	1.16	16.0	4.10	14.5	3.45	80.1	82.5
5	41	15	150	90	0	112	1.13	17.0	4.25	14.7	3.58	84.4	84.1
6	39	25	150	90	0	131	1.24	15.3	4.0	15.8	3.28	78.2	80.9

7	1	15	150	140	0	107	1.06	18.0	4.31	15.3	3.69	85.3	85.1
8	21	25	150	140	0	129	1.19	15.8	4.07	16.2	3.40	79.8	82.1
9	19	20	80	90	0	131	1.27	16.2	4.1	16.5	3.45	81.4	82.1
10	50	20	220	90	0	123	1.19	17.2	4.17	13.0	3.56	82.7	83.6
11	66	20	80	140	0	124	1.18	16.6	4.14	16.0	3.52	82.2	82.9
12	59	20	220	140	0	115	1.09	17.8	4.25	14.1	3.63	83.5	84.8
13	46	20	150	115	0	121	1.12	17.5	4.22	16.0	3.61	83.1	84.1
14	13	20	150	115	0	121	1.12	17.5	4.22	16.0	3.61	83.1	84.1
15	10	20	150	115	0	121	1.12	17.5	4.22	16.0	3.61	83.1	84.1
16	11	20	150	115	0	121	1.12	17.5	4.22	16.0	3.61	83.1	84.1
17	25	20	150	115	0	121	1.12	17.5	4.22	16.0	3.61	83.1	84.1
18	15	15	80	115	1	119	1.07	17.8	4.29	16.9	3.66	85.7	87.0
19	43	25	80	115	1	135	1.28	15.0	4.03	15.8	3.36	81.8	84.0
20	33	15	220	115	1	104	1.04	18.4	4.38	13.7	3.72	86.3	87.4

21	5	25	220	115	1	125	1.15	16.1	4.11	14.6	3.46	82.4	84.8
22	67	15	150	90	1	111	1.12	17.1	4.26	14.9	3.60	85.3	86.6
23	49	25	150	90	1	130	1.23	15.5	4.01	15.9	3.30	81.3	83.6
24	12	15	150	140	1	105	1.05	18.1	4.32	15.4	3.70	86.1	87.2
25	17	25	150	140	1	127	1.18	15.9	4.08	16.3	3.42	82.0	84.4
26	28	20	80	90	1	130	1.25	16.4	4.11	16.3	3.47	83.5	85.3
27	52	20	220	90	1	122	1.18	17.3	4.18	13.1	3.58	83.9	86.4
28	27	20	80	140	1	123	1.16	16.8	4.15	15.9	3.53	84.0	85.9
29	77	20	220	140	1	114	1.08	17.9	4.26	14.2	3.65	84.7	87.1
30	34	20	150	115	1	119	1.11	17.6	4.23	16.1	3.62	84.4	86.9
31	79	20	150	115	1	119	1.11	17.6	4.23	16.1	3.62	84.4	86.9
32	8	20	150	115	1	119	1.11	17.6	4.23	16.1	3.62	84.4	86.9
33	60	20	150	115	1	119	1.11	17.6	4.23	16.1	3.62	84.4	86.9
34	22	20	150	115	1	119	1.11	17.6	4.23	16.1	3.62	84.4	86.9

35	30	15	80	115	2	118	1.06	18.0	4.30	16.8	3.67	85.8	87.1
36	62	25	80	115	2	133	1.27	15.2	4.04	15.7	3.38	82.0	84.1
37	61	15	220	115	2	108	1.03	18.5	4.39	13.9	3.73	86.4	87.5
38	37	25	220	115	2	124	1.14	16.2	4.12	14.7	3.48	82.5	85.1
39	54	15	150	90	2	110	1.11	17.3	4.27	15.0	3.62	85.5	86.8
40	64	25	150	90	2	129	1.21	15.7	4.01	16.0	3.31	81.4	83.7
41	81	15	150	140	2	104	1.04	18.2	4.34	15.6	3.71	86.2	87.4
42	55	25	150	140	2	126	1.17	16.0	4.08	16.4	3.43	82.1	84.5
43	38	20	80	90	2	129	1.23	16.5	4.12	16.2	3.49	83.6	85.5
44	40	20	220	90	2	121	1.17	17.4	4.19	13.3	3.59	84.1	86.6
45	83	20	80	140	2	122	1.14	17.1	4.16	15.8	3.55	84.1	86.0
46	26	20	220	140	2	112	1.07	18.0	4.27	14.4	3.67	84.8	87.2
47	78	20	150	115	2	117	1.10	17.7	4.24	16.2	3.63	84.5	87.0
48	75	20	150	115	2	117	1.10	17.7	4.24	16.2	3.63	84.5	87.0

49	84	20	150	115	2	117	1.10	17.7	4.24	16.2	3.63	84.5	87.0
50	2	20	150	115	2	117	1.10	17.7	4.24	16.2	3.63	84.5	87.0
51	74	20	150	115	2	117	1.10	17.7	4.24	16.2	3.63	84.5	87.0
52	3	15	80	115	3	116	1.05	18.1	4.31	16.6	3.69	86.0	87.3
53	85	25	80	115	3	131	1.25	15.4	4.05	15.6	3.40	82.1	84.3
54	51	15	220	115	3	102	1.02	18.6	4.40	14.1	3.74	86.5	87.6
55	23	25	220	115	3	122	1.13	16.3	4.13	14.8	3.49	82.7	85.2
56	56	15	150	90	3	109	1.09	17.5	4.28	15.2	3.64	85.6	86.9
57	82	25	150	90	3	128	1.20	15.8	4.02	16.1	3.33	81.6	83.9
58	32	15	150	140	3	103	1.03	18.3	4.35	15.7	3.72	86.3	87.5
59	68	25	150	140	3	125	1.16	16.1	4.09	16.5	3.45	82.3	84.6
60	7	20	80	90	3	127	1.21	16.6	4.13	16.0	3.51	83.8	85.7
61	35	20	220	90	3	120	1.16	17.5	4.20	13.4	3.60	84.3	86.8
62	57	20	80	140	3	121	1.12	17.2	4.17	15.7	3.56	84.2	86.1

63	24	20	220	140	3	110	1.05	18.1	4.28	14.5	3.68	85.0	87.3
64	80	20	150	115	3	116	1.09	17.8	4.25	16.3	3.64	84.6	87.1
65	76	20	150	115	3	116	1.09	17.8	4.25	16.3	3.64	84.6	87.1
66	42	20	150	115	3	116	1.09	17.8	4.25	16.3	3.64	84.6	87.1
67	73	20	150	115	3	116	1.09	17.8	4.25	16.3	3.64	84.6	87.1
68	36	20	150	115	3	116	1.09	17.8	4.25	16.3	3.64	84.6	87.1
69	20	15	80	115	4	114	1.04	18.2	4.32	16.4	3.71	86.2	87.4
70	16	25	80	115	4	129	1.23	15.5	4.06	15.5	3.41	82.2	84.5
71	45	15	220	115	4	101	1.01	18.7	4.41	14.2	3.75	86.6	87.7
72	71	25	220	115	4	121	1.12	16.4	4.14	14.9	3.51	82.8	85.3
73	18	15	150	90	4	107	1.07	17.7	4.29	15.4	3.66	85.8	87.1
74	72	25	150	90	4	127	1.18	15.9	4.03	16.3	3.35	81.8	84.1
75	6	15	150	140	4	102	1.03	18.5	4.36	15.9	3.73	86.4	87.6

APPENDIX E: RESULTS OF SINGLE SCREW EXTRUSION OF SPROUTED PROSO MILLET-CORN MEAL BLEND

No	Run	Moisture Content	Screw Speed	Die Temperature	Corn meal Ratio	Germination time	Bulk Density	Hardness	Water Solubility Index	Water Absorption Index	Total Color Difference ΔE	Expansion ratio	Protein digestibility	Starch digestibility
		%w.b.	**rpm**	**°C**	**%**	**Day**	**kg/m^3**	**N**	**%**				**%**	**%**
1	1	15	80	115	15	Day 0	117	3.58	1.10	16.7	3.57	17.47	81.2	81.3
2	13	25	80	115	15	Day 0	143	3.10	1.57	13.5	2.79	14.97	80.9	80.0
3	34	15	220	115	15	Day 0	113	3.70	0.92	17.5	3.78	15.87	83.2	83.5
4	49	25	220	115	15	Day 0	130	3.14	1.22	13.9	2.57	16.57	82.4	81.8
5	5	20	150	90	0	Day 0	135	3.19	1.21	13.5	2.81	19.57	83.7	83.4
6	77	20	150	140	0	Day 0	128	3.34	1.32	13.8	2.85	16.57	83.9	84.0
7	116	20	150	90	30	Day 0	123	3.54	1.01	12.6	3.49	13.77	78.6	78.6

8	23	20	150	140	30	Day 0	114	3.66	0.94	13.6	3.78	15.67	79.5	80.5
9	37	15	150	115	0	Day 0	126	3.52	1.13	16.0	3.41	20.37	83.8	84.7
10	4	25	150	115	0	Day 0	143	2.74	1.43	12.9	2.55	15.67	84.2	83.2
11	113	15	150	115	30	Day 0	105	3.87	0.88	19.7	3.86	13.47	78.9	77.8
12	108	25	150	115	30	Day 0	126	3.29	1.11	14.6	3.78	13.77	78.5	78.7
13	124	20	80	90	15	Day 0	127	3.29	1.20	13.0	3.41	14.97	79.3	78.7
14	66	20	220	90	15	Day 0	125	3.38	1.19	14.3	3.61	15.67	79.7	80.2
15	79	20	80	140	15	Day 0	128	3.23	1.20	12.6	3.58	16.27	80.9	80.5
16	115	20	220	140	15	Day 0	118	3.51	1.10	13.9	3.66	16.77	79.1	81.7
17	43	15	150	90	15	Day 0	118	3.56	1.11	16.6	3.55	17.17	80.5	80.4
18	53	25	150	90	15	Day 0	128	3.10	1.59	13.0	2.79	16.57	79.1	77.7
19	119	15	150	140	15	Day 0	110	3.76	1.02	18.1	3.74	16.37	81.4	82.8
20	84	25	150	140	15	Day 0	126	3.23	1.20	13.9	2.57	16.77	82.7	82.2
21	132	20	80	115	0	Day 0	143	2.97	1.25	12.5	2.57	16.87	83.3	81.6

22	18	20	220	115	0	Day 0	130	3.14	1.22	14.6	2.79	16.97	84.2	84.5
23	61	20	80	115	30	Day 0	122	3.57	1.07	12.8	3.58	14.57	78.5	79.4
24	105	20	220	115	30	Day 0	114	3.62	0.98	13.1	3.66	15.37	79.8	79.6
25	85	20	150	115	15	Day 0	122	3.49	1.14	13.6	3.61	15.67	79.4	81.2
26	30	20	150	115	15	Day 0	122	3.49	1.14	13.6	3.61	15.67	79.4	81.2
27	35	20	150	115	15	Day 0	122	3.49	1.14	13.6	3.61	15.67	79.4	81.2
28	103	15	80	115	15	Day 1	116	3.59	1.09	16.8	3.58	17.37	82.8	82.2
29	129	25	80	115	15	Day 1	141	3.11	1.56	13.7	2.81	15.37	78.5	80.0
30	74	15	220	115	15	Day 1	112	3.72	0.91	17.6	3.79	15.77	84.1	84.4
31	65	25	220	115	15	Day 1	128	3.16	1.20	14.0	2.59	16.37	82.5	82.0
32	51	20	150	90	0	Day 1	134	3.22	1.19	13.7	2.83	19.37	85.1	84.4
33	52	20	150	140	0	Day 1	126	3.37	1.29	14.0	2.87	16.37	85.3	84.9
34	60	20	150	90	30	Day 1	122	3.55	1.00	12.7	3.50	13.87	78.9	78.7
35	122	20	150	140	30	Day 1	113	3.67	0.93	13.6	3.79	15.97	79.6	80.6

36	71	15	150	115	0	Day 1	124	3.54	1.11	16.3	3.43	20.17	85.6	85.5
37	59	25	150	115	0	Day 1	141	2.77	1.40	13.1	2.57	15.47	85.2	84.3
38	33	15	150	115	30	Day 1	104	3.89	0.87	19.8	3.87	13.37	80.2	80.0
39	111	25	150	115	30	Day 1	125	3.32	1.11	14.8	3.79	13.87	78.6	79.0
40	10	20	80	90	15	Day 1	126	3.32	1.19	13.1	3.44	15.37	79.4	79.0
41	2	20	220	90	15	Day 1	124	3.40	1.17	14.4	3.64	15.97	79.8	80.4
42	91	20	80	140	15	Day 1	127	3.26	1.19	12.7	3.61	16.47	78.5	80.8
43	14	20	220	140	15	Day 1	117	3.55	1.09	14.0	3.68	17.07	79.3	82.0
44	92	15	150	90	15	Day 1	116	3.57	1.10	16.7	3.56	17.07	81.8	81.7
45	121	25	150	90	15	Day 1	126	3.11	1.58	13.1	2.81	16.37	79.3	77.8
46	97	15	150	140	15	Day 1	109	3.77	1.01	18.2	3.75	16.27	83.0	84.1
47	62	25	150	140	15	Day 1	125	3.26	1.19	14.0	2.59	17.07	82.9	82.4
48	89	20	80	115	0	Day 1	141	3.01	1.22	12.7	2.59	16.67	84.3	83.1
49	99	20	220	115	0	Day 1	128	3.16	1.20	14.8	2.81	16.77	85.5	85.2

50	112	20	80	115	30	Day 1	121	3.58	1.06	12.9	3.61	14.67	78.6	79.5
51	80	20	220	115	30	Day 1	113	3.63	0.97	13.2	3.68	15.47	79.9	79.7
52	75	20	150	115	15	Day 1	121	3.52	1.13	13.7	3.65	15.97	79.6	81.3
53	45	20	150	115	15	Day 1	121	3.52	1.13	13.7	3.65	15.97	79.6	81.3
54	46	20	150	115	15	Day 1	121	3.52	1.13	13.7	3.65	15.97	79.6	81.3
55	44	15	80	115	15	Day 2	115	3.60	1.08	16.9	3.59	17.27	82.9	82.3
56	32	25	80	115	15	Day 2	140	3.12	1.55	13.8	2.82	15.47	78.6	80.2
57	98	15	220	115	15	Day 2	111	3.74	1.00	17.7	3.80	15.67	84.2	84.5
58	94	25	220	115	15	Day 2	127	3.17	1.19	14.1	2.60	16.27	82.6	82.1
59	8	20	150	90	0	Day 2	132	3.23	1.18	13.8	2.84	19.27	85.2	84.5
60	12	20	150	140	0	Day 2	125	3.38	1.27	14.1	2.88	16.27	85.4	85.0
61	106	20	150	90	30	Day 2	121	3.56	0.99	12.8	3.51	13.97	79.0	78.8
62	130	20	150	140	30	Day 2	112	3.68	0.92	13.7	3.80	16.07	79.7	80.7
63	78	15	150	115	0	Day 2	122	3.55	1.10	16.4	3.44	20.07	85.7	85.6

64	54	25	150	115	0	Day 2	140	2.78	1.38	13.2	2.58	15.37	85.3	84.4
65	83	15	150	115	30	Day 2	103	3.91	0.86	19.9	3.88	13.27	80.3	80.2
66	28	25	150	115	30	Day 2	126	3.33	1.11	14.9	3.80	13.97	78.7	79.1
67	47	20	80	90	15	Day 2	125	3.33	1.18	13.2	3.45	15.47	79.5	79.1
68	38	20	220	90	15	Day 2	123	3.41	1.16	14.5	3.65	16.07	79.9	80.5
69	39	20	80	140	15	Day 2	126	3.27	1.18	12.8	3.63	16.57	78.6	80.9
70	6	20	220	140	15	Day 2	116	3.56	1.08	14.1	3.69	17.17	79.4	82.1
71	96	15	150	90	15	Day 2	115	3.58	1.09	16.8	3.57	16.97	81.9	81.8
72	110	25	150	90	15	Day 2	125	3.12	1.57	13.2	2.82	16.27	79.4	77.9
73	135	15	150	140	15	Day 2	108	3.79	1.00	18.3	3.76	16.17	83.1	84.2
74	25	25	150	140	15	Day 2	124	3.27	1.18	14.1	2.60	17.17	82.1	82.5
75	50	20	80	115	0	Day 2	140	3.02	1.21	12.8	2.60	16.57	84.4	83.2
76	93	20	220	115	0	Day 2	127	3.17	1.19	14.9	2.82	16.67	85.6	85.3
77	76	20	80	115	30	Day 2	120	3.59	1.05	13.0	3.63	14.77	78.7	79.6

78	127	20	220	115	30	Day 2	112	3.64	0.96	13.3	3.69	15.57	80.4	79.8
79	21	20	150	115	15	Day 2	120	3.53	1.12	13.8	3.66	16.07	79.7	81.4
80	107	20	150	115	15	Day 2	120	3.53	1.12	13.8	3.66	16.07	79.7	81.4
81	9	20	150	115	15	Day 2	120	3.53	1.12	13.8	3.66	16.07	79.7	81.4
82	101	15	80	115	15	Day 3	114	3.61	1.07	17.0	3.60	17.17	83.0	82.4
83	70	25	80	115	15	Day 3	139	3.13	1.54	13.9	2.83	15.57	78.7	80.3
84	128	15	220	115	15	Day 3	110	3.75	0.89	17.8	3.81	15.57	84.3	84.6
85	24	25	220	115	15	Day 3	126	3.18	1.18	14.2	2.61	16.17	82.7	82.2
86	104	20	150	90	0	Day 3	131	3.24	1.17	13.9	2.85	19.17	85.3	84.6
87	67	20	150	140	0	Day 3	124	3.39	1.26	14.2	2.89	16.17	85.5	85.1
88	72	20	150	90	30	Day 3	120	3.57	0.98	12.9	3.52	14.07	79.1	78.9
89	82	20	150	140	30	Day 3	111	3.69	0.91	13.7	3.81	16.17	79.8	80.8
90	69	15	150	115	0	Day 3	121	3.56	1.07	16.5	3.45	19.97	85.8	85.7
91	125	25	150	115	0	Day 3	139	2.79	1.36	13.3	2.59	15.27	85.3	84.5

92	48	15	150	115	30	Day 3	102	3.93	0.85	20.0	3.89	13.17	80.4	80.3
93	56	25	150	115	30	Day 3	125	3.34	1.10	15.0	3.81	14.07	78.8	79.2
94	15	20	80	90	15	Day 3	124	3.34	1.17	13.3	3.46	15.57	79.6	79.2
95	81	20	220	90	15	Day 3	122	3.42	1.15	14.6	3.66	16.17	80.1	80.6
96	64	20	80	140	15	Day 3	125	3.28	1.17	12.9	3.64	16.67	78.7	81.0
97	134	20	220	140	15	Day 3	115	3.57	1.07	14.2	3.70	17.27	79.5	82.2
98	3	15	150	90	15	Day 3	114	3.59	1.08	16.9	3.58	16.87	82.0	81.9
99	117	25	150	90	15	Day 3	124	3.13	1.56	13.3	2.83	16.17	79.5	78.0
100	131	15	150	140	15	Day 3	107	3.80	0.99	18.4	3.77	16.07	83.2	84.3
101	133	25	150	140	15	Day 3	123	3.28	1.17	14.2	2.61	17.27	83.1	82.6
102	42	20	80	115	0	Day 3	139	3.03	1.20	12.9	2.61	16.47	84.5	83.3
103	126	20	220	115	0	Day 3	126	3.18	1.18	15.0	2.83	16.57	85.7	85.4
104	88	20	80	115	30	Day 3	119	3.60	1.04	13.1	3.64	14.87	78.8	79.7
105	86	20	220	115	30	Day 3	111	3.65	0.95	13.4	3.70	15.67	80.7	79.9

106	31	20	150	115	15	Day 3	119	3.54	1.11	13.9	3.67	16.17	79.8	81.5
107	63	20	150	115	15	Day 3	119	3.54	1.11	13.9	3.67	16.17	79.8	81.5
108	40	20	150	115	15	Day 3	119	3.54	1.11	13.9	3.67	16.17	79.8	81.5
109	90	15	80	115	15	Day 4	113	3.62	1.06	17.1	3.61	17.07	83.1	82.5
110	17	25	80	115	15	Day 4	138	3.14	1.53	14.0	2.84	15.67	78.8	80.4
111	102	15	220	115	15	Day 4	109	3.77	0.88	17.9	3.82	15.47	84.4	84.7
112	29	25	220	115	15	Day 4	125	3.19	1.17	14.3	2.62	16.07	82.8	82.3
113	36	20	150	90	0	Day 4	130	3.25	1.16	14.0	2.86	19.07	85.4	84.7
114	41	20	150	140	0	Day 4	123	3.40	1.25	14.4	2.90	16.07	85.6	85.2
115	57	20	150	90	30	Day 4	119	3.58	0.97	13.0	3.53	14.17	79.2	79.0
116	87	20	150	140	30	Day 4	110	3.70	0.90	13.7	3.82	16.27	79.9	80.9
117	123	15	150	115	0	Day 4	120	3.57	1.08	16.6	3.46	19.87	85.9	85.8
118	68	25	150	115	0	Day 4	138	2.80	1.34	13.4	2.60	15.17	85.4	84.6
119	11	15	150	115	30	Day 4	100	3.96	0.84	20.1	3.90	13.07	80.5	80.4

120	120	25	150	115	30	Day 4	124	3.35	1.10	15.1	3.82	14.17	78.9	79.3
121	19	20	80	90	15	Day 4	123	3.35	1.16	13.4	3.47	15.67	79.7	79.3
122	55	20	220	90	15	Day 4	121	3.43	1.14	14.7	3.67	16.27	80.2	80.7
123	95	20	80	140	15	Day 4	124	3.29	1.16	13.0	3.65	16.77	78.8	80.4
124	22	20	220	140	15	Day 4	114	3.58	1.06	14.3	3.71	17.37	79.6	82.3
125	26	15	150	90	15	Day 4	113	3.61	1.07	17.0	3.59	16.77	82.1	82.0
126	58	25	150	90	15	Day 4	123	3.14	1.55	13.4	2.84	16.07	79.6	78.1
127	114	15	150	140	15	Day 4	105	3.82	0.98	18.5	3.78	15.97	83.3	84.4
128	16	25	150	140	15	Day 4	122	3.29	1.16	14.3	2.62	17.37	83.2	82.7
129	27	20	80	115	0	Day 4	138	3.04	1.19	13.0	2.62	16.37	84.6	83.4
130	73	20	220	115	0	Day 4	125	3.19	1.17	15.1	2.84	16.47	85.8	85.5
131	100	20	80	115	30	Day 4	118	3.61	1.03	13.2	3.65	14.97	78.9	79.8
132	7	20	220	115	30	Day 4	110	3.66	0.94	13.5	3.71	15.77	80.8	80.0
133	109	20	150	115	15	Day 4	118	3.55	1.10	14.0	3.68	16.27	79.9	81.6

134	118	20	150	115	15	Day 4	118	3.55	1.10	14.0	3.68	16.27	79.9	81.6
135	20	20	150	115	15	Day 4	118	3.55	1.10	14.0	3.68	16.27	79.9	81.6

APPENDIX F: RESULTS OF TWIN-SCREW EXTRUSION OF SPROUTED PROSO MILLET

No	Run	Moisture Content	Screw Speed	Die Temperature	Germination time	Bulk Density	Hardness	Water Solubility Index	Water Absorption Index	Total Color Difference ΔE	Expansion ratio	Protein digestibility	Starch digestibility
		%w.b.	rpm	°C	Day	kg/m³	N	%				%	%
1	62	20	80	220	3	139	1.33	19.0	3.59	20.4	3.34	83.5	83.7
2	52	15	80	150	3	131	1.24	20.0	3.69	19.3	3.48	84.4	84.0
3	36	25	80	150	2	158	1.51	16.8	3.41	22.4	3.07	80.3	82.5
4	85	20	110	150	4	131	1.26	19.7	3.67	19.6	3.43	84.1	84.0
5	24	15	110	220	1	125	1.18	20.6	3.74	18.7	3.57	84.8	84.6
6	45	20	80	220	2	140	1.35	18.9	3.58	20.5	3.32	83.4	83.6
7	19	25	80	150	1	159	1.52	16.6	3.39	22.6	3.05	80.2	82.4

8	40	25	110	80	2	152	1.45	17.6	3.48	21.8	3.16	81.0	82.8
9	12	20	140	220	0	132	1.27	19.6	3.67	19.7	3.43	82.1	82.5
10	74	25	110	80	4	150	1.43	17.8	3.50	21.6	3.18	82.2	83.0
11	69	15	80	150	4	129	1.22	20.1	3.71	19.1	3.50	84.5	84.2
12	63	20	140	220	3	129	1.24	19.9	3.70	19.4	3.46	84.2	84.1
13	83	20	110	150	4	131	1.26	19.7	3.67	19.6	3.43	84.1	84.0
14	32	20	110	150	1	134	1.29	19.4	3.64	19.9	3.40	83.8	83.7
15	35	15	80	150	2	133	1.26	19.8	3.67	19.5	3.46	84.2	83.8
16	21	25	140	150	1	146	1.40	18.2	3.53	21.2	3.22	82.5	83.1
17	48	20	110	150	2	133	1.28	19.5	3.65	19.8	3.41	83.9	83.8
18	23	25	110	80	1	153	1.46	17.5	3.46	21.9	3.15	80.9	82.6
19	70	25	80	150	4	154	1.47	17.2	3.45	22.0	3.11	80.7	82.7
20	11	20	80	220	0	142	1.38	18.6	3.55	20.8	3.29	79.6	81.3
21	43	20	80	80	2	145	1.41	18.4	3.52	21.1	3.26	83.0	83.1

22	64	20	110	150	3	132	1.27	19.6	3.66	19.7	3.42	84.0	83.9
23	38	25	140	150	2	145	1.39	18.3	3.54	21.1	3.23	82.6	83.2
24	77	20	80	80	4	141	1.37	18.7	3.56	20.7	3.30	83.3	83.5
25	27	20	140	80	1	137	1.32	19.1	3.61	20.2	3.36	83.6	83.4
26	30	20	110	150	1	134	1.29	19.4	3.64	19.9	3.40	83.8	83.7
27	13	20	110	150	0	135	1.30	19.3	3.63	20.0	3.39	81.6	82.1
28	54	15	140	150	3	120	1.14	21.0	3.79	18.2	3.62	85.3	85.0
29	34	20	110	150	1	134	1.29	19.4	3.64	19.9	3.40	83.8	83.7
30	82	20	110	150	4	131	1.26	19.7	3.67	19.6	3.43	84.1	84.0
31	76	25	110	220	4	146	1.40	18.2	3.53	21.2	3.21	82.5	83.2
32	53	25	80	150	3	156	1.49	17.0	3.43	22.2	3.09	80.5	82.6
33	46	20	140	220	2	130	1.25	19.8	3.69	19.5	3.45	84.1	84.0
34	14	20	110	150	0	135	1.30	19.3	3.63	20.0	3.39	81.6	82.1
35	72	25	140	150	4	143	1.37	18.5	3.56	20.9	3.25	82.8	83.4

36	18	15	80	150	1	134	1.27	19.6	3.65	19.7	3.45	84.0	83.7
37	3	15	140	150	0	123	1.17	20.7	3.76	18.5	3.59	83.6	83.3
38	58	15	110	220	3	123	1.16	20.8	3.76	18.5	3.59	85.0	84.8
39	41	15	110	220	2	124	1.17	20.7	3.75	18.6	3.58	84.9	84.7
40	9	20	80	80	0	148	1.45	18.0	3.48	21.5	3.21	79.3	80.6
41	80	20	140	220	4	128	1.23	20.0	3.71	19.3	3.47	84.3	84.2
42	49	20	110	150	2	133	1.28	19.5	3.65	19.8	3.41	83.9	83.8
43	42	25	110	220	2	148	1.42	18.0	3.51	21.4	3.19	82.3	83.0
44	31	20	110	150	1	134	1.29	19.4	3.64	19.9	3.40	83.8	83.7
45	44	20	140	80	2	136	1.31	19.2	3.62	20.1	3.37	83.7	83.5
46	50	20	110	150	2	133	1.28	19.5	3.65	19.8	3.41	83.9	83.8
47	67	20	110	150	3	132	1.27	19.6	3.66	19.7	3.42	84.0	83.9
48	1	15	80	150	0	136	1.30	19.4	3.62	19.9	3.42	82.3	82.1
49	22	15	110	80	1	128	1.21	20.2	3.71	19.1	3.53	84.6	84.1

50	68	20	110	150	3	132	1.27	19.6	3.66	19.7	3.42	84.0	83.9
51	79	20	80	220	4	138	1.32	19.1	3.60	20.3	3.35	83.6	83.8
52	4	25	140	150	0	147	1.41	18.1	3.52	21.3	3.21	79.6	81.4
53	6	25	110	80	0	155	1.48	17.3	3.44	22.1	3.13	79.1	80.4
54	17	20	110	150	0	135	1.30	19.3	3.63	20.0	3.39	81.6	82.1
55	51	20	110	150	2	133	1.28	19.5	3.65	19.8	3.41	83.9	83.8
56	84	20	110	150	4	131	1.26	19.7	3.67	19.6	3.43	84.1	84.0
57	33	20	110	150	1	134	1.29	19.4	3.64	19.9	3.40	83.8	83.7
58	8	25	110	220	0	150	1.45	17.8	3.49	21.7	3.17	79.4	80.9
59	60	20	80	80	3	143	1.39	18.5	3.54	20.9	3.28	83.1	83.3
60	5	15	110	80	0	129	1.23	20.1	3.70	19.2	3.52	82.8	82.6
61	75	15	110	220	4	122	1.15	20.9	3.77	18.4	3.60	85.1	84.9
62	59	25	110	220	3	147	1.41	18.1	3.52	21.3	3.2	82.4	83.1
63	57	25	110	80	3	151	1.44	17.7	3.49	21.7	3.17	82.1	82.9

64	15	20	110	150	0	135	1.30	19.3	3.63	20.0	3.39	81.6	82.1
65	61	20	140	80	3	135	1.30	19.3	3.63	20.0	3.38	83.8	83.6
66	26	20	80	80	1	146	1.42	18.2	3.51	21.3	3.24	82.8	82.9
67	39	15	110	80	2	127	1.20	20.3	3.72	19.0	3.54	84.7	84.3
68	66	20	110	150	3	132	1.27	19.6	3.66	19.7	3.42	84.0	83.9
69	25	25	110	220	1	149	1.43	17.9	3.50	21.5	3.18	82.2	82.8
70	7	15	110	220	0	127	1.19	20.5	3.73	18.8	3.56	83.1	83.0
71	29	20	140	220	1	131	1.26	19.7	3.68	19.6	3.44	84.0	83.9
72	56	15	110	80	3	126	1.19	20.4	3.73	18.9	3.55	84.8	84.4
73	65	20	110	150	3	132	1.27	19.6	3.66	19.7	3.42	84.0	83.9
74	71	15	140	150	4	119	1.13	21.1	3.80	18.1	3.63	85.4	85.1
75	55	25	140	150	3	144	1.38	18.4	3.55	21.0	3.24	82.7	83.3
76	73	15	110	80	4	125	1.18	20.5	3.74	18.8	3.56	84.9	84.5
77	47	20	110	150	2	133	1.28	19.5	3.65	19.8	3.41	83.9	83.8

78	81	20	110	150	4	131	1.26	19.7	3.67	19.6	3.43	84.1	84.0
79	28	20	80	220	1	141	1.36	18.8	3.57	20.6	3.31	83.3	83.5
80	78	20	140	80	4	134	1.29	19.4	3.64	19.9	3.39	83.9	83.7
81	20	15	140	150	1	122	1.16	20.8	3.77	18.4	3.60	85.1	84.8
82	10	20	140	80	0	138	1.33	19.0	3.60	20.4	3.35	80.0	81.7
83	16	20	110	150	0	135	1.30	19.3	3.63	20.0	3.39	81.6	82.1
84	2	25	80	150	0	161	1.55	16.4	3.36	22.8	3.02	78.7	79.9
85	37	15	140	150	2	121	1.15	20.9	3.78	18.3	3.61	85.2	84.9

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