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Understanding the Effect of Extrusion Processing Parameters on Physical, Nutritional and Rheological Properties of Soy White Flakes Based Aquafeed in a Single Screw Extruder

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UNDERSTANDING THE EFFECT OF EXTRUSION PROCESSING PARAMETERS ON PHYSICAL, NUTRITIONAL AND RHEOLOGICAL PROPERTIES OF SOY WHITE FLAKES BASED AQUAFEED IN A SINGLE SCREW EXTRUDER

BY

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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 dissertation does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

K. Muthukumarappan, Ph.D. Date
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I would like to dedicate this dissertation to:

- My Family Members
- Poonam Singha

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TABLE OF CONTENTS

ABBREVIATIONS

a*	-	Redness/Greenness
ANFs	-	Anti-nutritional factors
ANOVA	-	Analysis of variance
b*	-	Yellowness/Blueness
BD	-	Bulk density
CCRD	-	Central composite rotatable design
C-DDGS	-	Conventional distiller's dried grain with solubles
CDS	-	Condensed distiller's solubles
CF	-	Crude fat
CF_b	-	Crude fiber
CFD	-	Computational fluid dynamics
CGM	-	Corn gluten meal
CP	-	Crude Protein
D	-	Diameter of die nozzle
db	-	dry basis
DDG	-	Distiller's dried grain
DDGS	-	Distiller's dried grain with solubles
DDS	-	Distiller's dried solubles
df	-	degree of freedom
DM	-	Dry matter
EAA	-	Essential amino acids
EAAI	-	Essential amino acid index

ER	-	Expansion ratio
FEM	-	Finite element meshing
FM	-	Fish meal
GLM	-	General linear model
HP-DDG	-	High protein distiller's dried grains
K	-	Consistency factor
L	-	Length of die nozzle
L*	-	Brightness/Darkness
LSD	-	Least significant difference
Lys	-	Lysine
MC	-	Moisture content
MFR	-	Mass flow rate
MS	-	Mean square
n	-	Flow behavior ondex
NFE	-	Nitrogen free extract
NSP	-	Non starch polysaccharides
PDI	-	Pellet durability index
Q	-	Volumetric flow rate
R^2	-	Coefficient of determination
rpm	-	Revolution per minute
RSM	-	Response surface methodology
SBM	-	Soybean meal
SME	-	Specific mechanical energy

SPC	-	Soy protein concentrate
SPI	-	Soy protein isolate
SWF	-	Soy white flakes
T	-	Barrel temperature
TIA	-	Trypsin inhibitor activity
UD	-	Unit density
WAI	-	Water absorption index
wb	-	wet basis
WSI	-	Water solubility index
ΔP	-	Pressure drop
η	-	Apparent viscosity
τ	-	Shear stress
$\dot{\gamma}$	-	Shear rate
$\dot{\gamma}_{app}$	-	Apparent shear rate
$\dot{\gamma}_t$	-	True shear rate

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

UNDERSTANDING THE EFFECT OF EXTRUSION PROCESSING PARAMETERS ON PHYSICAL, NUTRITIONAL AND RHEOLOGICAL PROPERTIES OF SOY WHITE FLAKES BASED AQUAFEED IN A SINGLE SCREW EXTRUDER

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The rapid expansion of aquaculture has increased the demand for aquafeed. As fishmeal is expensive, alternative plant based protein sources such as soybean has shown to be a good alternative for aquafeed production. The present study was done to investigate the effect of soy white flakes (SWF) as an alternative source of protein in the production of aquaculture feed through extrusion processing. Ingredient blends containing different levels of SWF along with distiller dried grains, corn flour, corn gluten meal, fish meal, vitamin and mineral mix with net protein adjusted to 32% protein were formulated. The ingredient blends were extruded in single screw extruders and the properties of extrudates were studied. The experiments conducted includes: 1) effect of changing the level of SWF (10, 20 and 30%) along with other ingredients, moisture content (15, 25 and 35%db) and barrel temperature (110, 135 and 170^{o}C) on properties of extrudates such as color, pellet durability, bulk density, water absorption and solubility indices, unit density and expansion ratio; 2) effect of changing levels of L/D ratio of die nozzle (3.33, 5.83 and 7.25), SWF (21, 29, 40, 52 and 59%) along with other ingredients,

moisture content (15, 19, 25, 31 and 35%db) and barrel temperature (100, 110, 125, 140 and 150^oC) on properties of extrudates such as pellet durability, bulk density, water absorption and solubility indices and expansion ratio; 3) effect of changing levels of screw speed (100, 150 and 200 rpm), SWF (20, 35 and 50%) along with other ingredients, moisture content (20, 25 and 30%) and barrel temperature (110, 125, 140^oC) on properties of extrudates such as pellet durability index, bulk density, water absorption and solubility indices and mass flow rate; 4) effect of changing levels of SWF along with other ingredients, moisture content, barrel temperature and screw speed on nutritional properties of extrudates such as protein content, fat, fiber, ash content, lysine content and trypsin inhibitor activity and also to optimize the processing conditions to maximize the nutritional quality; 5) development of a viscosity model incorporating a correction factor for SWF along with correction factor for temperature and the effect of extrusion processing parameters on mass flow rate, torque, specific mechanical energy and viscosity of dough and 6) rheological characterization and CFD simulation of SWF based dough.

In the initial study, increasing the level of SWF from 10 to 30% resulted in increase in water absorption index and unit density but decrease in expansion ratio. The interaction effect of SWF content, moisture content and temperature were significant for color, pellet durability index, bulk density and expansion ratio. All the extrudates showed relatively high pellet durability and inclusion of SWF produced less expanded and more compact textured extrudates.

In the experiment to determine the effect of die dimensions, response surface methodology was used to find the effect of screw speed, SWF level, moisture content and barrel temperature on the physical properties of extrudates. Quadratic polynomial regression equations were developed to correlate the product responses and process variables as well as to obtain the response surfaces plots. The independent variables had significant ($P< 0.05$) effects on physical properties of extrudates: (i) higher soy white flakes content increased the pellet durability index and water absorption index, but decreased the water solubility index, (ii) higher temperature decreased pellet durability index, bulk density and water solubility index, (iii) increase in L/D ratio from 3.33 to 7.25 increased the pellet durability index, expansion ratio but decreased the bulk density of the extrudates.

In the experiments with different screw speed level (100 to 200 rpm) and SWF levels (20 to 50%), it was observed that increasing screw speed resulted in increase in pellet durability and mass flow rate but decrease in water absorption index of the extrudates. The results of the experiments shows that aquaculture feed with high durability, lower bulk density and lower water absorption and higher solubility indices can be produced with ingredients mix containing 40% SWF net protein content adjusted to 32% using single screw extruder.

In the experiment to determine the effect SWF content, moisture content, barrel temperature and screw speed on the nutritional properties of the extrudates, response surface methodology was used. Response surface regression models were established to correlate the nutritional properties of extrudates to the process variables. SWF was the most significant variable with quadratic effects on most of the nutritional properties. As level of SWF content was increased from 20 to 50%, there was an increase in lysine content, TIA, protein content, ash content and decrease in fat and fiber content.

Increasing temperature resulted in decrease in lysine content and trypsin inhibitor activity of extrudates.

It was also observed that increase in the soy white flakes content resulted in a higher mass flow rate, torque and specific mechanical energy and a decrease in the apparent viscosity. The specific mechanical energy, mass flow rate increased and viscosity decreased with increase in screw speed. Higher barrel and die temperature led to decrease in the apparent viscosity of the dough, torque and specific mechanical energy.

The flow of SWF based dough in a single screw extruder was simulated by using computational fluid dynamics (CFD). Process conditions considered were screw speeds (40, 80, 120, 160, 200 rpm), barrel temperature (100, 120, 140^{o}C) and SWF contents (30, 40, 50%db). Simulation results were validated quantitatively by experimental data. The results showed good agreement between experimental and computational results. Moreover, the flow profiles in the extruder were analyzed by using the influence of screw speed and level of soy white flakes content. Qualitative behavior of local shear rate and viscosity along the screw were analyzed and comparisons of different process conditions have been presented.

CHAPTER 1

Introduction and Background

1.1 Introduction

Aquaculture is one of the fastest growing animal food industries. Global fish production has grown steadily in the last five decades with food fish supply increasing at an average annual rate of 3.2%, outpacing world population growth at 1.6%. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (FAO, 2014).

The expansion of aquaculture production has also increased the demand for aquafeed supply. Considering the techno-economic feasibility of aquaculture production, feed cost is a primary concern (Pritchard, 1976). The protein component of aquaculture diets is the single most important and expensive dietary nutrient which comes largely from ground, ocean-caught fishmeal due to its easy availability and ideal nutritional value and biological qualities (Aquaculture Development and Coordination Programme, 1983; Bautista et al., 1994). As the demand for quality fish protein increases, the available wild resources of fish meal for aquafeed production would be limited. The rapid expansion of global demand for aquaculture products, coupled with the depletion of wild fish stocks has elevated the cost of fishmeal, and consequently, diet prices (Amaya et al., 2007; Lim and Lee, 2009). Production of a quality aquafeed is greatly related to the feed ingredients' functionality, biological qualities, pretreatment of the ingredient, and the technological treatment which is applied to make feed.

Recent researches have focused on the use of alternative protein sources for aquafeed (Hardy, 2010; Hardy and Masumoto, 1990; Rumsey et al., 1993). The ultimate goal is to eliminate the fish meal inclusion in aqua diets by substituting appropriate alternative protein sources. Plant-derived protein source appeared to be attractive alternatives due to its sustainability, availability and relatively low cost. However, the major challenges faced by aquaculture industry in replacing fish meal with plant-based protein are poor functionality during the production, presence of some anti-nutritional factors and lack of essential nutrients.

Many research works on feeding trials using aquafeed with partial replacement of fishmeal with different animal by-product and plant feedstuff have shown optimum growth results for various species. An ideal alternative ingredient for fishmeal should have low starch and fibre content, absence of anti-nutritional factors and high protein content with balanced amino acid profile, high nutrient digestibility and good palatability.

Plant feedstuffs include coproduct of oilseed processing such as soybean meal, corn gluten meal etc. and coproduct of ethanol production such as distillers dried grains with solubles. United States of America is the major producer of soybean comprising 35% of the total production. Soybean production covers 6% of the world's arable land. The global soybean production is projected at 320.5 million tons (USDA, 2016). Soybean meal, a co product of soybean oil production is easily available and can be used as a replacement of fishmeal in aquafeed (Amaya et al., 2006; Fallahi et al., 2012; Kaushik et al., 1995; Webster et al., 1992). The nutritional profile of soybean is close to that of fish meal. The limitation of using soybean is high fiber content and anti-nutritional factors present in soybean meal. Soy protein isolate and soy protein concentrate do not have anti-nutrients but are expensive. Further the presence of phytates decrease the digestibility of this product. However phytase enzyme can be used to degrade phytates. Attempts have been made such as heat treatment to reduce the anti-nutritional factors of conventional soybean products and to improve soy utilization in feed industry.

As mentioned above, the other important factor in the production of a quality aquafeed is the technology of feed manufacturing. Extrusion is commonly used processing method in food and feed industries. It is a unit operation involving cooking, forming, mixing and shaping. Its performance is influenced by several variables. The interaction of high shear and temperature developing inside the extruder with water of the ingredients drastically affects the molecular structure of the material, mainly due to gelatinization of starch and denaturation of protein components of the feed blend, which influences the resulting physiochemical and nutritional properties of the extrudates (Guy, 2001). The main advantages of extrusion processing are: energy efficiency, continuous high throughput, improved textural and flavor characteristics and expanded products form. Extruded aquafeed are designed to float or sink based on the fish species requirement. The advantages of using extruded aquafeed over pelleted ones are presence of less fines, better durability and floatability.

Soy based aquafeed have shown good results on feeding trial of different fish species. Dersjant-Li (2002) reported that soy protein isolate can be used to replace 40-100% fish meal without negative impact on growth performance of shrimp. While research has been done on feeding trials with soy based diets, there is still more to work on extrusion processing effects of soy based diets for aqua feed production. The effect of soy co-products like white flakes in feed composition on the extrudate properties needs to be investigated.

1.2 Objectives

The main objective of this study was to maximize the amount of soy white flakes that could be incorporated in the production of aquaculture feed through extrusion technology and to understand the significant impacts of extrusion processing on physical and nutritional properties of SWF based aquafeed.

The specific objectives of this study were:

1. To study the effect of changing the levels moisture content, temperature profile in the barrel and SWF content in an ingredient blend containing 31.5% protein on the properties of extrudates such as color, pellet durability index, bulk density, water absorption index, water solubility index, unit density and expansion ratio in a single screw extruder.
2. To study the effect of level moisture content, die dimensions and temperature profile in the barrel and SWF content in an ingredient blend containing 32.5% protein on the extrudate properties such as pellet durability index, bulk density, water absorption and solubility indices and expansion ratio in a single screw extruder.
3. To study the effect of changing the levels moisture content, temperature, screw speed and SWF content in an ingredient blend containing 32.3% protein on the extrudate properties such as pellet durability, bulk density, water absorption index, water solubility index, mass flow rates of extrudates and to optimize the processing conditions in a single screw extruder.
4. To study the effect of level of moisture content, screw speed, temperature profile in the barrel and SWF content on extrudate nutritional properties such as nutrient

content, lysine content and trypsin inhibitor activity during extrusion processing in a single screw extruder.

5. To develop a viscosity model by incorporating SWF and temperature as independent factors in a single screw extruder.
6. To characterize the rheological properties of soy white flakes based dough in a single screw extruder online by capillary die rheometer and to simulate the rheological complex fluid flow inside the extruder.

This dissertation is based on the following manuscripts of the research papers:

Chapter 2: Singh, S.K. and Muthukumarappan, K., 2015. Single screw extrusion processing of soy white flakes based Catla feed. *Journal of Food Research*, *4*(1), p.1. DOI: 10.5539/jfr.v4n1p1

Chapter 3: Singh, S.K. and Muthukumarappan, K., 2014. Effect of different extrusion processing parameters on physical properties of soy white flakes and high protein distillers dried grains-based extruded aquafeeds. *Journal of Food Research*, *3*(6), p.107. DOI: 10.5539/jfr.v3n6p107

Chapter 4: Singh, S.K. and Muthukumarappan, K., 2015. Effect of feed moisture, extrusion temperature and screw speed on properties of soy white flakes based aquafeed: a response surface analysis. *Journal of the Science of Food and Agriculture*, 96(6), p. 2220-2229. DOI: 10.1002/jsfa.7339

Chapter 5: Singh, S.K. and Muthukumarappan, K., 2016. Modeling and optimizing the effect of extrusion parameters on nutritional properties of soy white flakes-based extrudates using response surface methodology. *Journal of the Science of Food and Agriculture*. (Manuscript under preparation)

Chapter 6: Singh, S.K. and Muthukumarappan, K., 2016. A viscosity model for soy white flakes based aquafeed dough in a single screw extruder. *Journal of Food Process Engineering.* DOI: 10.1111/jfpe.12357

Chapter 7: Singh, S.K. and Muthukumarappan, K., 2016. Rheological characterization and CFD simulation of soy white flakes based dough in a single screw extruder. *Journal of Food Process Engineering*. DOI: 10.1111/jfpe.12368

1.3 Literature Review

1.3.1 Protein Sources in Fish Feed

Protein sources are categorised into animal origin and plant origin. Animal proteins sources such as fishmeal (FM), meat bone-meal, blood meal (>60%) have higher protein content than plant protein sources such as soybean meal (>42%). Animal proteins are considered superior sources to plant proteins because of their balanced amino acid profile (Li et al., 2004).

The main protein sources used in aqua feed production was primarily fishmeal. With the rising prices of fishmeal, aquaculture industry is now focusing on plant proteins as inexpensive source of proteins to minimise production cost.

1.3.1.1 Alternative Plant Based Protein Sources

1.3.1.1.1 Soy White Flakes

The majority of soy product produced comes in the intermediate form of defatted soy white flakes (SWF). SWF are produced by cleaning, heating and cracking soybeans, removing the hulls by aspiration; flaking the chips to about 0.25-0.30 mm thickness and extracting the oil by hexane to 0.5-1.0% oil. While the oil is removed by extraction, the carotenes are removed as well and the extracted residue gets a typical white color, hence the name white flakes. SWF can then be ground to make soy flour, toasted to make soybean meal (SBM), or further processed to produce soy protein concentrate (SPC) or soy protein isolate (SPI). Defatted soy flour usually contains a minimum of 50% protein on a wet basis (Lusas and Riaz, 1995). SWF can have extremely high protein dispersibility index value of 95, but can also be produced with protein dispersibility index values of 20, 70, or 90, depending on the thermal exposure (Lusas and Riaz, 1995). Therefore, adequate thermal treatments of SWF are recommended for use in carnivorous fish feeds to reduce protein dispersibility index and anti-nutritional factors.

There is little information about the use of white flakes in aquafeed. Romarheim et al. (2006) studied the effect of extrusion on nutritional value of soybean meal and white flakes to replace fishmeal in the extruded diets of rainbow trout. It was reported that the trypsin inhibitor activity levels in the SBM and SWF diets were reduced to 0.6 and 2.1mg (g/dry matter) after extrusion. Feed intake and feed conversion ratio of trout was lower for SBM and SWF diets than FM diet. Apparent digestibility of nitrogen was highest for FM followed by SBM and lastly SWF diets.

1.3.1.1.2 Distiller's Grain (HP-DDG and DDGS)

Distillers grains is the co-product of ethanol industries. Distillers dried grains and distillers dried grains with solubles are rich in nutrient content compared to whole corn. During ethanol making process the residue remaining after distillation is separated into ethanol and stillage. The stillage contains a solid portion known as distillers grains and soluble portion known as solubles. The soluble portion also contains a lot of nutrient. Hence usually in ethanol plant, the soluble fraction is mixed with solid portion and dried. The dried product is sold as distillers dried grains with solubles (DDGS).

During the dry-milling process, three types of co-products including distillers dried grains (DDG), distillers dried solubles (DDS), and distillers dried grains with solubles (DDGS) are produced after the starch fermentation and distillation of fermented mash. The soluble portion of the residual co-product is evaporated and condensed distillers solubles (CDS) are produced. The solid portion of the remaining fermentation residue and condensed distillers solubles are both subjected to drying and produce either distillers dried grains (DDG) or distillers dried solubles (DDS) (Singh et al., 2005). When these two co-products mixed and dried, distillers dried grains with solubles (DDGS) is produced.

Recently bio-refinery plants have employed a new technique to increase the ethanol production yield. In the new dry-milling process, whole corn is fractionated into several fractions including germ, bran, and endosperm. Germ fraction contains high fat and phosphorous concentrations in addition to a better essential amino acids profile. Endosperm fraction is subjected to fermentation and ethanol recovery. Indeed, pre-removal of the non-fermentable fraction of corn is the main reason for the ethanol yield increase (Singh et al., 2005). The co-product of this process is DDG, which is high in protein but low in fat. Typically, conventional DDGS (C-DDGS) contains approximately 30% protein (Rosentrater and Muthukumarappan, 2006; Spiehs et al., 2002). The protein content of DDG is almost 1.5 times that of C-DDGS; hence, it is called HP-DDG (Robinson et al., 2008). Moreover, using HP-DDG provides higher available phosphorous content thus reducing the need for phosphorous supplementation. Robinson et al. (2008) believed that HP-DDG nutritional values are much more consistent than those of C-DDGS.

1.3.1.1.3 Corn Gluten Meal

Corn gluten meal (CGM) is a co-product of wet milling of corn, in the process of producing corn syrup. Corn gluten meal is the dried residue from corn after the larger part of the starch and germ have been removed and the bran separated by the process employed in the wet milling manufacture of corn starch or syrup, or by enzymatic treatment of the endosperm (Raven and Walker, 1980).

Corn gluten is an excellent protein source for aquafeed with at least 60% protein (Morales et al., 1994) which is 97% digestible by trout (Sugiura et al., 1998). Corn gluten meal can replace 25–40 % of fish meal without adverse impact on growth performance of trout (Hardy, 2000; Morales et al., 1994). In general, CGM can be added up to 10% in trout diets. At higher level, it tends to impart yellow color to fish flesh. However, CGM can be added up to 22.5% in salmon and trout commercial diets by adding canthaxanthin or astaxanthin which masks the yellow color in fillets and gives natural pink color (Skonberg et al., 1998).

Pereira and Oliva-Teles (2003) reported that CGM can replace up to 60% fish meal protein in diets for gilthead sea bream juveniles without any adverse effect on growth performance. It is reported that CGM can replace up to 40% of fishmeal in the diet of juvenile Japanese flounder (Kikuchi, 1999). Robinson et al. (2001) reported that up to 50% CGM can be efficiently utilized by channel catfish, *Ictalurus punctatus*, without adverse effect on feed palatability, weight gain, or feed efficiency. Wu et al. (1995) evaluated the use of corn gluten meal as protein source in tilapia diet and found that the diets having CGM (and soybean, with or without fishmeal and lecithin) with 32%

protein showed better weight gain, higher protein efficiency ratio and better or equal feed conversion ratios than a commercial feed containing 36% protein with fishmeal.

1.3.2 Fish Feed Formulation

The average protein component in the fish body is 65-75% and is the basic building nutrient in the fish feed. The dietary protein requirement of fish is much more than the terrestrial animals. Dietary protein content balancing is the primary importance in the fish feed formulation. The cost of protein sources accounts more than half of the feed ingredient cost.

The best quality of fish feed producing maximum growth in fish depends on

1. Quantitative protein content
2. Qualitative protein content
3. Total energy content and digestible energy content
4. Level of intake, feeding method & physiological state of fish

1.3.2.1 Quantitative Protein Requirement

In fish feed formulation two important factors to be considered are percentage of protein in the ingredient and protein cost. In fish cultivation, as the protein content increases, the growth rate also increases and reaches a maximum beyond which no further increase in growth rate was observed. Hence an optimum level of dietary protein is determined by the relationship between cost of the feed ingredient, nature of farming operations, capital investment and fish market trends. The optimum level of protein requirement depending on the physiological state of catla fish is given in the Table 1.1.

1.3.2.2 Qualitative Protein Requirement

The qualitative protein requirement is determined by the ratio of the essential amino acid required by the fish, to the quantity of essential amino acid present in the feed ingredients. The amino acid requirement of different fish species is given in the Table 1.2. The qualitative requirement of any fish feed is represented by the Essential Amino Acid Index (EAAI) and is calculated using the equation:

$$EAAI = \sqrt[n]{\frac{100a}{a_e} \times \frac{100b}{b_e} \times \frac{100c}{c_e} \ldots\ldots\ldots\ldots \frac{100j}{j_e}} \qquad (1.1)$$

where a .... j are the percentage of EAA in the diet

a_e ....j_e are the percentage of the EAA required

n is the number of terms in the expression

If any of the terms of the form $100a/a_e$ is greater than 100, it is reduced to 100 for the calculation of *EAAI* (Jauncey and Ross, 1982).

1.3.2.3 Total and Digestible Energy Content

The total energy content of any fish feed is determined based on the fraction of protein, lipid and carbohydrate contributing to the dietary energy. The total energy content is calculated based on the energy content of fractions namely, 4.5 kcal/g for protein, 9.1 kcal/g for lipid and 4.1 kcal/g for carbohydrate. The digestible energy content is calculated based on the ratio of the percentage of the different factions really available to the fish for digestion in the digestive system.

1.3.2.3.1 Protein Energy

Protein is the most expensive source of energy in the fish feed. Hence alternative economical dietary protein is to be identified for minimizing the feed cost. DDGS is an important ingredient considered for minimizing the feed cost based on its availability and future scope. The relationship between the protein and energy levels of the feed is expressed as Protein to Energy ratio (P: E) and usually expressed in mg of protein per kcal of energy.

1.3.2.3.2 Lipid Energy

The lipid in the fish feed has two principal functions as an energy source and as a source of essential fatty acid. Fish cannot synthesize ω-3 and ω-6 fatty acids required for growth and have to be supplemented in the feed. The ω-3 and ω-6 fatty acid content of some commonly used oil used in the fish feed are given in the Table 1.3.

1.3.2.3.3 Carbohydrate Energy

The carbohydrate content of feedstuff can be divided into digestible carbohydrates and fiber. Fiber is composed of complex polysaccharides and in the case of plant material mainly cellulose. Fish do not have digestible enzyme cellulase and thus cannot utilize cellulose. Hence for fish feed purpose, dietary fiber is considered to be unavailable to fish as an energy source.

1.3.2.4 Level of Intake, Feeding Method and Physiological State

Depending on the feeding method the fish feeding can be divided into three namely, programmed feeding, ad libitum feeding and demand feeding. Generally protein requirement of fries are much higher than the grownup fish. Hence protein content and size of pellets in a feed material is decided based on the physiological state of fish. For example, for catla (*Catla catla*), depending on the physiological state, the commercially available feeds are manufactured as per Table 1.4.

1.3.3 Soy White Flakes as a Base Material for Fish Feed

Soybean products have been of particular interest as a FM replacer due to the dense nutritional content; however, indigestible, deficient, and anti-nutritional factors (ANF) components limit the bioavailability of nutrients in soy and other plant products (Francis et al., 2001; Sinha et al., 2011; Tacon and Jackson, 1985).

Defatted soybean meal (SBM) is one of the most commonly used alternatives to FM, due to a well-balanced amino acid profile, moderately high protein content, consistent quality, relatively low cost, and high domestic availability (Sales, 2009). Compared to FM, SBM has lower methionine, which is considered to be the first limiting amino acid for fish (Storebakken et al., 2000), while lysine and threonine are also known to be limiting amino acids in SBM (Gatlin et al., 2007). SBM and other processed (mechanical, chemical, and/or microbiological) soy products have been used to replace FM in aquaculture feeds for several species with varying degrees of success (Brown, 2008; Kaushik et al., 1995; Refstie et al., 1997). Experiments which utilized carnivorous species have revealed limited inclusion levels of dietary SBM (Baeverfjord and Krogdahl, 1996; Olli et al., 1994b) SPC and SPI with reduced anti-nutritional factors (such as trypsin inhibitors and phytic acid) and bioavailable protein have been successful, but high processing costs limits their use as complete FM replacements (Gatlin et al., 2007). Soy bioprocessing technologies are being optimized to increase protein content, reduce indigestible components (e.g., oligosaccharides), reduce ANF’s, or improve digestibility (Refstie et al., 2005).

1.3.4 Extrusion Technology

Extruder is a high temperature short time bioreactor that transforms a variety of raw materials into modified intermediate and finished products. It combines several unit operations including mixing, cooking, kneading, shearing, shaping and forming (Riaz, 2000, 2007). The advantages of the extrusion cooking are 1) continuous high throughput processing, 2) energy efficient, 3) processing of relatively dry viscous material, 4) improves textural and flavor characteristics of food, 5) control of the thermal changes of food constituents, 6) use of unconventional ingredients and 7) process scale-up.

The extruders used in the food and feed industries can be widely divided into two namely single screw extruder and twin screw extruder.

1.3.4.1 Single Screw Extruder

The main advantage of single screw extruder over twin screw extruder is, they are mechanically very simple and therefore costs 1/4th to ½ of price of similar sized twin screw extruder. Due to this advantage single screw extruders are used wherever possible in the industry. Single screw extruder relies on drag flow for conveyance. Hence the mass must stick to the wall for proper movement inside the barrel. In general higher the friction forces more efficient is the extruder. An important difficulty faced while using the single screw extruder is its poor mixing.

The movement and transformation of material in the extruder can be divided into three sections: feeding, kneading or transition and final cooking zones. The feeding zone receives preconditioned material and is conveyed to processing zone where free-flowing amorphous material is worked into dough. The compression ratio is increased to assist in blending in kneading zone. In the cooking zone, the thermal and mechanical energy input plasticises the material above its melting point. The final screw element reduces the volumetric displacement and adds compression (Riaz, 2000).

1.3.4.2 Twin Screw Extruder

Based on the direction of rotation the twin screw extruder is divided into counter rotating and co-rotating extruder. Based on the screw configuration and degree of intermeshing, twin screw extruder can be divided into fully intermeshing, partially intermeshing and non-intermeshing extruder (Janssen, 1989; Riaz, 2007).

The main advantage of twin screw extruder over single screw extruder is its transport mechanism. In a twin screw extruder the material is positively transported by the ‘C’ shaped channels formed in the screw. This allows the twin screw extruder to use wide variety of raw materials to obtain wide variations in product properties.

1.3.5 Aquafeed Technology

One of the oldest technologies that have been used for aquafeed production is pellet milling. Expanders are also being used for aquafeed production. Extruder technology has been used in aquafeed production for half a century. It has been suggested that extrusion offers better flexibility in operation than pelletizing technology (Martín, 1999). The advantages of extrusion processing over the pelletizing and expanders are: 1) controlling the physical properties of the aquafeed such as buoyancy and density of the feed, water stability, density and porosity, 2) increased bulk density, 3) increased nutrient density, 4) increased nutrient palatability, 5) inactivation of microorganisms, 6) enhancing the ingredient digestibility, 7) inactivating anti-nutritional factors and 8) versatility in production of high protein and fat contents diets.

Quality of the food and feed products are related to both physical and nutritional characteristics of the product. In general extrusion processing involves in several independent variables (such as temperature, moisture content, blend composition, screw speed, die size and configuration, screw configuration, etc.) and dependent variables (such as specific mechanical energy, residence time, torque, pressure, etc.), and thus interaction effects of these variables induce chemical and physical changes to the processing ingredients and thus transfers the state of the materials at both macroscopic and microscopic scale. The complexity of the process indicates that there is a strong interrelationship between the effect of extrusion processing on physical and nutritional properties of the feed/food.

Various authors have studied the effect of extruder parameters like screw configuration, screw speed, temperature of cooking and feed parameters like moisture content, particle size distribution, starch content, protein content and fat content on the extrudate quality.

1.3.6 Effect of Feed Parameters

1.3.6.1 Raw Materials Composition

The most used raw materials in the extrusion process are starch and protein-based materials. The structure of the extruded products may be formed from starch or protein polymer transformations during the process. The physicochemical changes in biopolymers that can occur during extrusion cooking include: binding, cleavage, loss of native conformation, fragmentation, re-association, and thermal degradation. Physical losses may change the composition of the raw ingredients such as water evaporation

(Riaz, 2000). Most of the chemical reactions take place in the high-pressure zone of the barrel and at the die.

1.3.6.1.1 Starch

The effect of starch quality on the extrudate quality was studied extensively. The starch granule consists of two glucose polymers: amylose and amylopectin; both play crucial roles in physicochemical and functional properties of the starch (Caldwell et al., 2000). According to Colonna et al. (1989), during extrusion processing, amylopectin is more prone to shear. According to Case et al. (1992), increased screw speed and barrel temperature significantly influenced the degree of starch gelatinization. Mercier et al. (1989a), suggested that the interaction effect of application of heat, shear and water content on the starch granule can destroy the organized molecular structure, also resulting in less integrity and molecular hydrolysis of the material. Starch gelatinization affects many extrudate properties, such as water stability, digestibility, and expansion ratio. However, the extent of starch gelatinization by itself depends on starch type, particle size, and conditions of the extrusion process (Rokey and Plattner, 2003). The micro and macro structures and physiochemical alterations of cooked, double extrusion cooked and retrograded corn starch during a single-screw extrusion processing was studied by Chinnaswamy et al. (1989).

The micro structural, physiochemical and macro molecular changes in extrusion cooked and retrograded corn starch was studied by Chinnaswamy et al. (1989). The experiments were conducted at a constant temperature of 140°C at die section and 80°C at feed section in a laboratory single screw Brabender extruder. The water solubility, shear strength, micro structure, x-ray difractograms were studied on a single extrusion cooked, double extrusion cooked and retro graded starch. The expansion ratio dropped from 12.9 to 4.6 and the gas cell size decreased considerably with retrograded starch compared to other two starches. Water solubility decreased from 28.7% to 4.7% and the shear strength increased from 0.64 to 5.24 MPa in retrograded starch compared to other two starches.

The influence of process condition namely, screw speed, temperature at the metering zone, and temperature at the die with the wheat flour components such as wheat starch, vital gluten and wheat flour solubles on extrudate quality was studied by Paton and Spratt (1984) in a single screw extruder. The interaction effects of the process condition with wheat flour components were studied in relation to torque, extrudate viscosity, water absorption index and water solubility index. They reported that the integral structure of wheat flour particles imparts a greater resistance to water penetration and cooking than when the individual components exists at random in a composite formulation. The composite of all three components gave better performance than the wheat flour alone.

Chinnaswamy and Hanna (1990) conducted experiments at different moisture content (10-30%) and barrel temperature (110-200°C) with starchy material. They studied functional properties of extrudates like radial expansion, shear strength, water solubility of starch, apparent viscosity, macro molecular structure by differential scanning calorimetry and gel permeation chromatography. They reported that maximum expansion ratio of 16.4 was obtained with 50% natural amylase. Sodium bicarbonate degraded the starch molecules to a greater extent compared to sodium chloride and urea. The physicochemical properties like density, solid density, apparent solid density, expansion ratio, open and closed pore volumes, water absorption index, and water solubility index of commercially available starch based breakfast cereal was studied by Jones et al. (2000). The density was measured by volume displacement method; solid density was measured by helium comparison pycnometer. The expansion ratio was ranged from 1.0 to 17.3 units. The densities of the product ranged from 0.08 to 1.44 g/cm^3. The water solubility and water absorption index ranged from 6.3 to 86.4% and 2.5 to 6.3 g/g respectively. The effect of feed rate, screw speed and barrel temperature on the starch gelatinization in a twin screw extruder was studied by Ibanoglu et al. (1996) and they concluded that barrel temperature had the most pronounced effect on the starch gelatinization followed by feed rate and screw speed.

1.3.6.1.2 Protein

Proteins have a larger number of chemical groups when compared to polysaccharides. This indicates that proteins are more reactive (Mitchel and Arêas, 1992) and undergo many changes during the extrusion process, with the most important being denaturation (Camire, 2000). Proteins in general are classified, with respect to their solubility in water, saline solution, alcohol solution and acid or alkaline solutions, respectively (Pereda et al., 2005).

Thomas et al. (1997) studied the effect of water and steam added in the preconditioner on the protein quality through protein dispersibility index, nitrogen solubility index and trypsin inhibitor activity (TIA) of the extruded product. The authors used a preconditioner to increase temperature and mixing steam and water, so that the protein quality was maintained after extruding in the extruder. They found that significant interaction between the water and steam content on protein quality in all the experiments.

The screw speed has no significant effect on the protein quality parameters. In another study conducted by Dahl and Villota (1991) on the effect of extrusion on texturization of alcohol modified soy flour, less contribution of protein in expansion was reported.

1.3.6.1.3 Lipids

During extrusion processing, lipids exhibit lubrication effects and reduce the friction between particles in the mix and between the screw and barrel surfaces and the fluid melt (Guy, 2001). The effect of lipids and processing conditions on degree of starch gelatinization of extruded pet food was studied by Lin et al. (1997) and found that high lipid content of the extruding blend can adversely affect the starch gelatinization. The adverse effect of high moisture content on expansion of the extrudates was also reported. The same authors, Lin et al. (2000) studied the effect of lipids, processing conditions on the sensory characteristics of extruded dry pet food and reported that high lipid content results in lipid oxidation and lower the sensory quality of the extrudates during the storage. Colonna et al. (1989) reported that reduction of moisture content can control the expansion of materials containing high lipid content. Some other researchers believed that the type of starch and lipid present in the raw material greatly influences the formation of the amylose-lipid complex, with free fatty acids and mono-glycerides (Camire, 2000; Mitchel and Arêas, 1992).

1.3.6.1.4 Fibers

Dietary fiber consists of fractions of vegetable cells, polysaccharides, lignin, and associated substances, which are resistant to hydrolysis by enzymes present in the digestive system of humans. It has been reported that, extrusion cooking of fibers can produce changes in their structural characteristics and physicochemical properties of fibers and converts the insoluble fibers into soluble fibers (Camire et al., 1990; Guillon et al., 1992; Larrea et al., 2005).

According to Wang et al. (1993), production of soluble fiber is due to the rupture of covalent and non-covalent bonds between carbohydrates and proteins associated to the fiber. In turn it reduces the molecular size that would be more soluble (Fornal et al., 1987; Wang et al., 1993). Many researchers reported that high dietary fiber content in extruding materials reduces expansion index of the extrudates (Hsieh et al., 1989; Ilo and Berghofer, 1999; Vernaza et al., 2009). One of the reasons of this reduction in expansion index can be due to reduction of starch content as a result of high fiber content in the extruding materials formula (Colonna et al., 1989). According to Camire et al. (1990), fibers may bind water more strongly than proteins and starch during extrusion and thus inhibits water loss at the die exit of the extruder, reducing expansion index.

1.3.6.2 Effect of Moisture Content

Extrusion at low moisture content tends to produce a dense unexpanded product. As the feed moisture content increase, there will be more expansion due to cooking of available starch and will produce lighter product. However, as the moisture level increases to a high level, it reduces the viscosity of the material through the barrel and makes it more difficult to expand the product (Plattner, 2007b).

Thiébaud et al. (1996) suggested that at a given temperature, higher moisture contents resulted in less texturized extrudates. High moisture content can reduce the melt viscosity and weaken the protein–protein interactions. They reported that protein could be texturized at 140 to 180°C and relatively low moisture content.

Extrusion at low moisture content can affect the specific mechanical energy. According to Guy (2001), moisture content of 15 to 30% increases mechanical energy and results extrudates with lower density, whereas increased moisture from 50 to 70% results in products with higher density.

Shukla et al. (2005) reported that when DDGS based feed was extruded in single screw extruder, the product showed maximum water absorption index (WAI) and water stability index at 15% initial moisture content of feed than 20% and 11% feed.

1.3.6.3 Effect of Particle Size Distribution

Particle size is important attribute that affect product characteristics. A uniform particle size ensures adequate hydration and uniform cooking during extrusion. If the particle size is larger, it does not get cooked properly and degrade the product quality. In addition to that, it may cause plugging of the die during extrusion. For die opening less than 3mm, it is recommended to grind the ingredients small enough such that the largest particle is less than one-third of the die orifice. The benefits of a proper final particle size are: improved product appearance, reduced chances of die plugged, good water stability and absorption, lower bulk densities, higher throughput, improved digestibility and palatability, and improved oil retention. The effect of particle size on bulk density of dog food extruded in a single screw extruder is illustrated in Table 1.5 (Rokey, 2007).

1.3.7 Effect of Extruder Parameters

1.3.7.1 Effect of Screw Configuration

Several studies have been conducted using single and twin-screw extruders to evaluate the effect of extruder parameters on extrudate properties. Sokhey et al. (1994) used corn starch to study the effect of different kinds of screws (screws without mixing elements, screws with one mixing elements and screws with two mixing elements) in a Brabender single screw extruder. They have studied the performance of the extruder with specific mechanical energy, radial expansion and over all expansion. They reported that the screw configuration had no significant effect on specific mechanical energy, and overall expansion. But significant difference in radial expansion was observed between the extruded and re-extruded corn starches with different screws.

In another study conducted by Barres et al. (1990), they studied the effect of 5 different screw configurations and varying levels of feeding rate in two different twin-screw extruders, namely Clextral BC 45 and 72, on water solubility index (WSI) of starch-based extrudates. It was found that screw with reverse elements greatly influenced the WSI of the extrudates.

The effect of mixing element on the water solubility and water absorption indices of cross linked starch was studied by Seker et al. (2004). The mixing element increased the specific mechanical energy dissipation in to the starch but there was no significant effect on the water solubility and water absorption indices of cross linked starch in a single screw extruder.

1.3.7.2 Effect of Barrel Temperature

Extruder barrel temperature is reported to have profound effect on the product properties depending on the degree of cooking. Extrusion cooking helps to inactivate heat labile anti-nutrients present in plant proteins (Lucht, 2007). Temperature also plays significant role in properties of the extrudates mainly due to its effect on expansion. Generally, expansion does not occur at temperature of less than 100°C.

Various researchers studied the effect of temperature on water absorption and solubility indices of an aquafeed containing distillers dried grains with solubles (DDGS) during single screw extrusion processing. They reported that increase in processing temperature from 100 to 150°C resulted in significant increase in water solubility index and water absorption index of the DDGS based diet extruded in single screw extruder. Extrusion of similar types of feed was studied by Kannadhason et al. (2009). They observed that increase in processing temperature from 100-150°C increased the WSI but decreased WAI and PDI in curvilinear fashion for DDGS and tapioca based diet. In another study, a decrease in bulk density and increase in radial expansion ratio were reported with extrusion processing temperature of 120°C (Chevanan et al., 2010).

1.3.7.3 Effect of Screw Speed

The extrusion processing is quite complex and interaction effect of several variables influence the products of this process. Therefore, in most of the cases, it is not possible to explain the effect of each variable separately. It is well known that screw speed governs the mechanical energy input. Increasing screw speed elevates the friction between the product and screw and thus more mechanical energy is produced. On the other hand increased screw speed results in inadequate cooking due to shorter residence time. According to Plattner (2007a), increased SME due to increased screw speed decreases bulk density. In a single-screw extrusion study for a DDGS-based aquadiet, similar results were observed by Rosentrater et al. (2009b). The author reported that increasing the screw speed from 100-150 rpm reduced bulk density and pellet durability index.

1.3.7.4 L/D ratio

The die L/D ratio also affects the extrudate properties. Chevanan et al. (2007a) reported that increase in the L/D ratio (3.33, 3.33, 3.43, 4.81, 5.83, 7.25 and 10.00) of the die nozzle resulted in an increase in bulk density, L*, a*, and torque, but a decrease in unit density, pellet durability, water-absorption index and sinking velocity of the DDGS based extrudates. However, there was no regular trend for other properties like unit density, bulk density and pellet durability index evaluated.

1.3.8 Fish Feed Extrusion

Fish feed requires 26-50 % protein depending on the physiological state of fish. Hence the formulated feed contains high amounts of both starch and protein. Bandyopadhyay and Rout (2001) studied the effect of flow rate, L/D ratio of barrel and barrel temperature on the water stability and expansion ratio of the sinking marine shrimp feed. The best shrimp feed had 90.3% water stability, 0.99 expansion ratio and 1347.9 kg/m^3 true density. Rolfe et al. (2001) studied the water stability index, pellet durability and buoyancy of extruded cat fish feed. Increasing the moisture content resulted in increased water stability and pellet durability. Reducing the particle size from 700 to 100 μm resulted in increased degree of gelatinization, pellet durability and water stability.

1.3.8.1 Properties of Aquaculture Feed Extrudates

1.3.8.1.1 Physical Properties

The understanding of physical properties of aquafeed is important for formulation of feed and manipulation of processing variables to get the desired property of aquafeed. Some of the important physical properties in relation to aquaculture feed are described below:

1.3.8.1.1.1 Bulk Density and Unit Density

The bulk density is defined as mass per unit volume of the material including the void space. It gives useful information for designing storage containers for extrudates and raw materials (Ayadi et al., 2011). From an economic point of view, the higher the bulk density, the better, because it reduces the packaging, storage and transportation costs for a certain mass of product. The unit density of extrudate depends on the degree of expansion during extrusion and is an important parameter determining floatability of aquafeed. Tumuluru and Sokhansanj (2008) reported that the effect of extrusion processing conditions such as screw speed, barrel temperature, die dimension, and feed moisture content were significant on bulk density and unit density of the extrudates.

1.3.8.1.1.2 Expansion Ratio

Expansion ratio is defined as the ratio of extrudate diameter to the die diameter (Onwulata et al., 2001; Zazueta-Morales et al., 2002). Expansion occurs in both radial and axial directions, at different degrees, depending on the viscoelastic properties of the melt. During the vaporization of moisture and cooling steps the extrudate experiences a phase transition from a molten to a rubbery state (Barrett, 2003).

The expansion of the extrudates is related to the state of damage of starch and gelatinization and protein denaturation. The quantity of starch and protein in the blend affects expansion property of extrudates (Plattner, 2007b). The pre-processing history of ingredients in the blend also affects expansion of extrudates; heat denatured proteins looses the stability to expand (Sørensen et al., 2009).

1.3.8.1.1.3 Pellet Durability

Fish feed are subject to several mechanical forces during handling and transportation. These forces may be classified as impact, compression, and shear. Impact shatters the pellet surface and any natural cleavage planes in the pellet; compression forces crush the pellet; shear forces cause abrasion of the edges and surface of the pellet (Winowiski, 1995). Physiochemical transformation of starch component of the feed blend in response to the variation of extrusion processing condition significantly impacts the cohesiveness and durability of the extruded blends against the external pressures (Chevanan et al., 2007b; Colonna et al., 1989; Rosentrater et al., 2009a).

The durability of extruded pellet is measured in terms of pellet durability index and is the measure of pellet's ability to withstand breakage and disintegration in a device with a tumbling motion (Tumbling Can Tester) or one that simulates vigorous pneumatic handling (Holmen Pellet Tester). The whole pellets retained by sieving after subjecting a measured quantity of pellets to the standard testing procedure gives the measure of pellet durability index (Evans, 1999).

1.3.8.1.1.4 Water Absorption and Solubility Indices

WAI can be defined as the amount of occupied volume (due to starch content of the material) after swelling up in water. In other words, WAI indicates the part of the starch which was not affected by the extrusion cooking and maintained its internal structure (Mason and Hoseney, 1986). Thus, changing the WAI with increasing the screw speed could be explained by structural modifications of the feed compositions, such as starch gelatinization and protein denaturation (Badrie and Mellowes, 1991; Rosentrater et al., 2009a). Water solubility index, on the other hand, can be defined as the portion of starch that was converted during the extrusion cooking process. Ng et al. (1999)

suggested that WAI is inversely related to WSI. In our study, an inverse relationship between the WAI and WSI was observed.

Jones et al. (2000) used the following procedure to determine the WSI and WAI. To determine WAI, 2.5 g of finely ground sample was suspended in 30 mL of distilled water at 30°C in a 50 mL tarred centrifuge tube. The content was stirred intermittently over a period of 30 min, and then centrifuged at 3000 x g for 10 min. The supernatant water was transferred into tarred aluminum dishes. The mass of the remaining gel was weighed, and WAI was calculated as the ratio of gel mass to the sample mass.

WSI was determined as the water soluble fraction in the supernatant, expressed as percent of dry sample. The WSI was determined from the amount of dried solids recovered by evaporating the resulting supernatant in an oven at 135°C for 2 h; it was determined as the mass of solids in the extract to the original sample mass (%).

1.3.8.1.2 Nutritional Properties

Extrusion cooking can cause both beneficial and detrimental effect on nutritional properties of the feed. According to Singh et al. (2007), the thermo-mechanical effect of extrusion parameters and processing variables can destruct the anti-nutritional factors, gelatinize the starch, increase solubility of dietary fiber, and even reduce lipid oxidation.

Bhattacharya et al. (1988) reported that increasing extrusion temperature from 100 to 140˚C improved the degree of inactivation of protease inhibitors in wheat flour. They also found that the effect of length to diameter ratio and screw speed on protein digestibility values were insignificant ($P < 0.05$). In another study, it was reported that increased screw speed could increase the protein digestibility of extruded corn-gluten, because the increase in shear forces in the extruder denatured the proteins more easily (Bhattacharya and Hanna, 1985).

Fish require some main nutrients such as protein, fat, carbohydrate, vitamins and minerals; however, these requirements vary by species and age. According to Fenerci and Şener (2005), fish use proteins as their energy source, but because of the high cost of proteins, fats and carbohydrates are preferred as energy source in feeds. Proteins must be used only for growth in fish (Demir, 1996; Şener and Yıldız, 1998).

However, Maillard reactions between protein and sugars can reduce the nutritional value of the protein, depending on the raw material types, their composition and process conditions. Several reports had attempted to relate Maillard reaction and discoloration or browning to loss of lysine (Asp and Björck, 1989; Björck and Asp, 1983; Cheftel, 1986; Hurrel and Carpenter, 1977). Furthermore, lysine loss has been related to extrusion process parameters such as raw material, feed moisture, screw speed, extrusion temperature, die diameter, feed rate, screw compression ratio, torque and pressure, energy input and pH (Asp and Björck, 1989; Camire et al., 1990).

1.3.8.1.2.1 Anti-Nutritional Factors

Numerous substances are naturally present in plants, not needed for normal growth but may have protective functions, are called secondary metabolites. These secondary plant metabolites are also referred to as anti-nutritional factors (ANFs). These ANFs adversely affect the growth performance of animals when fed on them. They may affect by reducing protein digestibility, binding to various nutrients or damaging the intestinal wall, thereby lowering digestive efficiency (Mosenthin and Jezierny, 2010). They can be divided into two categories: heat-labile and heat-stable ANFs. Heat-labile

ANFs include trypsin inhibitors, phytates, lectins, goitrogens and antivitamins. Heat-stable ANFs include carbohydrate or soluble fiber, saponins, estrogens, allergins, and lysinoalanine.

The type and content of these ANFs varies considerably among different feedstuffs: Protease inhibitors and lectins are most significant for legume seeds (soybeans, peas, faba beans, lupins), tannins are present in rapeseed, faba beans and peas, whereas glucosinolates and sinapins dominate in rapeseed, alkaloids are important in lupins, and pyrimidine glycosides can generally be found in faba beans (Mosenthin and Jezierny, 2010). These ANFs exert various deleterious effects on monogastric animals, including fish (Table 1.6).

There are number of physical and chemical means of processing methods to remove the anti-nutrients in plant proteins. These include: heat treatment (cooking, steaming), soaking, germination, decortication (dehulling), fermentation, selective extraction, irradiation, enzymatic treatment (Deshpande, 2002; Mosenthin and Jezierny, 2010). Heat-labile anti-nutrients such as protease inhibitors, lectins can be reduced to safe level by proper thermal processing. However excessive heating can adversely affect the protein quality of feed.

The major anti-nutritional factor of challenge in soybean is trypsin inhibitor which is a protease enzyme. To avoid any nutritional problem in animals, it is necessary to remove at least 85% of the trypsin inhibitor units. This can be easily done by heat treatment. Moist extrusion (MC>18%), can destroy up to 95% of the trypsin inhibitor with significant loss of lysine. However, dry extrusion has deleterious effect on lysine. Moist extrusion is better than dry extrusion and traditional roasting (Rokey, 2007).

1.3.8.1.2.2 Available Lysine

According to Singh et al. (2007), lysine is the most limiting essential amino acid in extruded products. Iwe et al. (2004) reported that increased screw speed (80– 140 rpm) and a decreased die diameter (10–6 mm) enhanced available lysine retention. They suggested increasing screw speed increased shear, leading to more severe conditions, but the reduction in residence time shortened the duration of heat treatment, which resulted in higher lysine retention. It has been reported that excessive heating leads to a decrease in amino acid availability, specifically Lysine. The effect of heat treatment on available lysine of DDGS and SBM were studied by Fernandez and Parsons (1996); McGinnis and Evans (1947); Warnick and Anderson (1968). In another study conducted for extrusion of wheat flour (150^{o}C mass temperature, 5 mm die diameter, 150 rpm screw speed), increasing in feed rate (from 200 to 350 gmin^{-1}) led to a significant increase in lysine retention (Björck and Asp, 1983).

In terms of moisture content, several studies reported conflicting results. According to Cheftel (1986), to maximize lysine retention, for MC <15%, product temperature should be kept below 180˚C. Björck and Asp (1983) reported that higher moisture content (15–25%) significantly improves lysine retention. According to Singh et al (2007), these changes might not be related to a single factor. They suggested that the role of feed moisture content and the interactions of other parameters on the protein nutritional quality need further investigation. The effect of extrusion processing variables on lysine availability is presented in Table 1.7.

1.3.9 Modeling and Simulation of Extrusion Studies

Extrusion processing is a very complex process, involving numerous interdependent input parameters (both process, system parameters) and output parameters. Process parameters are the operating conditions that can be controlled and manipulated directly such as raw material characteristics, moisture content, screw speed, screw configuration, barrel temperature etc. System parameters are influenced by the process parameters and subsequently affect the output parameters such as residence time, specific mechanical energy, pressure build up, viscosity of dough etc. Output parameters includes expansion, bulk density, mechanical properties such as breaking strength, pellet durability etc., chemical properties such as water solubility index, water absorption index, nutrient content etc. Various models have been developed to understand the extrusion process to obtain required product characteristics.

Generally, regression modeling and response surface modeling were employed to predict output parameters form process parameters in extrusion processing. All the mathematical modeling by response surface and regression technique is product specific and machine specific. Response surface modeling is an empirical model building technique where the physical relationships are not known (Box and Draper, 1987) and is used to determine the optimum conditions for obtaining the maximum or minimum response within the operating conditions. Regression modeling is also widely used to determine the relationship between the input and output variables in extrusion processing. The relationship between the input and output variables are mostly non-linear in nature. Hence the non-linear regression equations will contain many cross product terms and higher order terms. Due to this there is possibility of very large error when there is little variation in the operating conditions. In regression modeling, the mathematical relationships are established to obtain good results through approximation without understanding the actual process.

Harper et al. (1971) developed a viscosity model where they incorporated moisture and temperature effects. Many researchers used this same model to study the viscosity of dough in extruders (Altomare et al., 1992; Bhattacharya and Hanna, 1986; Harmann and Harper, 1974; Jao et al., 1978; Luxenburg et al., 1985; Remsen and Clark, 1978). Lam and Flores (2003) introduced a correction factor for the particle size distribution of the raw ingredients in the viscosity model of dough inside the barrel of a single screw extruder.

Computational fluid dynamics (CFD) simulation of flow characteristics inside the extruder may be used to improve the understanding of underlying mechanisms and provide an insight in to the effects of process parameters. Many researchers have designed a single screw extruder using Finite Element Meshing (FEM) and CFD. Dhanasekharan and Kokini (2003) analyzed a computational method based on numerical simulations to obtain simultaneous scale-up of mixing and heat transfer for single screw extrusion of wheat dough. Connelly and Kokini (2007) used CFD to study and compare the mixing ability of single and co-rotating twin screw mixers. El-Sadi and Esmail (2005) did numerical simulation of flow to investigate the performance of micro pump using complex liquid. The dispersive mixing efficiency of the plasticized starch in a twin screw extruder was studied and simulated by Emin and Schuchmann (2013). Computer simulation of moistened defatted soy flour in a single screw extruder has been performed by Ghoshdastidar et al. (2000) to predict the dough behavior in an extrusion process.

Siregar et al. (2014b) designed and analyzed single screw extruder for processing of Jatropha seeds using FEM and CFD.

In order to maximize the SWF utilization for production of high quality aquaculture feed through processing, optimization of extrusion process parameters, operating conditions and ingredient combinations were very important. Even with advancement of technologies, extrusion processing is considered to be an art and understanding the effect of different process parameters on the quality of extruded feed is very important for scaling up operations and to produce consistent product in large scale production.

Table 1.1 Dietary nutrient requirement for catla (*Catla catla*) (% dry feed except otherwise mentioned).

Nutrient	Life stage/size class				
	Larvae	Fry	Fingerling	Grower	Broodstock
Crude protein	45%	35-45%	30-40%	30%	33%
Crude lipid	8-10%	8-10%	8-10%	7-9%	14%
Carbohydrate	26%	22-26%	<30%	22-26%	20%
Crude Fibre[†]	-	-	-	-	-

[†]Empirical data not available, general acceptable level 6-10%

Source: ICAR (2006); Singh et al. (2004); Sinha and Sinha (1994)

Table 1.2 Essential amino acid requirements of various species (% protein) [†]

Amino acid	Catla	Rohu	Tilapia	Common carp	Channel cat fish
Arginine	5.63	5.75	4.2	4.2	4.3
Histidine	2.38	2.25	1.7	2.1	1.5
Isoleucine	2.75	3.00	3.1	2.3	2.6
Leucine	4.38	4.63	3.4	3.4	3.5
Lysine	6.86	5.58	5.1	5.7	5.1
Methionine	3.00	2.88	-	-	-
Phenylalalanine	4.50	4.00	-	-	-
Threonine	4.50	4.28	3.6	3.9	2.0
Tryptophan	1.03	1.13	1.0	0.8	0.5
Valine	3.60	3.75	2.8	3.6	3.0

- Not required

[†]Source: Lovell (1989); Murthy (2002)

Table 1.3 ω-3 and ω-6 fatty acid content in different lipid sources used for fish feed formulation. [†]

Ingredient	ω-3	ω-6
Fish oil (Manhaden)	38%	4%
Safflower oil	3%	73%
Soya bean oil	7.7%	52.8%
Pollock liver oil	2.4%	0.8%
Cuttle fish liver oil	32.3%	2.1%
Beef Tallow	Not present	2%

[†]Source: Jauncey and Ross (1982)

Table 1.4 Recommended size of feed for catla (*Catla catla*) during different physiological states. †

Life stage	**Fish size (g)**	**Feed type**	**Feed size (mm)**	**Feeding rate (% body weight)**
Larvae	1.4 mg	Particle	<50 µ	400 & 800 in 1st & 2nd weeks, respectively
Fry	3-4 g	Crumble pellet	0.5 mm.	6-8, 5-6 & 3-4 in 1st, 2nd & 3rd months
Fingerling	10 g	Dry pellet	1.5-2.0 mm	3-5 & 1-3 in 1st & subsequent months
Grower	>50 g	Dry pellet	2.5-3.0 mm	1-3
Brood stock	2 years plus (3.0-5.5 kg)	Dry pellet	5 mm	1

†Source: ICAR (2006); Nandi et al. (2001)

Table 1.5 Effect of grind size on processing of extruded feed. †

Grind (hammer mill screen opening)	**Bulk density (g/l) at given rate**	**SME(kWh/t) at given rate and bulk density**	**Rate(kg/h) at given bulk density**
1.5mm	316	46.8	357
800µ	232	42.3	513

†Source: Rokey (2007)

Table 1.6 Anti- nutritional factors and their adverse effects in fish. [†]

Anti-nutrient	**Sources**	**Effects in fish**
Protease inhibitor	Soybean, pea, faba bean, cotton, rapeseed meal	Inhibit the activity of the proteolytic enzymes trypsin and chymotrypsin, and hinder feed protein utilization
Lectins or phytohaemagglutinins	Faba beans, pea,lupin, soybean meal	Affect intestinal mucosa
Glucosinolates	Rapeseed meal	Retard growth and disrupt the thyroid function
Phytates	Soybean, pea, mustard, rapeseed meal	Reduce availability of minerals like phosphorous, and decrease protein digestibility
Saponins	Sunflower oil meal, lupin seed meal, pea seed meal	Retard growth and damage intestinal mucosa of fish
Gossypol	Cottonseed	Growth depression, intestinal and other internal organ abnormalities, and unbalanced sex ratio in fish.
Tannins	Pea, rapeseed, mustard meal	Interfere with digestion and retard growth
Non starch polysaccharides (NSP) and oligosaccharides	Soybean meal	Decrease feed intake and digestibility

[†]Source: Francis and Becker (2002)

Table 1.7 Effects of processing variables on lysine retention. †

Processing Parameter	Effects on lysine retention	Food source	References
Screw speed	↑with increasing screw speed	Defatted soy flour and sweet potato flour mixture	Iwe et al. (2004)
Die diameter	↓with increasing die diameter	Defatted soy flour and sweet potato flour mixture	Iwe et al. (2004)
Feed rate	↑with increasing feed rate	Wheat flour	Björck and Asp (1983)
Feed moisture	↓with increasing moisture	Cowpea and mung bean	Pham and Del Rosario (1984)

↑, increase; ↓, decrease.

†Source: Singh et al. (2007)

CHAPTER 2 [†]

Single Screw Extrusion Processing of Soy White Flakes Based Catla Feed

2.1 Abstract

An initial investigation into the inclusion of soy white flakes (SWF) and high protein distillers dried grains (HP-DDG) in catla (*Catla catla*) diet, belonging to the family Cyprinidae, was conducted using a single screw extruder. Three isocaloric (302 kcal/100 g) ingredient blends containing graded levels of SWF in combination with HP-DDG and other required ingredients were formulated to contain a net protein content of 31.5% (db). Extrusion processing was then performed using three levels each of SWF content, moisture content, and temperature gradient keeping a constant screw speed and die diameter. Effects of these variables on extrudate physical properties including: color, pellet durability index, bulk density, water absorption index, water solubility index, unit density and expansion ratio were extensively analyzed. Increasing the level of SWF resulted in increase in water absorption index and unit density but decrease in expansion ratio. The interaction effect of SWF content, moisture content and temperature were significant for color, pellet durability index, bulk density and expansion ratio. All the extrudates showed relatively high pellet durability and inclusion of SWF produced less expanded and more compact textured extrudates.

2.2 Introduction

Extrusion is a versatile and very efficient technology that is widely used in food and feed processing including increasing numbers of ready-to-eat cereals, salty and sweet snacks, coextruded snacks, indirect expanded products, croutons for soups and salads, an

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expanding array of dry pet foods and fish foods, textured meat-like materials from defatted high-protein flours, nutritious precooked food mixtures for infant feeding, and confectionery products (Mercier et al., 1989a). Extrusion cooking is a high-temperature, short-time process in which starchy and/or proteinaceous food materials are plasticized, cooked, and in some cases expanded by a combination of moisture, pressure, heating, and mechanical shear, resulting in molecular transformation and chemical reactions. It provides a continuous high throughput processing and can be controlled automatically. It can be used to produce products with various shape, color, texture and appearance.

Protein is the most important nutrient which promotes growth in fishes. Depending on the fish species, fish feed generally requires protein content of 26% to 50% (Lovell, 1989). Commonly, high amount of ground marine caught fish as fish meal, are used to meet the requirement of protein in fishes which contributes significantly to variable production cost in aquaculture industry. However, decreasing fishmeal supply relative to demand and increasing costs threaten the sustainability and growth of the aquaculture industry. Approximately two to six pounds of marine fish are needed for the production of only one pound of farm fish (Marine Aquaculture Task Force, 2007). As protein is the costliest among various ingredients in preparation of fish feeds, it is necessary to search for the alternative protein sources in order to reduce the cost of feeds (FAO, 2004; Lunger et al., 2007; Renukaradhya and Varghese, 1986). Hence, the goal is to minimize fish meal inclusion in fish feed by substituting appropriate alternative protein sources (Hardy and Masumoto, 1990). A number studies of have been done regarding the efficacy of plant feedstuffs as alternative protein sources in fish feeds (Hossain and Jauncey, 1989).

High protein distillers dried grains (HP-DDG) and soy white flakes (SWF) can be used as an alternative source of protein. Distillers Dried Grains (DDG) and Distillers Dried Grains with Solubles (DDGS), a co-product from corn-based dry grind fuel ethanol manufacturing, is a viable protein source. Typically, DDGS contains approximately 30% protein (Rosentrater and Muthukumarappan, 2006; Spiehs et al., 2002) whereas DDG contain 1.5 times more protein that of DDGS and less fat; hence, it is called HP-DDG (Robinson and Li, 2008). Moreover, HP- DDG provides higher available phosphorous content thus reducing the need for phosphorous supplementation and its nutritional values are much more consistent than those of DDGS (Robinson et al., 2008). Fallahi et al. (2013) reported that inclusion of HP-DDG up to 40% led to the production of more expanded and floatable extrudates compared to those extrudates containing DDGS for rainbow trout. Soy is one of the most important protein-rich plants and a source of protein for aquafeeds (De Francesco et al., 2007; Karalazos et al., 2007; Morris et al., 2005). Use of soy products like full fatted soybean meal, defatted toasted soybean meal (SBM) and defatted untoasted soybean meal or soy white flakes (SWF) is becoming common (Fallahi et al., 2012). Romarheim et al. (2005) found that extrusion of SWF diet increased the digestibility of protein and all amino acids whereas fishmeal and SBM had no significant effect on amino acid digestibility. Dersjant-Li (2002) reported that soy protein isolate can be used to replace 40-100% fish meal without negative impact on growth performance of shrimp. To date, however, no trials of partial or complete replacement of fishmeal with SWF and HP-DDG for fish feeds have been conducted.

Therefore, the objectives of this study were to produce feed pellets for catla (*Catla catla*) with SWF and HP-DDG inclusions and to examine the effect of various levels of SWF content, moisture content and extruder barrel temperature on physical properties of the extruded feeds.

2.3 Materials and Methods

SWF were kindly donated by South Dakota Soybean Processors (Volga, SD). Corn flour was purchased from Cargill Dry Ingredients (Paris, IL). HP-DDG was obtained from the Dakota Ethanol LLC (Wentworth, SD). Corn gluten meal (CGM) and fishmeal were purchased from Consumer Supply Distributing Co. (Sioux City, IA). Vitamin-mineral premix was obtained from Lortscher Agri Service, Inc. (Bern, Kansas, USA). Soybean oil was obtained from USDA (Brookings, SD).

2.3.1 Blend Formulation

Three isocaloric (302 kcal/100g) blends were formulated to contain a net protein content of 31.5% (db) and a target fat content of ~ 4.2%. The total energy content for each blend was determined based on the fraction of protein, fat and carbohydrate contributing to the dietary energy. The total energy content was calculated based on the energy content of fractions namely, 4.5 kcal/g for protein, 9.1 kcal/g for lipid and 4.1 kcal/g for carbohydrate. The different ingredients in the blends include SWF (42.5% protein), HP-DDG (42% protein and 4.5% fat), corn gluten meal, corn flour, fish meal, soybean oil, and vitamin & mineral mix (Table 2.1). The ingredients were mixed in a laboratory scale Hobart mixer (Hobart Corporation, Troy, Ohio, USA) for 10 min and stored overnight at ambient temperature (25^{o}C) for moisture stabilization. The moisture balancing of the blends was done by adding required quantities of water during mixing.

2.3.2 Extrusion Processing

The extrusion processing was performed using a single screw extruder (Brabender Plasti-Corder, Model PL 2000, South Hackensack, NJ) which was powered by a 7.5 hp motor with an operating range of screw speeds from 0 to 210 rpm (0 to 22 rad/s). The extruder had a barrel with length-to-diameter ratio of 20:1 and a barrel diameter (D) of 19 mm. A uniform 19.05 mm pitch screw with compression ratio of 3:1 was used in the experiments. The clearance (H) between the inner wall of the barrel and screw at die section is 1.27 mm (0.05 in) and the clearance (3H) between the inner wall of the barrel and screw at feed section is 3.81mm (0.15 in). A typical screw of a single screw extruder is shown in Figure 2.1. The length and diameter of the die nozzle was 17.5 mm and 3 mm (L/D: 5.83), respectively. The extruder barrel was equipped with external band heaters with provisions to control the temperature of all three zones: feed zone, transition zone/melting zone, and die sections (Figure 2.2).

The raw materials were fed in feeding zone of the extruder through feed hopper. It got gelatinized and plasticized under thermal and mechanical stresses generated by the rotation of screws in melting zone of the extruder. The gelatinized material then enters the cooking zone where the extruder barrel is fully filled due to pressure generated at die nozzle. When the process reached the steady state, samples were collected at the die. All samples were left to dry at ambient temperature (25ºC) for 48 h prior to further analysis. During the experiment the screw speed of extruder was maintained at 150 rpm.

2.3.3 Experimental Design and Analysis

Experiments were conducted using a full factorial, three-level design, with SWF content, moisture content, and barrel temperature gradient levels being the independent variables. This resulted in 27 unique extrusion trials for different combinations of three levels each of SWF content (10%, 20%, and 30%), moisture content (15%, 25%, and 35% db), and temperature gradient (T1-T2-T3) in the barrel (45-110-110 ºC, 45-140-140 ºC, and 45-170-170 ºC), hereafter referred as temperature of 110, 140 and 170ºC. Each treatment was extruded once and three replicates were determined for all the extrudate physical properties, except unit density which was measured with ten replicates. All the collected data were analyzed with SAS v.9 (SAS, 2012). The Proc GLM procedure was used to determine the main, treatment and interaction effects using a Type I error rate (α) of 0.05. Post-hoc least significant differences (LSD) tests were used to identify where the significant differences occurred.

2.3.4 Measurement of Physical Properties

2.3.4.1 Color

A spectrophotometer (LabScan XE, HunterLab, Reston, VA) was used to determine extrudate color, where L* quantified the brightness/darkness, a* the redness/greenness and b* the yellowness/blueness of the extrudate samples.

2.3.4.2 Pellet Durability Index (PDI)

Approximately 100 g of extrudates from each blend were manually sieved (U.S.A. standard testing, ASTM E-11 specification, Daigger, Vernon Hills, IL) to remove initial fines, and then tumbled in a pellet durability tester (Model PDT-110, Seedburo Equipment Company, Chicago, IL) for 10 min. Afterwards, the samples were again sieved, and then weighed on an electronic balance. PDI was calculated as:

$$PDI = \left(\frac{M_a}{M_b}\right) \times 100 \tag{2.1}$$

where, M_a was the mass (g) after tumbling and M_b was the sample mass (g) before tumbling.

2.3.4.3 Bulk Density (BD)

Bulk density was determined as the ratio of the mass of extrudates that they filled up to a given bulk volume and measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by (USDA, 1999).

2.3.4.4 Water Absorption Index (WAI) and Water Solubility Index (WSI)

Extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was suspended in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminium cup and dried at 135^{o}C for 2 h (AACC, 2000). The weight of the gel remaining in the centrifuge tube was measured. The WAI and WSI were calculated by:

$$WAI(unitless) = \frac{W_g}{W_{ds}} \tag{2.2}$$

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

$$WSI(\%) = \left(\frac{W_{ss}}{W_{ds}}\right) \times 100 \tag{2.3}$$

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

2.3.4.5 Unit Density (UD) and Expansion Ratio (ER)

The extrudates were cut to a length of ~ 1 inch (25.4 mm) and weighed on an analytical balance (Adventurer™, Item No: AR 1140, Ohaus Corp. Pine Brook, NJ), then measured with a digital calliper (Digimatic calliper, Model No: CD-6C, Mitutoyo Corp., Tokyo, Japan) to determine their diameter. The unit density (UD, g/cm^3) was calculated as the ratio of the mass M (g) to the volume V (cm^3) of each measured and weighed extrudate sample, assuming a cylindrical shape for each extrudate (Rosentrater et al., 2005).

$$UD(g/cc) = \left(\frac{M}{V}\right) \quad (2.4)$$

The radial expansion ratio of the extrudates was measured as the ratio of the diameter of the extrudates to the diameter of the die orifice.

2.4 Results and Discussion

2.4.1 Color

Change in color of extrudates can be an indication of nutrients degradation during extrusion processing (Björck and Asp, 1983). Increasing the SWF content from 10% to 30% resulted in 1.2% increase in L* value but 6.9% decrease in a* value and 3.5% decrease in b* value (Table 2.2). Change in a* value can be due to the difference in color of the raw material used before extrusion. Decrease in yellowness of extrudate was expected because raw DDG was yellowish in color; thus, decrease in DDG content or increase in SWF content (Table 2.1) resulted in a significant decrease in yellowness of the extrudate. Increasing the moisture content of ingredient blends from 15% to 35%, led to significant decrease in L* and b* values by 28.0% and 23.0%, respectively. Increasing moisture content in blends had significant effect on a* value but no particular trend was observed (Table 2.2). Likewise, increasing extruder barrel temperature from 110 ºC to 170ºC resulted in significant change ($p<0.05$,

Table 2.3) in L*, a* and b* values but no specific trends were discernible.

2.4.2 Pellet Durability Index (PDI)

Pellet durability indicates the mechanical strength of the extrudates. (Rosentrater et al., 2005). In fact, the extent of heat treatment, along with the level of starch transformation, protein denaturation, and water content, during the extrusion processing, influence the pellet durability quality of the extrudates (Rosentrater et al., 2009a). The main effects of the independent variables on the extrudate pellet durability are presented in Table 2.2. The effect of changing the level of white flakes, moisture content and temperature on pellet durability of extrudates was found to be significant ($p<0.05$,

Table 2.3) but no definite pattern was observed. As depicted in Table 2.2, increasing SWF inclusion from 10% to 20%, moisture content from 15% to 25% and temperature from 110ºC to 140ºC decreased PDI by 2.6%, 8% and 3.0%, respectively. Whereas, further increasing SWF content from 20% to 30%, increasing moisture content from 25% to 35% and increasing temperature from 140ºC to 170ºC resulted in a significant increase ($\alpha=0.05$) by 5%, 12.3% and 2.5% in PDI, respectively. Maximum and minimum values of PDI were observed as 89.97% and 85.73% at 30% and 20% SWF content in ingredient blends respectively, 92.27% and 82.15% at 35% and 25% moisture content of ingredients respectively and 88.99% and 86.28% at 110^{o}C and 140^{o}C respectively (Table 2.2). Interaction effect of all independent variables on PDI was significant, $p<0.0001$ (

Table 2.3).

2.4.3 Bulk Density (BD)

Bulk density influences storage capacity required at the processing plant and during shipping. Increasing SWF content from 10% to 30% significantly changed ($p<0.05$,

Table 2.3) BD of the extrudates but no particular trend was observed. Changing the level of moisture content from 15% to 35% resulted in a 17% decrease and increasing the barrel temperature from 110ºC to 170ºC resulted in a 12% decrease in BD. This may be due to the reason that when the melt exits the die nozzle at high temperature it expands more and have more volume than the extrudates exiting at low temperature.

2.4.4 Water Absorption Index (WAI) and Water Solubility Index (WSI)

Water absorption index indicates the amount of water immobilized by the extrudate, while water solubility index indicates the amount of small molecules solubilized in water (Mezreb et al., 2003). WAI is related to the water activity and influences the storage stability. Main effects of independent variables on WAI and WSI are shown in Table 2.2. When percentage of SWF in ingredient mix was increased from 10 to 30%, a significant increase of 7.0% in WAI was found. As the moisture content of ingredient was increased from 15% to 35% and barrel temperature was increased from 110ºC to 170ºC, WAI increased by 37.5% and 12.3%, respectively (Table 2.2). A similar trend was observed by Anderson et al. (1969) with extruded sorghum grits. No significant change was observed for WSI as SWF content was increased from 10% to 30% in ingredient blends (Table 2.2). When moisture content was increased from 15% to 35% and barrel temperature was increased from 110ºC to 170ºC a decrease of 14% and an increase of 4.3% in WSI were observed, respectively (Table 2.2). This may be due to the reason that as the temperature increased, the extent of starch gelatinization increased. According to Harper (1981), WSI of the extrudate is directly related to the extent of starch gelatinization that occurs inside the extruder.

2.4.5 Unit Density (UD)

Unit density influences the floatability of the extrudates. As depicted in Table 2.2, a significant increase of 3.4% in UD of the extruded products was observed when level of SWF was raised from 10% to 30%. The maximum and minimum unit density values were 0.95 g/cm^3 and 0.85 g/cm^3 observed at 15% and 25% ingredient moisture content, respectively. The apparent viscosity of the ingredient melt inside the barrel and die is inversely proportional to the extruder barrel temperature (Bhattacharya and Hanna, 1986; Harper, 1981). When the ingredient melt having lower viscosity exits through the die, the produced extrudates tend to expand more, and thus have reduced UD. Increasing the barrel temperature from 110ºC to 170ºC resulted in a 28% decrease in UD (Table 2.2).

2.4.6 Expansion Ratio (ER)

Changes in the level of SWF content, moisture content of ingredient mix and temperature had a significant effect on ER of extrudates (

Table 2.3) but no particular trend was observed. Changing the level of SWF content from 10% to 20%, a significant decrease of 3.4% in expansion ratio was observed; further increasing of the SWF inclusion to 30% had no effect on ER of the extrudates (Table 2.2).

2.5 Conclusions

The goals of this study were to produce fish feed pellets with HP-DDG and SWF inclusions and to examine the effect of various levels of SWF, moisture content and extruder barrel temperature on physical properties of the extruded feeds. Changing the level of SWF significantly affected extrudate color, pellet durability, bulk density, water absorption index, unit density and expansion ratio ($p<0.05$). Increasing the level of SWF from 10% to 30%, significantly increased the value of WAI from 3.98 to 4.26 and UD from 0.89 g/cm3 to 0.92 g/cm3 but decreased the value of ER from 1.17 to 1.13 ($\alpha=0.05$). Also changing the level of moisture content and temperature had significant effect ($p<0.05$) on all physical properties. Increasing moisture content from 15% to 35% resulted in a 37.5% increase in WAI and 17% and 14% decrease in bulk density and WSI, respectively. As temperature increased from 110 to 170°C, WAI and WSI increased by 12.3% and 4.3%, respectively. But, there was a decrease in BD by 12% and UD by 28%. The interaction effect of SWF content, moisture content and temperature (SWF×MC×T) were found to be significant for color, PDI, BD and ER. All the extrudates showed relatively high pellet durability, which is important to retaining their physical structure during transportation and storage. This indicates that utilization of combined SWF and HP-DDG did not have detrimental effect on pellet durability. Increasing levels of SWF produced less expanded and more compact textured extrudates. Based on the results obtained, further research may be conducted to study the effects of increased level of SWF (more than 30%) with different die dimension on the extrudate quality.

Table 2.1 Ingredient composition of feed blends.

Feed ingredients	**Mass of ingredients (g/100g)**		
	Blend I	**Blend II**	**Blend III**
SWF	10	20	30
HP-DDG	40	30	20
Corn gluten meal	7	7	7
Corn flour	35	35	35
Fish meal	5	5	5
Soybean oil	1	1	1
Vitamin & mineral mix	2	2	2
Total	100	100	100

†SWF – Soy white flakes; HP-DDG – High protein distiller's dried grains

Table 2.2 Main effects of SWF content, moisture content of raw material and temperature profile (on extrudate physical properties).

Variable	**L* (-)**	**a* (-)**	**b* (-)**	**PDI (%)**	**BD (g/cc)**	**WAI (-)**	**WSI (%)**	**UD (g/cc)**	**ER (-)**
SWF (%)									
10	41.93^b	6.20^a	15.45^a	88.01^b	0.36^c	3.98^b	14.03^{ab}	0.89^b	1.17^a
	(6.11)	(0.88)	(1.67)	(5.30)	(0.05)	(0.60)	(1.51)	(0.15)	(0.07)
20	42.06^b	5.99^b	15.17^b	85.73^c	0.39^a	4.07^b	13.66^b	0.92^a	1.13^b
	(6.25)	(0.89)	(1.89)	(6.37)	(0.05)	(0.67)	(1.27)	(0.16)	(0.12)
30	42.42^a	5.77^c	14.91^c	89.97^a	0.38^b	4.26^a	14.21^a	0.92^a	1.13^b
	(6.63)	(0.92)	(2.10)	(4.62)	(0.04)	(0.63)	(1.79)	(0.19)	(0.15)
MC (% db)									
15	48.93^a	5.19^c	16.61^a	89.28^b	0.42^a	3.39^c	15.39^a	0.95^a	1.15^b
	(3.29)	(0.80)	(0.36)	(2.30)	(0.02)	(0.29)	(0.59)	(0.14)	(0.12)
25	42.31^b	6.61^a	16.19^b	82.15^c	0.36^b	4.25^b	13.29^b	0.85^b	1.20^a
	(1.76)	(0.58)	(0.71)	(5.33)	(0.04)	(0.38)	(1.04)	(0.12)	(0.11)
35	35.17^c	6.16^b	12.72^c	92.27^a	0.35^c	4.66^a	13.21^b	0.94^a	1.09^c
	(2.87)	(0.67)	(0.91)	(3.08)	(0.06)	(0.39)	(1.64)	(0.21)	(0.10)
T (°C)									
110	40.58^c	6.30^a	15.20^a	88.99^a	0.41^a	3.81^b	13.81^b	1.07^a	1.10^b
	(7.24)	(1.21)	(2.09)	(5.77)	(0.02)	(0.60)	(1.50)	(0.12)	(0.08)
140	43.34^a	5.72^c	14.95^b	86.28^c	0.37^b	4.22^a	13.67^b	0.90^b	1.23^a
	(6.85)	(0.81)	(2.14)	(6.61)	(0.04)	(0.55)	(1.62)	(0.13)	(0.11)
170	42.49^b	5.94^b	15.37^a	88.44^b	0.36^c	4.28^a	14.41^a	0.77^c	1.11^b
	(4.13)	(0.47)	(1.37)	(4.25)	(0.07)	(0.67)	(1.44)	(0.09)	(0.11)

[†]Means with different letters in a column within each independent variable are significantly different ($p<0.05$) for that independent variable at $p<0.05$; values in parentheses are standard deviation. SWF – Soy white flakes; MC – Moisture content of the blend; T-Barrel temperature.

Table 2.3 Interaction results for SWF content, moisture content of raw material and barrel temperature on extrudate physical properties (p values).

Variable	**L^* (-)**	**a^* (-)**	**b^* (-)**	**PDI (%)**	**BD (g/cc)**	**WAI (-)**	**WSI (%)**	**UD (g/cc)**	**ER (-)**
SWF	0.02	<.0001	<.0001	<.0001	<.0001	0.0004	0.1046	0.0113	<.0001
MC	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
SWF×MC	0.0039	<.0001	0.0004	<.0001	<.0001	0.0431	0.008	<.0001	<.0001
T	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0158	<.0001	<.0001
SWF×T	<.0001	<.0001	0.0153	<.0001	<.0001	0.0564	0.0016	0.0334	<.0001
MC×T	<.0001	<.0001	<.0001	<.0001	<.0001	0.4202	0.8013	<.0001	<.0001
SWF×MC×T	<.0001	<.0001	0.0526	<.0001	<.0001	0.714	0.2371	0.3335	0.0044

[†]SWF – Soy white flakes; MC – Moisture content of the blend; T- Barrel temperature.

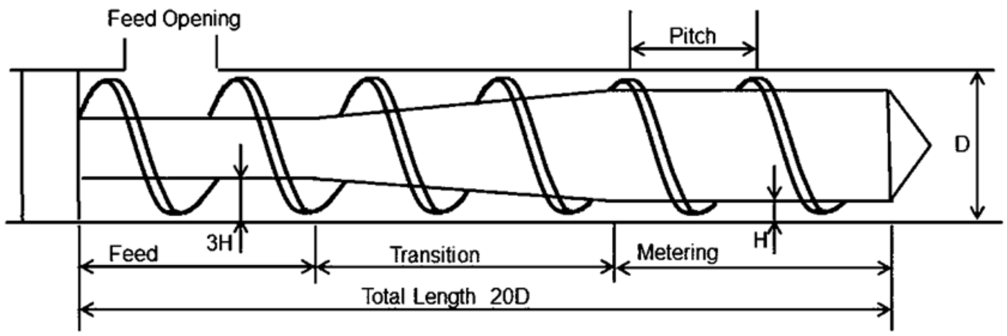

Figure 2.1 Schematic representation of screw in a single screw extruder

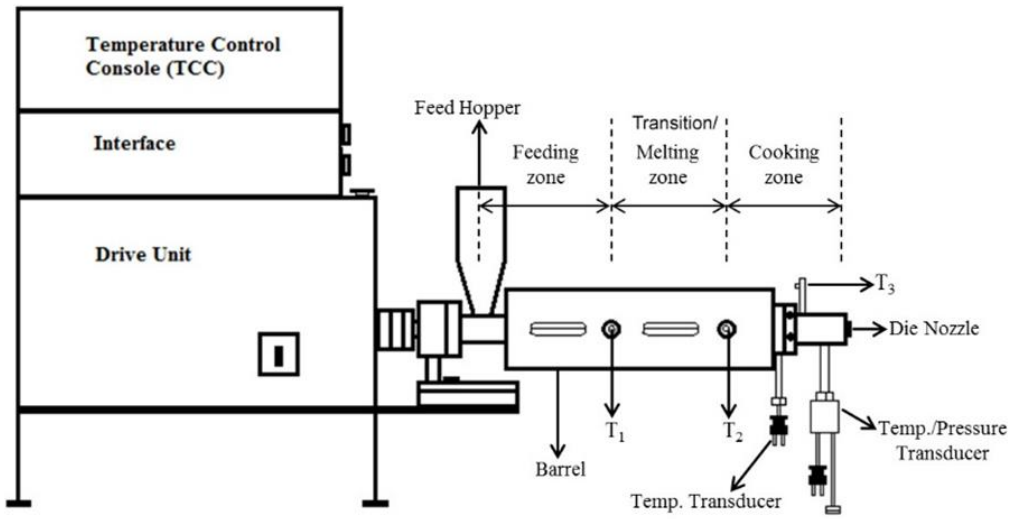

Figure 2.2 Schematic representation of laboratory extruder (Brabender Plasti-Corder, Model PL 2000)

CHAPTER 3 [†]

Effect of Extrusion Processing Parameters on Physical Properties of Soy White Flakes and High Protein Distillers Dried Grains-Based Extruded Aquafeeds

3.1 Abstract

Nutritionally balanced ingredient blends for catla (*Catla catla*), belonging to the family Cyprinidae, were extruded using single screw extruder. The extrusion was carried out at five levels of soy white flakes content (21%, 29%, 40%, 52%, and 59%db), five levels of moisture content (15, 19, 25, 31, and 35% db) and five levels of barrel temperature (100, 110, 125, 140, and 150ºC) using three different die nozzles (having L/D ratios 3.33, 5.83, and 7.25).Blends with net protein content of 32.5% contains soy white flakes, along with high protein distillers dried grains (HP-DDG), corn flour, corn gluten meal, fish meal, vitamin, and mineral mix. A central composite rotatable design (CCRD) and response surface methodology (RSM) was used to investigate the significance of independent and interaction effects of the extrusion process variables on the extrudates physical properties namely pellet durability index, bulk density, water absorption and solubility indices and expansion ratio. Quadratic polynomial regression equations were developed to correlate the product responses and process variables as well as to obtain the response surfaces plots. The independent variables had significant ($P <$ 0.05) effects on physical properties of extrudates: (i) higher soy white flakes content increased the pellet durability index and water absorption index, but decreased the water solubility index, (ii) higher temperature decreased pellet durability index, bulk density

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[†] Singh, S.K. and Muthukumarappan, K., 2014. Effect of different extrusion processing parameters on physical properties of soy white flakes and high protein distillers dried grains-based extruded aquafeeds. *Journal of Food Research*, *3*(6), p.107. DOI: 10.5539/jfr.v3n6p107

and water solubility index, (iii) increased L/D ratio from 3.33 to 7.25 increased the pellet durability index, expansion ratio but decreased the bulk density of the extrudates.

3.2 Introduction

In the food producing industries, aquaculture is one of the fastest growing sectors (FAO, 2012) and plays a pivotal role for the maintenance of commercial fishery markets (O'Mahoney et al., 2011). In aquaculture, diet is often the single largest operating cost item and can represent over 50% of the operating costs in intensive aquaculture (El-Sayed, 1999, 2004). Protein is the most important nutrient of the fish feed. The main protein source used in aquafeed production is primarily fish meal which is supplied through the consumption of wild fish stocks. Indubitably, with the increasing rate of farmed fish production (FAO, 2009) and consequently rising prices of fishmeal (Hardy, 2010), the continued use of fishmeal as the main protein source of the feed will no longer be ecologically and economically sustainable in the long run. Therefore, aquaculture industry now is focusing on alternative protein sources such as plant proteins as inexpensive source of protein to minimize production cost.

Soy white flakes and High Protein - Distiller Dried Grains (HP-DDG) contain significant amount of protein and are thus a possible alternative source of protein for aquaculture feeds (Kim et al., 1989; Wu et al., 1994; Wu et al., 1996). Use of soy products like full fatted soybean meal, defatted toasted soybean meal (SBM) and defatted untoasted soybean meal or soy white flakes is becoming common (Fallahi et al., 2012). Romarheim et al. (2005) found that extrusion of soy white flakes diet increased the digestibility of protein and all amino acids compared to the unextruded soy white flakes diet probably due to the reduction in trypsin inhibitor activity. Dersjant-Li (2002) reported that soy protein isolate can be used to replace 40-100% fish meal without negative impact on growth performance of shrimp. Distillers Dried Grains (DDG) and Distillers Dried Grains with Solubles (DDGS), a co-product from corn-based dry grind fuel ethanol manufacturing, is also a viable protein source. Research carried out by Wu et al. (1994); Wu et al. (1996) indicated that tilapia fish can be grown with DDGS, and can improve the economic viability of aquaculture farms.

Extrusion cooking is widely used in the food and feed industries because of versatility during processing and the ability to produce various final textural properties (Mercier et al., 1989b). Extruded aquafeed are designed to floater sink based on the fish species requirement. One of the important quality parameters for fish feed is floatability (Bandyopadhyay and Rout, 2001; Rolfe et al., 2001) which depends on the unit density of extrudates. During extrusion cooking, the extent of expansion affects the unit density of the extrudates. Expansion can be monitored by changing the nature and type of ingredients used and the extruder process parameters. In the food industry, puffed products are often produced by using starch based ingredients, while texturized products are often produced by using protein based ingredients (Kokini et al., 1992). Extrudate properties of starch based products depend on the extent of gelatinization occurring inside the extruder barrel. The formation of elastic melt inside the barrel depends on the extent of gelatinization. (Case et al., 1992; Ibanoglu et al., 1996; Ilo et al., 1996; Lin et al., 2000; Sokhey et al., 1994). Expansion occurs due to the flashing of water vapor when the elastic melt exits through the die nozzle. (Alves et al., 1999; Lam and Flores, 2003). On the other hand, ingredients with higher protein content shows limited degree of expansion due to plastic melt formation and protein denaturation inside the extruder barrel. The material is in plastic and homogeneous state and when it exits through the die nozzle there is a sudden pressure drop resulting in the formation of voids. Due to this void formation the final product becomes more porous and fibrous textured (Gwiazda et al., 1987; Prudêncio-Ferreira and Arêas, 1993; Singh et al., 1991). Depending on the type of species, aquaculture feed requires 26 to 50% protein content (Lovell, 1989).

Extrusion process depends on many factors which includes the pressure developed inside the die and the degree to which the screw is filled. These variables in combination with the type and composition of raw ingredients used, affects operational capabilities (Mercier et al., 1989b). Extruder die too have an impact on the processing conditions. For example, in case of circular dies, nozzle dimensions (i.e., nozzle diameter and length) will affect process conditions and performance (Chinnaswamy and Hanna, 1987). In a recent study, we performed initial investigation of the effect of inclusion of 30% soy white flakes for the production of feed pellets for catla (Singh and Muthukumarappan, 2014b).

The objective of this study was to examine the effect of varying level of soy white flakes as the fish meal replacer, barrel temperature, die aspect ratio, and moisture content on physical properties of soy white flakes and HP-DDG based extrudates.

3.3 Materials and Methods

3.3.1 Blends Preparation

Five isocaloric (302 kcal/100g) different blends were adjusted to a target protein content of ~ 32.5% db and a target fat content of ~ 3.5%.The total energy content for each blend was determined based on the fraction of protein, lipid and carbohydrate contributing to the dietary energy. The total energy content was calculated based on the energy content of fractions namely, 4.5 kcal/g for protein, 9.1 kcal/g for lipid and 4.1

kcal/g for carbohydrate. The ingredient components of the feed blends are provided in Table 3.1. Soy white flakes was kindly donated by South Dakota Soybean Processors, Volga, SD. HP-DDG was obtained from the Dakota Ethanol LLC (Wentworth, SD). Corn gluten meal and fishmeal were purchased from Consumer Supply Distributing Co. (Sioux City, IA). Corn flour was from Cargill Dry Ingredients (Paris, IL). Vitamin and mineral premix was obtained from Lortscher Agri Service, Inc. (Bern, Kansas, USA). Soybean oil was provided from USDA (Brookings, SD). The different ingredients were mixed in a laboratory model Hobart mixer (Hobart Corporation, Troy, Ohio, USA) for 10 minutes; the moisture content of the ingredient mix was adjusted by adding required quantities of water during mixing. The resulting blends were then stored at ambient temperature overnight until processing.

3.3.2 Extrusion Processing

Extrusion experiments were performed using a single screw extruder (Brabender Plasti-Corder, Model PL 2000, South Hackensack, NJ), which was powered by a 7.5 hp motor with an operating range of screw speeds from 0-210 rpm. The extruder had a barrel length-to-diameter ratio of 20:1 and a barrel diameter of 19 mm. A uniform 19.05 mm pitch screw with compression ratio of 3:1 was used in the experiments (Figure 3.1). The screw had a variable flute depth, with a depth at the feed portion of 19.05 mm, and near the die of 3.81 mm. The raw materials were fed manually to the extruder in constant quantities. Experiments were conducted using five levels of soy white flakes(21, 29, 40, 52, and 59% db), five levels of temperature gradient in the barrel (45-100-100^{o}C, 45-110-110^{o}C, 45-125-125^{o}C, 45-140-140^{o}C, and 45-150-150^{o}C) hereafter referred as temperature of 100, 110, 125, 140, and 150^{o}C, and five levels of moisture content (15, 19, 25, 31, and 35% db), for three different die nozzles with various L/D ratios (3.33, 5.83 and 7.25) (Table 3.2). During the experiment the screw speed of extruder was maintained at 150 rpm.

3.3.3 Experimental Design and Statistical Analysis

In the present study, a central composite rotatable design (CCRD) was used to evaluate the effect of soy white flakes, moisture content, temperature and L/D of die nozzle on the physical properties of the extrudate. Pellet durability index, bulk density, water absorption and solubility indices and expansion ratio of the extrudates were measured as the response/dependent variables. The measurements were completed in triplicate, except for expansion ratio, which were measured with ten replications. The collected data were then analyzed with Proc GLM procedure to determine the treatment combination effects using SAS v9.3 (SAS Institute, Cary, NC). Then post hoc LSD tests were used to determine where the specific differences occurred. The experimental design was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN), which consisted of 3 numerical independent variables of soy white flakes (X_1), moisture content (X_2) and T (X_3) each at five levels and one categorical variable of die nozzle configuration (X_4) at three levels. The experimental design points (in coded and actual values) are shown in Table 3.3. Using Eq. (3.1), the numerical independent variables in actual form (X_1, X_2) were converted to their coded form (x_1, x_2).

$$x_i = \frac{(X_i - X_o)}{\Delta X} \tag{3.1}$$

where x_i is the dimensionless coded value of the ith independent variable, and X_i, X_0, and ΔX correspond to the actual value, actual value at the center point, and the step change of the ith variable, respectively.

For each categorical variable, 20 experiments were performed in randomized order including six replications at the design center to obtain an accurate estimation of the experimental error (Table 3.4). The pellet durability index (Y_{PDI}), bulk density (Y_{BD}), water absorption index (Y_{WAI}), water solubility index (Y_{WSI}) and expansion ratio (Y_{ER}) were taken as the five responses of the designed experiments. The quadratic polynomial equation was used to describe the effect of the independent variables in terms of linear, quadratic and their interactions on the dependent variables as given by Eq. (3.2).

$$Y_i = b_o + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j + \varepsilon \qquad (3.2)$$

where Y_i is the predicted response; b_0 is the interception coefficient; b_i, b_{ii}, and b_{ij} are coefficients of the linear, quadratic, and interaction terms; $\varepsilon$is the random error; and X_i is the independent variables studied. 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) presented in Table 3.5 and Table 3.6. 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 F-test (Table 3.7). 3D response surfaces were used to visualize interactive effects of the independent variables.

3.3.4 Measurement of Physical Properties

3.3.4.1 Pellet Durability Index

Approximately 100g of extrudates from each blend were manually sieved (U.S.A. standard testing, ASTM E-11 specification, Daigger, Vernon Hills, IL) to remove initial fines, and then tumbled in a pellet durability tester (Model PDT-110, Seedburo Equipment Company, Chicago, IL) for 10 min. Afterwards, the samples were again sieved, and then weighed on an electronic balance (Explorer Pro, Model: EP4102, Ohaus, Pine Brook, NJ). Pellet durability index (PDI) was calculated following the Eq. (3.3):

$$PDI = \left(\frac{M_a}{M_b} \right) \times 100 \tag{3.3}$$

where, M_a was the mass (g) after tumbling and M_b was the sample mass (g) before tumbling.

3.3.4.2 Bulk Density

Bulk density was determined as the ratio of the mass of extrudates that they filled up to a given bulk volume and measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by USDA (1999).

3.3.4.3 Water Absorption Index and Water Solubility Index

Extrudates were ground to fine powders using a coffee grinder (Black & Decker® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was suspended in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminums cup and dried at 135ºC for 2 h (AACC, 2000). The weight of the gel remaining in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by Eq. (3.4) and (3.5), respectively:

$$WAI(unitless) = \frac{W_g}{W_{ds}} \tag{3.4}$$

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

$$WSI(\%) = \left(\frac{W_{ss}}{W_{ds}} \right) \times 100 \tag{3.5}$$

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

3.3.4.4 Expansion Ratio

The radial expansion ratio of the extrudates was measured as the ratio of the diameter of the extrudates to the diameter of the die orifice.

3.4 Results and Discussion

Response surface methodology (RSM) was used to analyze the relationship between the dependent and independent variables. The predictive models in coded terms (i.e., Y_{PDI}, Y_{BD}, Y_{WSI} and Y_{ER}) are presented in Table 3.7. On the contrary, the final equations in actual form are defined for each type of categorical factor separately. The final equations in actual form obtained for pellet durability index (Y_{PDI}), bulk density (Y_{BD}), water solubility index (Y_{WSI}) and expansion ratio (Y_{ER}) for each level of categoric variable (D1, D2 and D3) are given in Table 3.8.

Overall, changing the level of temperature content significantly affected (P<0.05) all the resulting physical properties. Changing the level of moisture content significantly affected (P<0.05) all the resulting physical properties except pellet durability index. Whereas, changing soy white flakes content significantly affected (P<0.05) pellet durability index, water absorption and solubility indices (Table 3.5 and Table 3.6). The behavior observed for the treatment combinations were produced due to the various competing interaction effects (Table 3.9 and Table 3.10).

3.4.1 Pellet Durability Index

The response surface plot presented (Figure 3.2) showed that for all L/D ratio, the pellet durability index of extrudates increased on increasing soy white flakes content and decreasing temperature. ANOVA showed that moisture content had no significant effect on pellet durability index and hence response surface plots of interaction effect involving moisture content are not shown. Lack of fit was not significant relative to the pure error, which meant the model was well fitted. The regression equation for pellet durability index in coded and actual form is shown in Table 3.7 and Table 3.8, respectively.

The values of pellet durability index of extruded products under experimental conditions are presented in Table 3.9 and Table 3.10. The maximum and minimum pellet durability index values were related to the treatments with 40% soy white flakes, 25% moisture content, 125°C barrel temperature, 3.33 L/D and 21% soy white flakes, 25% moisture content, 125°C barrel temperature, 5.83 L/D ratio, respectively.

3.4.2 Bulk Density

The response surface plot presented (Figure 3.3) showed that for all L/D ratio, the bulk density of extrudates decreased with an increasing of moisture content and temperature. A significant decrease in bulk density was observed when length to diameter ratio of die was increased from 3.33 to 7.25. ANOVA showed that soy white flakes had no significant effect on bulk density and hence response surface plots of interaction effect involving soy white flakes are not shown. The regression equation for bulk density in coded and actual form is shown in Table 3.7 and Table 3.8, respectively.

The treatment combination effects of soy white flakes, moisture content, temperature and L/D ratio on bulk density of extrudates are presented in Table 3.9 and Table 3.10. The lowest bulk density, 0.32 g/cm^3 , was recorded at 29% soy white flakes, temperature of 140ºC, moisture content of 31%, L/D of 7.25 and at 59% soy white flakes, temperature of 125ºC, moisture content of 25%, L/D ratio of 7.25; and the highest bulk density of 0.57 g/cm^3, was obtained at 40% soy white flakes, temperature of 125°C, moisture content of 15%, and L/D of 3.33.

3.4.3 Water Absorption Index

Soy white flakes, moisture content and temperature significantly affected the water absorption index through a linear model (response surface plots were prepared but not shown due to linear model and to reduce the manuscript size). ANOVA analysis demonstrated that the linear model was significant ($P< 0.05$). Increasing the soy white flakes content from 21% to 52%, there was a significant increase of in water absorption index. Water absorption index was found to increase when temperature was raised from 100ºC to 150ºC. With extruded corn grits, a similar trend was observed by (Anderson et al., 1969). Furthermore, water absorption index also increased when moisture content level of the feed was increased from 15% to 35%. This was in agreement with Williams et al. (1977) who observed that higher temperature and drier conditions could result in higher dextrinization, which could lead to a decreased extrudate water absorption index and higher extrudate water solubility index. According to Mason and Hoseney (1986), water absorption index indicates the part of the starch that was not affected by the extrusion cooking and maintained its internal structure. The experimental values of water absorption index of extrudates under different designed extrusion conditions are presented in Table 3.9 and Table 3.10.

3.4.4 Water Solubility Index

Changing the level of soy white flakes, moisture content of ingredient mix and barrel temperature had significant effect ($P< 0.05$) on water solubility index (Table 3.6). Williams et al. (1977) reported that there was an inverse relationship between the water absorption index and water solubility index values of the extrudates. Similarly, in this study the water solubility index at 15% moisture content was higher compared to the water solubility index at 35% moisture content. Increasing the barrel temperature from 100 to 150 ºC resulted in decrease in water solubility index. In another study, Chevanan et al. (2007a) reported that there was no significant change in water solubility index of the DDGS-based extrudates due to the change in extruder barrel temperature. Increasing the soy white flakes content initially led to a significant decrease in water solubility index and then further increase in soy white flakes level resulted in slight increase in water solubility index. An inverse relationship between the water solubility index and water absorption index values was observed. This observation was in agreement with what Anderson et al. (1970), Williams et al. (1977) and Fallahi et al. (2012); Fallahi et al. (2013) reported.

ANOVA analysis showed that quadratic effect of soy white flakes significantly affected water solubility index and all linear effects including soy white flakes, moisture content and temperature had significant effect on water solubility index. The interaction effect of soy white flakes, moisture content and temperature at L/D ratio at 7.25 on water solubility index was maximum and since L/D ratio does not significantly affect the water solubility index, the response surface plots of water solubility index at L/D ratio 7.25 are shown (Figure 3.4). Water solubility index is found to increase when temperature and soy white flakes content, temperature and moisture content, and moisture content and soy white flakes content are decreased (Figure 3.4). Lack of fit was not significant relative to the pure error, which meant the model was well fitted. The regression equation for the empirical relationship between water solubility index and the independent extrusion processing variables for each L/D ratio is shown in Table 3.8.

3.4.5 Expansion Ratio

The extent of puffing of extruded products is indicated by the expansion ratio. The response surface plot presented in Figure 3.5 showed that for all L/D ratio, the expansion ratio of extrudates increased with an increasing moisture content and after reaching a maximum the expansion ratio decreased with further increasing in moisture content. The expansion ratio of extrudates increased for L/D ratio 5.83 and 7.25 but decreased for L/D ratio 3.33 with increase in temperature. ANOVA showed that soy white flakes had no significant effect on expansion ratio and hence response surface plots of interaction effect involving soy white flakes are not shown. The regression equation for the relationship between expansion ratio and independent variables in terms of coded and actual form for each L/D ratio is shown in Table 3.7 and Table 3.8, respectively. Increasing the level of moisture content of ingredient mix and temperature had a significant effect on expansion ratio of extrudates.

The experimental values of expansion ratio of extrudates under different designed extrusion conditions are shown in Table 3.9 and Table 3.10. The maximum and minimum expansion ratio were achieved at 52% soy white flakes, 19% moisture content,140ºC, and 7.25 L/D ratio and 40% soy white flakes, 25% moisture content, 150ºC temperature, and 3.33 L/D ratio, respectively.

3.5 Conclusions

This experimental study was conducted to investigate the effect of various extrusion processing conditions on the soy white flakes and HP-DDG based extrudates.

Overall, it can be concluded that increasing the level of soy white flakes from 21% to 59%, resulted in increase of pellet durability and water absorption index. Increasing L/D ratio from 3.33 to 7.25 resulted in increase in pellet durability index, expansion ratio, but a decrease in bulk density of the extrudates. The increase in pellet durability indicates that the aquaculture feed could resist mechanical damage during transportation and storage. Significant decrease in bulk density ($P<0.001$) due to increase in L/D ratio is desirable for storage purpose. Further studies should aim for the production of aquaculture feed with incorporation of soy white flakes levels between 20% and 60% db at different screw speeds and should optimize processing conditions.

Table 3.1 Blend composition of feed.

Ingredients	**Blend 1**	**Blend 2**	**Blend 3**	**Blend 4**	**Blend 5**
Soy white flakes	21	29	40	52	59
HP-DDG	39	32	20	9	1
Corn gluten meal	2	2	2	2	2
Corn flour	30	30	30	30	30
Fish meal	5	5	5	5	5
Soybean oil	1	1	1	1	1
Vitamin & mineral mix	2	2	2	2	2

[†]HP-DDG – High protein distiller's dried grain

Table 3.2 Dimensions of die used in this study.

Die No.	**Diameter of nozzle (L) (mm)**	**Length of nozzle (D) (mm)**	**L/D ratio (-)**
D1	6	20.0	3.33
D2	3	17.5	5.83
D3	2	14.5	7.25

Table 3.3 Independent numerical and categorical variables and their levels.

Numerical variables	**Symbol**	**Coded variable levels**				
		-1.682	-1	0	1	1.682
Soy white flakes (%)	X_1	21	29	40	52	59
Moisture content (% db)	X_2	15	19	25	31	35
Temperature (^{o}C)	X_3	100	110	125	140	150
Categorical variable		**D1**	**D2**	**D3**		
L/D (-)	$X_4[1]$	1	0	-1		
	$X_4[2]$	0	1	-1		

†L – Length of nozzle; D – Diameter of nozzle

Table 3.4 Experimental design layout.

Run	**Coded variables**					**Actual variables**			
	x_1	x_2	x_3	x_4		X_1	X_2	X_3	X_4
				$x_4[1]$	$x_4[2]$	(%)	(% db)	(^{o}C)	(-)
1	0	0	-1.682	1	0	40	25	100	D1
2	0	0	0	0	1	40	25	125	D2
3	-1	-1	1	1	0	29	19	140	D1
4	1.682	0	0	1	0	59	25	125	D1
5	1	-1	-1	0	1	52	19	110	D2
6	0	0	0	1	0	40	25	125	D1
7	1	1	-1	0	1	52	31	110	D2
8	1	-1	1	0	1	52	19	140	D2
9	0	0	0	-1	-1	40	25	125	D3
10	1	-1	1	1	0	52	19	140	D1
11	-1	1	-1	0	1	29	31	110	D2
12	1	1	1	1	0	52	31	140	D1
13	0	0	1.682	0	1	40	25	150	D2
14	-1	-1	-1	0	1	29	19	110	D2
15	0	0	0	-1	-1	40	25	125	D3
16	1	-1	1	-1	-1	52	19	140	D3
17	-1	1	-1	1	0	29	31	110	D1
18	0	0	0	-1	-1	40	25	125	D3
19	-1.682	0	0	1	0	21	25	125	D1
20	-1	-1	-1	-1	-1	29	19	110	D3
21	0	0	0	1	0	40	25	125	D1
22	0	-1.682	0	0	1	40	15	125	D2
23	0	0	-1.682	-1	-1	40	25	100	D3
24	-1	-1	-1	1	0	29	19	110	D1
25	1.682	0	0	0	1	59	25	125	D2
26	0	0	0	-1	-1	40	25	125	D3
27	1	1	-1	1	0	52	31	110	D1
28	0	0	0	-1	-1	40	25	125	D3
29	-1.682	0	0	0	1	21	25	125	D2
30	0	0	0	0	1	40	25	125	D2
31	0	0	0	0	1	40	25	125	D2
32	-1	1	1	0	1	29	31	140	D2
33	1	-1	-1	1	0	52	19	110	D1
34	0	0	0	-1	-1	40	25	125	D3
35	1	1	-1	-1	-1	52	31	110	D3
36	0	0	-1.682	0	1	40	25	100	D2
37	0	1.682	0	1	0	40	35	125	D1
38	0	-1.682	0	1	0	40	15	125	D1
39	0	0	0	1	0	40	25	125	D1
40	-1	1	1	1	0	29	31	140	D1
41	0	0	0	0	1	40	25	125	D2
42	0	1.682	0	-1	-1	40	35	125	D3
43	1.682	0	0	-1	-1	59	25	125	D3
44	-1	-1	1	0	1	29	19	140	D2
45	0	0	0	0	1	40	25	125	D2
46	0	0	0	0	1	40	25	125	D2
47	0	0	0	1	0	40	25	125	D1
48	1	1	1	0	1	52	31	140	D2
49	0	0	0	1	0	40	25	125	D1
50	-1	1	-1	-1	-1	29	31	110	D3
51	-1.682	0	0	-1	-1	21	25	125	D3
52	0	0	1.682	-1	-1	40	25	150	D3
53	0	1.682	0	0	1	40	35	125	D2
54	0	-1.682	0	-1	-1	40	15	125	D3
55	1	1	1	-1	-1	52	31	140	D3
56	0	0	0	1	0	40	25	125	D1
57	1	-1	-1	-1	-1	52	19	110	D3
58	-1	-1	1	-1	-1	29	19	140	D3
59	-1	1	1	-1	-1	29	31	140	D3
60	0	0	1.682	1	0	40	25	150	D1

Table 3.5 Analysis of Variance (ANOVA) for pellet durability index and bulk density.

Source	df	Pellet durability index				Bulk density			
		SS	MS	F value	*P*-value	SS	MS	F value	*P*-value
Model	17	702.94	41.35	6.41	< 0.0001	1.93×10^{-1}	1.13×10^{-2}	16.54	< 0.0001
X_1	1	77.83	77.83	12.06	0.0012	7.18×10^{-4}	7.18×10^{-4}	1.05	0.3117
X_2	1	17.18	17.18	2.66	0.1102	9.79×10^{-3}	9.79×10^{-3}	14.30	0.0005
X_3	1	203.67	203.67	31.56	< 0.0001	2.61×10^{-2}	2.61×10^{-2}	38.06	< 0.0001
X_4	2	198.72	99.36	15.40	< 0.0001	1.24×10^{-1}	6.19×10^{-2}	90.37	< 0.0001
X_1^2	1	0.58	0.58	0.09	0.7663	5.95×10^{-6}	5.95×10^{-6}	0.01	0.9262
X_2^2	1	105.47	105.47	16.35	0.0002	1.28×10^{-2}	1.28×10^{-2}	18.66	< 0.0001
X_3^2	1	5.40	5.40	0.84	0.3654	4.65×10^{-5}	4.65×10^{-5}	0.07	0.7956
X_1X_2	1	10.60	10.60	1.64	0.2071	5.00×10^{-4}	5.00×10^{-4}	0.73	0.3979
X_1X_3	1	1.64	1.64	0.25	0.6169	1.23×10^{-5}	1.23×10^{-5}	0.02	0.8939
X_1X_4	2	32.82	16.41	2.54	0.0907	5.14×10^{-3}	2.57×10^{-3}	3.75	0.0316
X_2X_3	1	1.55	1.55	0.24	0.6270	2.96×10^{-4}	2.96×10^{-4}	0.43	0.5146
X_2X_4	2	7.67	3.84	0.59	0.5565	6.50×10^{-4}	3.25×10^{-4}	0.47	0.6254
X_3X_4	2	40.41	20.20	3.13	0.0540	1.27×10^{-2}	6.36×10^{-3}	9.29	0.0005
Residual	42	271.01	6.45	-	-	2.88×10^{-2}	6.85×10^{-4}	-	-
Lack of fit	27	202.96	7.52	1.66	0.1536	2.37×10^{-2}	8.76×10^{-4}	2.58	0.0291
Pure error	15	68.05	4.54	-	-	5.10×10^{-3}	3.40×10^{-4}	-	-

†df – degree of freedom, SS – Sum of squares, MS – Mean square.

Table 3.6 Analysis of Variance (ANOVA) for water solubility index and expansion ratio.

Source	df	Water solubility index				Expansion ratio			
		SS	**MS**	**F value**	***P*-value**	**SS**	**MS**	**F value**	***P*-value**
Model	17	22.25	1.31	2.77	0.0037	4.20×10^{-1}	2.50×10^{-2}	13.22	< 0.0001
X_1	1	2.91	2.91	6.15	0.0172	3.38×10^{-3}	3.38×10^{-3}	1.82	0.1842
X_2	1	3.64	3.64	7.71	0.0082	2.20×10^{-2}	2.20×10^{-2}	11.65	0.0014
X_3	1	6.22	6.22	13.17	0.0008	2.50×10^{-2}	2.50×10^{-2}	13.71	0.0006
X_4	2	0.25	0.12	0.26	0.7700	2.30×10^{-1}	1.10×10^{-1}	61.92	< 0.0001
X_1^2	1	6.12	6.12	12.95	0.0008	5.14×10^{-3}	5.14×10^{-3}	2.77	0.1035
X_2^2	1	1.27	1.27	2.68	0.1090	3.10×10^{-2}	3.10×10^{-2}	16.76	0.0002
X_3^2	1	0.19	0.19	0.41	0.5277	2.20×10^{-2}	2.20×10^{-2}	11.94	0.0013
X_1X_2	1	0.21	0.21	0.45	0.5055	5.12×10^{-3}	5.12×10^{-3}	2.76	0.1039
X_1X_3	1	0.61	0.61	1.30	0.2604	8.62×10^{-4}	8.62×10^{-4}	0.46	0.4991
X_1X_4	2	0.05	0.03	0.06	0.9452	1.10×10^{-2}	5.74×10^{-3}	3.10	0.0557
X_2X_3	1	0.00	0.00	0.00	0.9927	5.55×10^{-4}	5.55×10^{-4}	0.30	0.5871
X_2X_4	2	1.35	0.68	1.43	0.2501	3.13×10^{-3}	1.57×10^{-3}	0.84	0.4371
X_3X_4	2	0.08	0.04	0.09	0.9160	6.50×10^{-2}	3.30×10^{-2}	17.58	< 0.0001
Residual	42	19.84	0.47	-	-	7.80×10^{-2}	1.85×10^{-3}	-	-
Lack of fit	27	15.87	0.59	2.22	0.0541	6.60×10^{-2}	2.45×10^{-3}	3.16	0.0114
Pure error	15	3.97	0.26	-	-	1.20×10^{-2}	7.76×10^{-4}	-	-

[†]df – degree of freedom, SS – Sum of squares, MS – Mean square.

Table 3.7 Final equation in terms of coded factors after excluding the insignificant terms for pellet durability index, bulk density, water solubility index and expansion ratio.

Coded model equations	R^2	AdjR^2	PredR^2	**Adeq. precision**
$Y_{PDI} = +89.91+1.38x_1-2.23x_3-1.86x_4[1]-0.61x_4[2]+1.56x_2^2$	0.72	0.61	0.36	9.98
$Y_{BD} = +0.42 - 0.015x_2 - 0.025x_3 + 0.058x_4[1] - 4.89\times10^{-3}x_4[2] + 1.92\times10^{-3}x_1x_4[1]+0.013x_1x_4[2] + 0.02x_3x_4[1] + 2.87\times10^{-3}x_3x_4[2]+ 0.017x_2^2$	0.87	0.82	0.69	19.03
$Y_{WSI} = +14.07 - 0.27x_1 - 0.30x_2 - 0.39x_3 + 0.38x_1^2$	0.53	0.34	-0.09	6.52
$Y_{ER} = +1.19 - 0.023x_2 + 0.025x_3 - 0.081x_4[1] + 0.013x_4[2] - 0.056x_3x_4[1] + 0.023x_3x_4[2] - 0.027x_2^2 - 0.023x_3^2$	0.84	0.78	0.62	14.85

†PDI – Pellet durability index; BD – Bulk density; WAI – Water absorption index; WSI – Water solubility index; ER – Expansion ratio.

Table 3.8 Best-fit response surface models for extrudate physical properties.

L/D	Response Surface Model	R^2	Std. Deviation	*F* Statistic
D1	$Y_{PDI} = +155.64 + 0.10X_1 - 3.14X_2 - 0.46X_3 + 0.01X_1X_2 - 1.51\times10^{-3}X_1X_3 + 2.94\times10^{-3}X_2X_3 - 8.74\times10^{-4}X_1^2 + 0.05X_2^2 + 1.57\times10^{-3}X_3^2$	0.72	2.54	6.41
	$Y_{BD} = +0.81 + 1.51\times10^{-3}X_1 - 0.02X_2 - 6.48\times10^{-4}X_3 - 6.90\times10^{-5}X_1X_2 + 4.15\times10^{-6}X_1X_3 - 4.07\times10^{-5}X_2X_3 + 2.80\times10^{-6}X_1^2 + 5.20\times10^{-4}X_2^2 + 4.61\times10^{-6}X_3^2$	0.87	0.026	16.54
	$Y_{WSI} = +30.07 - 0.17X_1 - 0.41X_2 - 0.07X_3 + 1.42\times10^{-3}X_1X_2 - 9.28\times10^{-4}X_1X_3 - 1.50\times10^{-5}X_2X_3 + 2.84\times10^{-3}X_1^2 + 5.18\times10^{-3}X_2^2 + 2.96\times10^{-4}X_3^2$	0.53	0.69	2.77
	$Y_{ER} = -0.97 + 0.02X_1 + 0.04X_2 + 0.02X_3 - 2.21\times10^{-4}X_1X_2 - 3.47\times10^{-5}X_1X_3 + 5.58\times10^{-5}X_2X_3 - 8.24\times10^{-5}X_1^2 - 8.11\times10^{-4}X_2^2 - 1.01\times10^{-4}X_3^2$	0.84	0.043	13.22
D2	$Y_{PDI} = +172.09 + 0.23X_1 - 3.29X_2 - 0.59X_3 + 0.01X_1X_2 - 1.51\times10^{-3}X_1X_3 + 2.94\times10^{-3}X_2X_3 - 8.74\times10^{-4}X_1^2 + 0.05X_2^2 + 1.57\times10^{-3}X_3^2$	0.72	2.54	6.41
	$Y_{BD} = +0.82 + 2.45\times10^{-3}X_1 - 0.02X_2 - 1.80\times10^{-3}X_3 - 6.90\times10^{-5}X_1X_2 + 4.15\times10^{-6}X_1X_3 - 4.07\times10^{-5}X_2X_3 + 2.80\times10^{-6}X_1^2 + 5.20\times10^{-4}X_2^2 + 4.61\times10^{-6}X_3^2$	0.87	0.026	16.54
	$Y_{WSI} = +27.68 - 0.17X_1 - 0.33X_2 - 0.06X_3 + 1.42\times10^{-3}X_1X_2 - 9.28\times10^{-4}X_1X_3 - 1.50\times10^{-5}X_2X_3 + 2.84\times10^{-3}X_1^2 + 5.18\times10^{-3}X_2^2 + 2.96\times10^{-4}X_3^2$	0.53	0.69	2.77
	$Y_{ER} = -1.56 + 0.02X_1 + 0.04X_2 + 0.03X_3 - 2.21\times10^{-4}X_1X_2 - 3.47\times10^{-5}X_1X_3 + 5.58\times10^{-5}X_2X_3 - 8.24\times10^{-5}X_1^2 - 8.11\times10^{-4}X_2^2 - 1.01\times10^{-4}X_3^2$	0.84	0.043	13.22
D3	$Y_{PDI} = +184.83 + 0.05X_1 - 3.31X_2 - 0.61X_3 + 0.01X_1X_2 - 1.51\times10^{-3}X_1X_3 + 2.94\times10^{-3}X_2X_3 - 8.74\times10^{-4}X_1^2 + 0.05X_2^2 + 1.57\times10^{-3}X_3^2$	0.72	2.54	6.41
	$Y_{BD} = +1.09 + 7.74\times10^{-5}X_1 - 0.02X_2 - 3.51\times10^{-3}X_3 - 6.90\times10^{-5}X_1X_2 + 4.15\times10^{-6}X_1X_3 - 4.07\times10^{-5}X_2X_3 + 2.80\times10^{-6}X_1^2 + 5.20\times10^{-4}X_2^2 + 4.61\times10^{-6}X_3^2$	0.87	0.026	16.54
	$Y_{WSI} = +28.31 - 0.17X_1 - 0.36X_2 - 0.06X_3 + 1.42\times10^{-3}X_1X_2 - 9.28\times10^{-4}X_1X_3 - 1.50\times10^{-5}X_2X_3 + 2.84\times10^{-3}X_1^2 + 5.18\times10^{-3}X_2^2 + 2.96\times10^{-4}X_3^2$	0.53	0.69	2.77
	$Y_{ER} = -1.62 + 0.02X_1 + 0.04X_2 + 0.03X_3 - 2.21\times10^{-4}X_1X_2 - 3.47\times10^{-5}X_1X_3 + 5.58\times10^{-5}X_2X_3 - 8.24\times10^{-5}X_1^2 - 8.11\times10^{-4}X_2^2 - 1.01\times10^{-4}X_3^2$	0.84	0.043	13.22

†L – Length of nozzle; D – Diameter of nozzle

Table 3.9 Treatment combination effects for soy white flakes, moisture content of raw material, temperature and die on extrudate physical properties.

Treatment	PDI (%)	BD (g/cc)	WAI (unitless)	WSI (%)	ER (unitless)
1	90.09±0.18[16-18]	0.46±0.01[12-14]	3.62±0.05[23-25]	14.97±0.11[4-8]	1.06±0.02[27-29]
2	87.49±0.02 [23]	0.39±0.00[21-22]	4.70±0.11 [5]	13.50±0.39[18-23]	1.22±0.01[7-11]
3	85.30±0.10[26-27]	0.49±0.01[4-6]	3.72±0.10[21-25]	14.83±0.41[4-11]	1.06±0.01[27-29]
4	85.93±0.79 [25]	0.47±0.01[9-11]	5.14±0.11[3-4]	13.56±0.20[17-22]	1.12±0.01[18-23]
5	97.03±0.13[2-3]	0.48±0.01[6-9]	4.34±0.12[7-9]	14.48±0.25[7-14]	1.131±0.00[8-22]
6	89.75±0.28[17-18]	0.48±0.00[8-10]	4.81±0.10 [5]	13.15±0.21[22-24]	1.09±0.02[23-27]
7	95.61±0.47[4-5]	0.44±0.01[14-16]	4.64±0.14[5-6]	14.29±0.40[10-16]	1.07±0.02[26-28]
8	92.01±0.16 [14]	0.49±0.01[4-5]	5.39±0.06 [2]	13.31±0.18[21-24]	1.16±0.02[15-18]
9	90.94±0.14 [15]	0.33±0.00 [25]	4.15±0.12[10-15]	13.85±0.38[15-21]	1.32±0.01 [2]
10	88.00±0.30[22-23]	0.50±0.02 [4]	4.23±0.07[8-12]	14.43±0.18[7-16]	1.03±0.01[30-31]
11	90.51±0.63[15-16]	0.45±0.01[13-15]	3.54±0.14[25-26]	14.88±0.32[4-10]	1.08±0.02[26-28]
12	92.36±0.11[13-14]	0.49±0.01[4-7]	5.16±0.08[3-4]	13.40±0.32[19-24]	0.96±0.01 [32]
13	81.75±0.15 [30]	0.33±0.01 [25]	5.30±0.05[2-3]	12.83±0.23 [24]	1.29±0.02[2-3]
14	90.80±0.17 [15]	0.38±0.01 [22]	4.29±0.14[7-11]	13.83±0.26[16-21]	1.10±0.01[21-26]
15	92.01±0.21 [14]	0.34±0.01[24-25]	5.04±0.14 [4]	12.94±0.34[23-24]	1.28±0.02[3-5]
16	90.21±0.22[16-17]	0.34±0.01[24-25]	5.59±0.05 [1]	12.91±0.11[23-24]	1.41±0.02 [1]
17	90.17±0.42[16-17]	0.47±0.00[8-11]	3.71±0.08[22-25]	14.57±0.25[7-13]	1.05±0.00[28-31]
18	93.13±0.25[10-12]	0.36±0.01 [23]	4.47±0.01[6-7]	13.262±0.25[1-24]	1.28±0.02[3-4]
19	84.29±0.41 [28]	0.46±0.00[11-13]	3.10±0.13[28-29]	15.19±0.36[3-6]	1.08±0.00[25-27]
20	97.54±0.19 [2]	0.47±0.01[10-12]	3.15±0.07[28-29]	15.27±0.28[3-5]	1.15±0.01[16-19]
21	90.16±0.22[16-17]	0.50±0.01 [4]	3.93±0.14[16-20]	14.25±0.18[11-16]	1.10±0.01[21-26]
22	96.71±0.14 [3]	0.49±0.00[4-6]	3.74±0.07[20-23]	14.99±0.07[4-7]	1.14±0.01[17-20]
23	98.11±0.37 [1]	0.44±0.01[14-16]	3.37±0.09[26-27]	15.01±0.08[4-7]	1.13±0.01[18-21]
24	88.91±0.38[20-21]	0.54±0.00 [2]	3.03±0.02 [29]	15.70±0.12[2-3]	1.09±0.01[23-27]
25	92.25±0.18 [14]	0.44±0.00[16-18]	4.04±0.12[13-18]	15.77±0.33[1-3]	1.19±0.02[11-14]
26	93.29±0.05[10-12]	0.38±0.01[21-22]	4.12±0.05[11-15]	14.15±0.25[12-17]	1.25±0.01[4-7]
27	94.84±0.13 [6]	0.52±0.00 [3]	4.03±0.09[14-18]	14.46±0.12[7-15]	1.02±0.01 [31]
28	92.87±0.31[11-13]	0.38±0.00 [22]	4.34±0.15[7-10]	14.35±0.18[9-16]	1.23±0.02[6-9]
29	83.23±0.18 [29]	0.41±0.01[19-20]	3.07±0.09 [29]	16.26±0.18[1-2]	1.19±0.02[11-14]
30	89.92±0.36[17-18]	0.43±0.01 [18]	4.11±0.12[11-16]	14.04±0.35[13-18]	1.19±0.01[12-15]

[†]The values with the same superscript for a given property are not significantly different ($P < 0.05$). PDI – Pellet durability index; BD – Bulk density; WAI – Water absorption index; WSI – Water solubility index; ER – Expansion ratio.

Table 3.10 Treatment combination effects for soy white flakes, moisture content of raw material, temperature and die on extrudate physical properties (continued).

Treatment	**PDI (%)**	**BD (g/cc)**	**WAI (unitless)**	**WSI (%)**	**ER (unitless)**
31	85.77±0.18$^{25-26}$	0.43±0.01 ^{18}	3.91±0.10$^{17-21}$	14.34±0.16$^{9-16}$	1.23±0.02$^{6-10}$
32	85.12±0.43 ^{27}	0.40±0.01$^{20-21}$	4.15±0.14$^{10-15}$	14.31±0.33$^{9-16}$	1.21±0.02$^{8-12}$
33	93.55±0.07$^{9-10}$	0.53±0.01 ^{2}	3.36±0.08$^{26-27}$	15.68±0.07$^{2-3}$	1.09±0.01$^{23-27}$
34	92.81±0.50$^{12-13}$	0.35±0.01 ^{24}	4.03±0.04$^{14-18}$	14.34±0.16$^{9-16}$	1.24±0.01$^{5-8}$
35	96.72±0.47 ^{3}	0.44±0.01$^{15-17}$	4.16±0.08$^{9-14}$	14.37±0.40$^{8-16}$	1.12±0.02$^{19-24}$
36	96.03±0.11 ^{4}	0.46±0.00$^{11-13}$	3.42±0.01$^{26-27}$	15.79±0.21$^{1-3}$	1.09±0.01$^{22-27}$
37	86.19±0.96$^{24-25}$	0.47±0.01$^{10-12}$	4.36±0.19$^{7-8}$	13.84±0.22$^{16-21}$	1.09±0.01$^{23-27}$
38	94.59±0.35$^{6-7}$	0.57±0.01 ^{1}	3.43±0.07$^{26-27}$	15.35±0.25$^{3-4}$	1.12±0.01$^{18-23}$
39	88.43±0.06$^{21-22}$	0.48±0.02$^{5-8}$	3.90±0.06$^{17-22}$	14.35±0.17$^{9-16}$	1.12±0.01$^{19-25}$
40	92.02±0.16 ^{14}	0.47±0.00$^{9-11}$	4.07±0.04$^{12-18}$	13.34±0.35$^{20-24}$	1.03±0.01$^{29-31}$
41	93.06±0.18$^{10-12}$	0.45±0.01$^{14-16}$	3.55±0.06$^{24-26}$	14.73±0.27$^{5-12}$	1.16±0.00$^{15-18}$
42	93.91±0.29$^{8-9}$	0.37±0.00 ^{23}	4.08±0.15$^{12-17}$	14.41±0.18$^{7-16}$	1.12±0.01$^{20-25}$
43	93.50±0.09$^{9-10}$	0.32±0.01 ^{26}	4.11±0.17$^{11-16}$	15.34±0.23$^{3-5}$	1.26±0.01$^{3-6}$
44	90.18±0.20$^{16-17}$	0.40±0.00$^{20-21}$	3.38±0.02$^{26-27}$	15.62±0.44 ^{3}	1.17±0.00$^{14-17}$
45	87.75±0.47 ^{23}	0.41±0.01 ^{19}	4.05±0.14$^{12-18}$	14.05±0.28$^{13-18}$	1.19±0.02$^{10-14}$
46	89.81±0.25$^{17-18}$	0.43±0.01$^{17-18}$	3.97±0.03$^{15-19}$	14.13±0.19$^{12-17}$	1.18±0.02$^{13-17}$
47	83.37±0.66 ^{29}	0.47±0.00$^{8-11}$	3.63±0.05$^{23-25}$	14.59±0.21$^{6-13}$	1.13±0.01$^{18-22}$
48	92.09±0.06 ^{14}	0.39±0.01$^{21-22}$	4.46±0.04$^{6-7}$	14.01±0.34$^{13-18}$	1.17±0.01$^{13-17}$
49	89.61±0.25$^{18-19}$	0.48±0.01$^{7-10}$	3.53±0.03$^{25-26}$	14.48±0.14$^{7-14}$	1.08±0.02$^{26-28}$
50	94.88±0.57 ^{6}	0.44±0.01$^{16-18}$	3.39±0.06$^{26-27}$	15.35±0.43$^{3-4}$	1.06±0.01$^{27-30}$
51	94.58±0.10$^{6-7}$	0.40±0.01$^{19-20}$	3.01±0.08 ^{29}	16.26±0.21$^{1-2}$	1.17±0.02$^{13-17}$
52	86.59±0.27 ^{24}	0.33±0.01 ^{25}	3.89±0.04$^{18-22}$	14.57±0.21$^{7-13}$	1.28±0.02$^{3-4}$
53	93.40±0.22$^{9-11}$	0.44±0.00$^{15-16}$	4.39±0.01$^{7-8}$	13.98±0.26$^{13-19}$	1.03±0.01$^{29-31}$
54	97.46±0.18 ^{2}	0.44±0.00$^{14-16}$	3.26±0.04$^{27-28}$	16.33±0.38 ^{1}	1.18±0.01$^{13-17}$
55	95.11±0.31$^{5-6}$	0.33±0.01 ^{25}	5.02±0.03 ^{4}	14.36±0.35$^{8-16}$	1.18±0.02$^{12-16}$
56	88.81±0.24$^{20-21}$	0.46±0.00$^{12-14}$	3.92±0.04$^{17-20}$	14.41±0.30$^{7-16}$	1.09±0.02$^{24-27}$
57	98.37±0.61 ^{1}	0.44±0.00$^{14-16}$	3.74±0.02$^{20-24}$	15.32±0.21$^{3-5}$	1.20±0.01$^{9-13}$
58	94.24±0.19$^{7-8}$	0.34±0.01 ^{25}	3.78±0.02$^{19-23}$	14.92±0.37$^{4-9}$	1.24±0.01$^{6-9}$
59	85.88±0.22 ^{25}	0.32±0.00 ^{26}	4.23±0.09$^{8-13}$	14.22±0.07$^{12-16}$	1.25±0.02$^{4-7}$
60	89.17±0.43$^{19-20}$	0.48±0.00$^{8-10}$	4.37±0.04$^{7-8}$	13.95±0.18$^{14-20}$	0.90±0.01 ^{33}

†The values with the same superscript for a given property are not significantly different ($P< 0.05$). PDI – Pellet durability index; BD – Bulk density; WAI – Water absorption index; WSI – Water solubility index; ER – Expansion ratio.

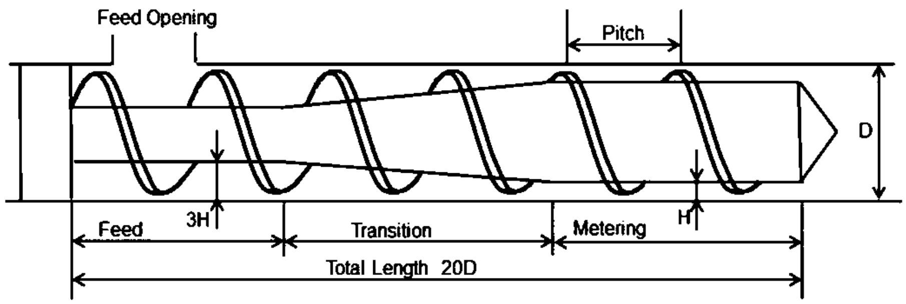


Figure 3.1 Schematic representation of screw in a single screw extruder

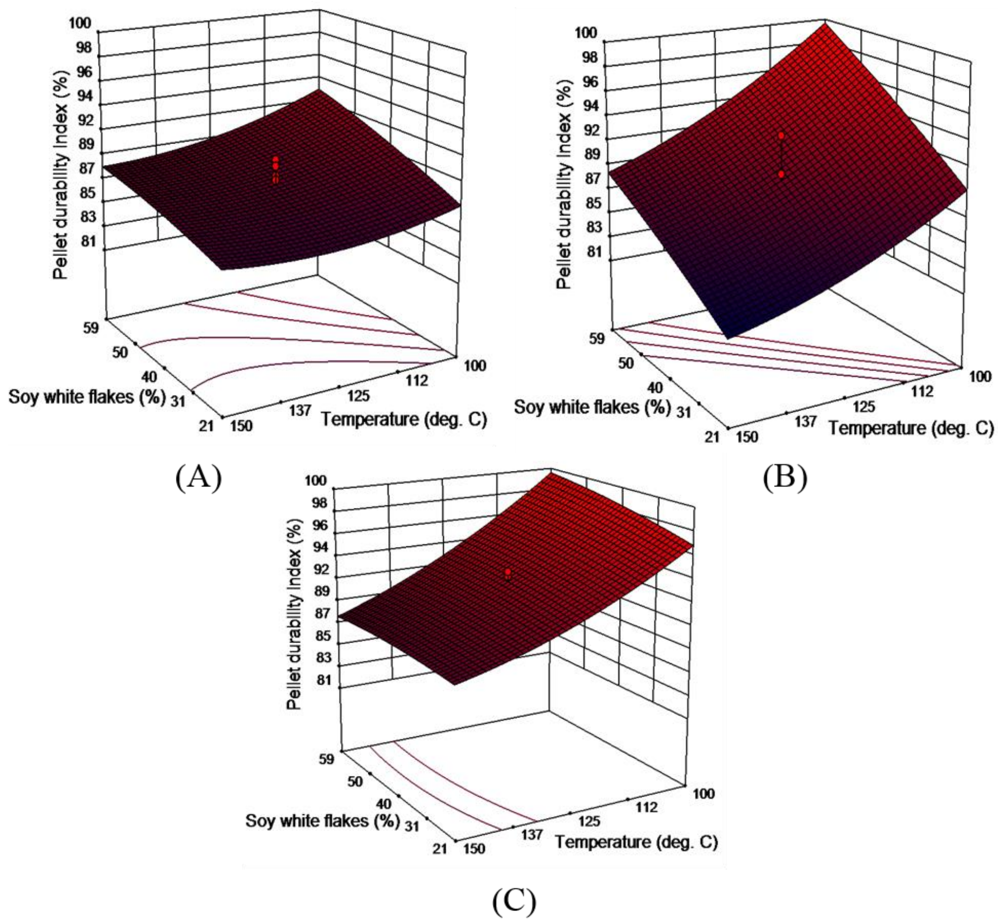


Figure 3.2 Response surface plots of pellet durability index for the effect of soy white flakes content and temperature at 25 % db moisture content at different die aspect ratio (L/D), (A) 3.33, (B) 5.83, and (C) 7.25

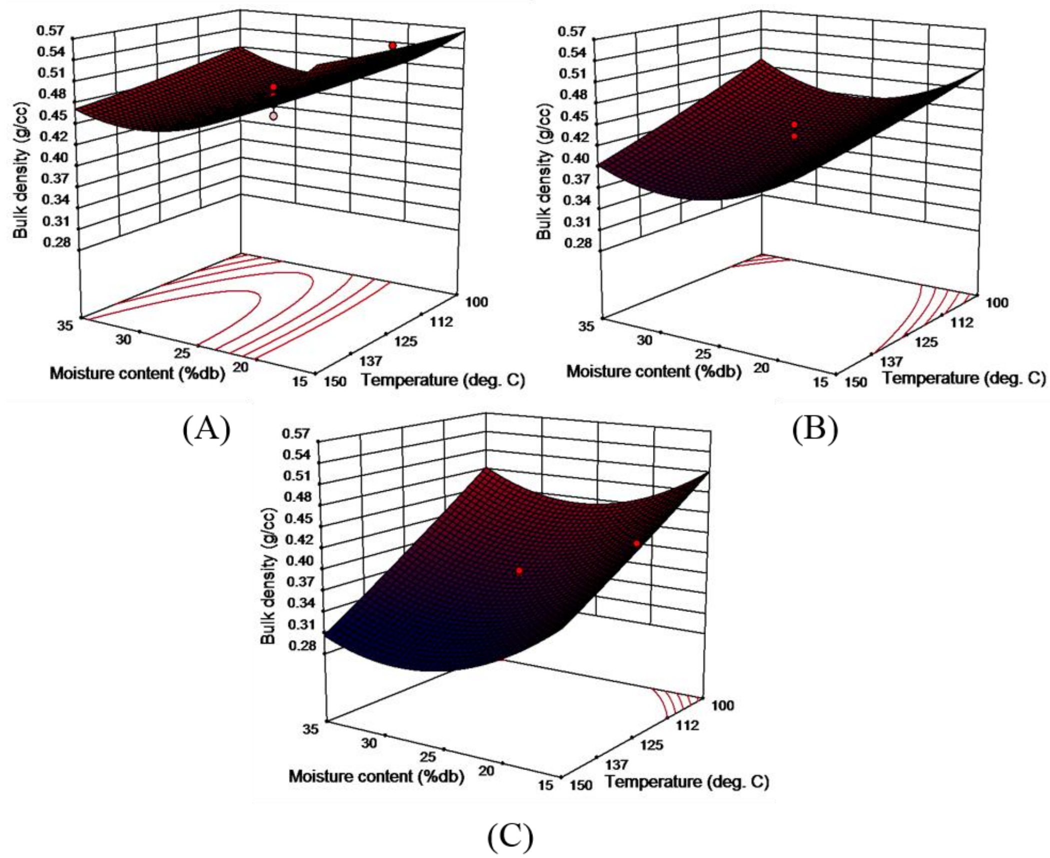
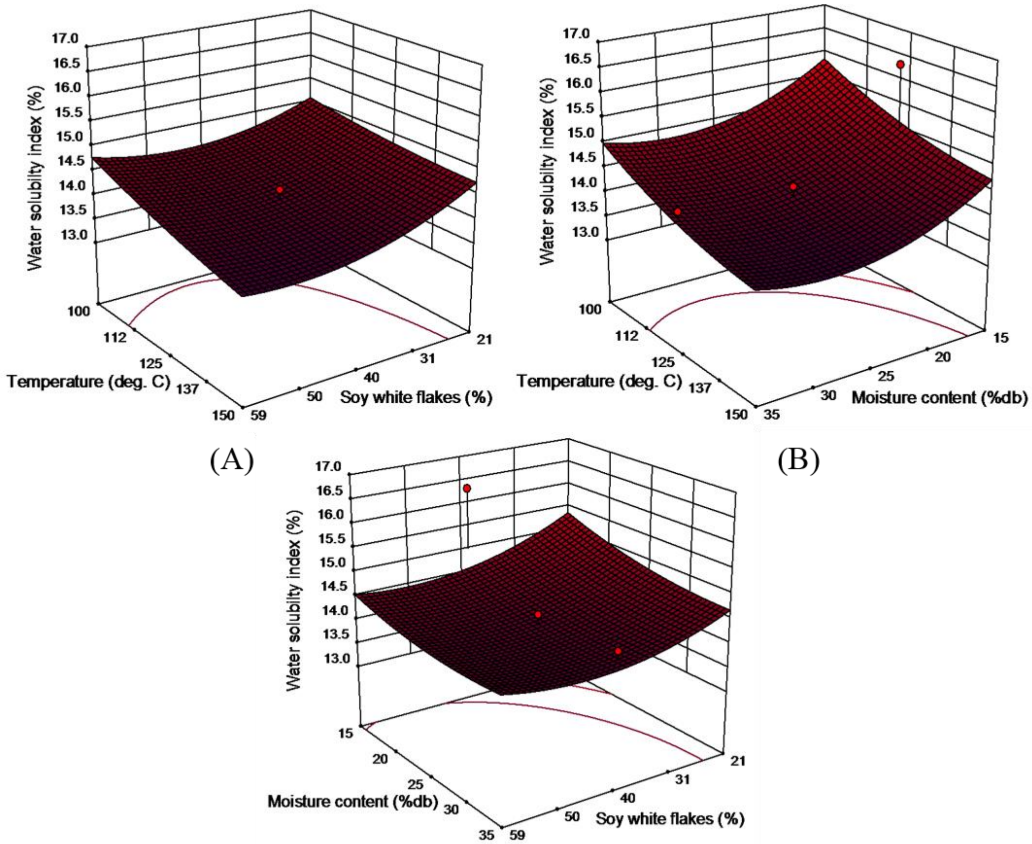
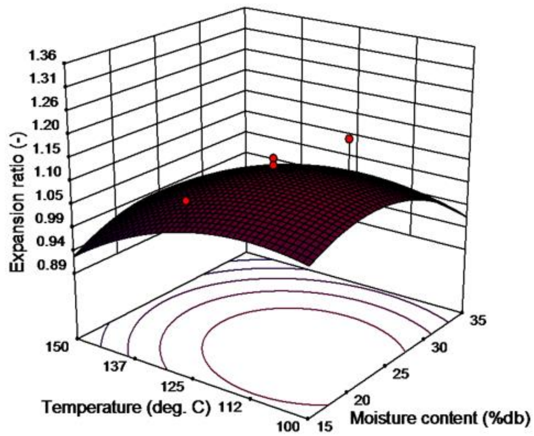


Figure 3.3 Response surface plots of bulk density for the effect of temperature and moisture content at 40% db soy white flakes at different die aspect ratio (L/D), (A) 3.33, (B) 5.83, and (C) 7.25

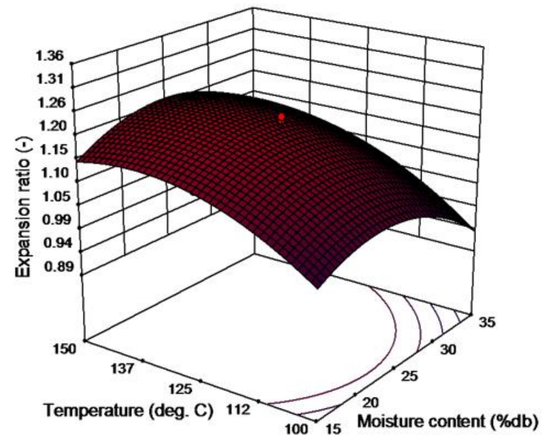


(C)

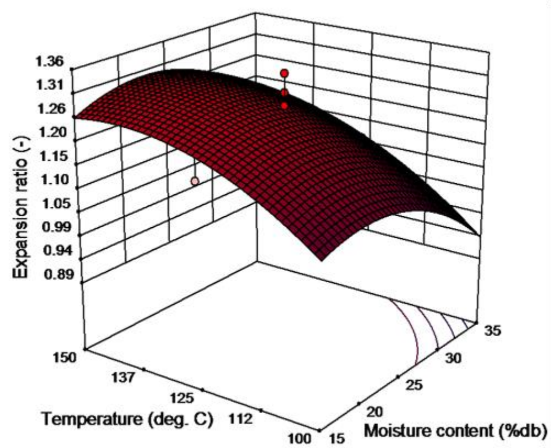
Figure 3.4 Response surface plots of water solubility index at die aspect ratio (L/D) of 7.25 for the effect of (A) temperature and soy white flakes content, (B) temperature and moisture content and (C) moisture and soy white flakes content



(A)



(B)



(C)

Figure 3.5 Response surface plots of expansion ratio for the effect of temperature and moisture content at 40% db soy white flakes at different die aspect ratio (L/D), (A) 3.33, (B) 5.83, and (C) 7.25.

CHAPTER 4 †

Effect of Feed Moisture, Extrusion Temperature and Screw Speed on Properties of Soy White Flakes Based Aquafeed: A Response Surface Analysis

4.1 Abstract

Soy white flakes (SWF) is an intermediate product during soy bean processing. It is untoasted inexpensive product and contains around 51% of crude protein. It can be a potential source of protein to replace fish meal for developing aquafeed. Extrusion process is versatile and is used for the development of aquafeed. Our objective was to study the effects of inclusion of SWF (up to 50%) and other extrusion processing parameters such as barrel temperature and screw speed on the properties of aquafeed extrudates using a single-screw extruder.

Extrudate properties including pellet durability index, bulk density, water absorption and solubility indices and mass flow rate, were significantly ($P<0.05$) affected by the process variables. SWF was the most significant variable with quadratic effects on most of the properties. Increasing temperature and screw speed resulted in increase in durability and mass flow rate of extrudates. Response surface regression models were established to correlate the properties of extrudates to the process variables.

SWF was used as an alternate protein source of fish meal. Our study shows that aquafeed with high durability, lower bulk density and lower water absorption and higher solubility indices can be obtained by adding SWF up to 40%.

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4.2 Introduction

Extrusion cooking is an important food and feed processing operation (Harper, 1981; Paton and Spratt, 1984) and is often used to manufacture aquafeeds. In aquaculture feed production, the important characteristics which affect the quality of fish feed include pellet size, shape, bulk density, water absorption and solubility, hardness or softness (Kazamzadeh, 1989). Extrusion increases the feed digestibility, destroys anti-nutritional compounds and pathogenic microorganisms in the feed and thus, provides feed manufacturers with the means to improve the quality of their products (Williams, 1991). During extrusion processing of starch based products, a relatively elastic melt is formed inside the barrel which results in a more expanded and crispy product (Alves et al., 1999; Ilo et al., 1996; Lin et al., 1997). During extrusion processing of protein based products, a plastic melt is formed inside the barrel and a more porous and textured product is formed (Gwiazda et al., 1987; Prudêncio-Ferreira and Arêas, 1993; Singh et al., 1991). The extent of gelatinization of starch based products and the formation of more plastic melt of protein based products depends on various machine parameters like cooking temperature, rate of shear, screw speed and residence time inside the barrel (Chevanan, 2007).

Aquaculture is one of the fastest growing sector of agriculture and the most rapidly expanding markets for manufacturing fish feeds (Riaz, 1997). Aquaculture feed requires protein content of 26 % to 50 % (Lovell, 1989) and hence formulated feed contains high amounts of both protein and starch. The main protein sources used in aqua feed production is primarily fishmeal which is supplied through the consumption of wild fish stocks. Indubitably, with the increasing rate of farmed fish production (FAO, 2009) and consequently rising prices of fishmeal (Hardy, 2010), the continued use of fishmeal as the main protein source of the feed will no longer be ecologically and economically sustainable in the long run. Soy white flakes and High Protein - Distiller Dried Grains (HP-DDG) contains high amount of protein and are thus a possible alternative source of protein for aquaculture feeds (Kim et al., 1989; Singh and Muthukumarappan, 2014a, b; Wu et al., 1994; Wu et al., 1996). Use of soy products like full fatted soybean meal, defatted toasted soybean meal (SBM) and defatted untoasted soybean meal or white flakes (WF) is becoming common (Fallahi et al., 2012; Singh and Muthukumarappan, 2014a, b). Romarheim et al. (2005)_ENREF_163 found that extrusion of WF diet increased the digestibility of protein and all amino acids whereas extrusion of fishmeal and SBM had no significant effect on amino acid digestibility. Distillers Dried Grains (DDG) and Distillers Dried Grains with Solubles (DDGS), a co-product from corn-based dry grind fuel ethanol manufacturing, is also viable protein source. Mjoun and Rosentrater (2011) used DDGS for the development of good quality extruded aquafeed. Research carried out by Wu et al. (1994); Wu et al. (1996) indicated that tilapia fish can be grown with DDGS, and can improve the economic viability of aquaculture farms. Extensive research has been carried out on the effect of moisture content on the extrudate properties with various kinds of starch or protein based feed materials (Kim et al., 1989; Lin et al., 2000; Shukla et al., 2005; Sokhey et al., 1994). The screw speed has a definite effect on residence time; the extent of pressure developed inside the barrel affects the physical properties of extrudates (Case et al., 1992). Extensive research work has been carried out on the effect of feed ingredients parameters and machine parameters on either starch based or protein based products. Our preliminary study on the effect of soy white flakes inclusion (up to 30%) and varying barrel temperature from 110^{o}C to 170^{o}C at constant screw speed (150 rpm) resulted in aqua feed with favorable properties (Singh and Muthukumarappan, 2014b). In another study, we increased the level of soy white flakes up to 60%. The effect of soy white flakes content and different die nozzles at constant speed (150 rpm) were evaluated. A positive effect on the durability and expansion ratio of aquafeed with increase in die aspect ratio of nozzle was observed (Singh and Muthukumarappan, 2014a). However, the effect of various levels of screw speed on the properties of aquafeed was not studied.

Therefore, the objectives of our present study were to understand the effect of extrusion process parameters such as screw speed, moisture content and barrel temperature using RSM and adopting Box Behnken design on the properties of aquafeed for Catla (*Catla catla*) produced using soy white flakes as a major protein source.

4.3 Materials and Methods

Soy white flake was donated by South Dakota Soybean Processors, Volga, SD. Corn flour was purchased from Cargill Dry Ingredients (Paris, IL). HP-DDG was obtained from the Dakota Ethanol LLC (Wentworth, SD). Corn gluten meal and fishmeal was purchased from Consumer Supply Distributing Co. (Sioux City, IA). Vitamin and mineral premix was obtained from Lortscher Agri Service, Inc. (Bern, Kansas, USA). Soybean oil was obtained from USDA (Brookings, SD).

4.3.1 Feed Blends Formulation

Three isocaloric (304 cal g^{-1}) blends were formulated with a net protein content of 32.3% db. The different ingredients in the blends include HP-DDG, soy white flakes, corn gluten meal (CGM), corn flour, fish meal, soybean oil, and vitamin-mineral mix (Table 4.1). The ingredients were mixed in a laboratory scale Hobart mixer (Hobart

Corporation, Troy, Ohio, USA) for 10 min and stored overnight at ambient temperature for moisture stabilization. The moisture balancing of the blends was done by adding required quantities of water during mixing.

4.3.2 Extrusion Processing

The extrusion processing was performed using a single screw extruder (Brabender Plasti-Corder, Model PL 2000, South Hackensack, NJ) which was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 210 rpm (0 to 22 rad s^{-1}). A schematic representation of laboratory scale single screw extruder is shown in Figure 4.1. The extruder had a barrel with length-to-diameter ratio of 20:1 and a barrel diameter of 19 mm. A uniform 19.05 mm pitch screw with compression ratio of 3:1 was used in the experiments. The screw had a variable flute depth, with depths at the feed portion of 19.05 mm, and near the die of 3.81 mm. The center of the die assembly was conical, and tapered from an initial diameter of 6.0 mm to an exit diameter of 3 mm, respectively, at the discharge opening. The length and diameter of the die was 17.5 mm and 3 mm (l/d = 5.83), respectively. The extruder barrel was equipped with external band heaters with provisions to control the temperature of all three zones: feed zone, transition zone/melting zone, and die sections. The material gets gelatinized and plasticized under thermal and mechanical stresses generated by the rotation of screws in melting zone of the extruder. The gelatinized material then enters the cooking zone where the extruder barrel is fully filled due to pressure generated at die nozzle. When the process reached the steady state, samples were collected at the die. All samples were stored in a conditioned room prior to further analysis.

4.3.3 Experimental Design and Statistical Analysis

Experiments were conducted using Box-Behnken design which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN), consisting four numerical independent variables namely soy white flakes (X_1), moisture content (X_2), temperature (X_3) and screw speed (X_4) each at three levels as shown in Table 4.2. Pellet durability index, bulk density, water absorption and solubility indices and mass flow rate of the extrudates were measured as the response/dependent variables. All measurements were done in triplicate. Using Eq. (4.1), the numerical independent variables in actual form (X_1, X_2) were converted to their coded form (x_1, x_2).

$$x_i = \frac{(X_i - X_o)}{\Delta X} \tag{4.1}$$

where x_i is the dimensionless coded value of the i^{th} independent variable, and Xi, X_0, and ΔX correspond to the actual value, actual value at the center point, and the step change of the i^{th} variable, respectively.

Twenty nine experiments were performed in randomized order including five replications at the design center to obtain an accurate estimation of the experimental error (Table 4.3). The pellet durability index (Y_{PDI}), bulk density (Y_{BD}), water absorption index (Y_{WAI}), water solubility index (Y_{WSI}) and mass flow rate (Y_{MFR}) were taken as the five responses of the designed experiments. A second order polynomial equation was used to describe the effect of the independent variables in terms of linear, quadratic and their interactions on the dependent variables as given by Eq. (4.2).

$$Y_i = b_o + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j + \varepsilon \tag{4.2}$$

where Y_i is the predicted response; b_0 is the interception coefficient; b_i, b_{ii}, and b_{ij} are coefficients of the linear, quadratic, and interaction terms; ε is the random error; and X_i is the independent variables studied. 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) presented in Table 4.4 and Table 4.5. 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 F-test (Table 4.6). 3D response surfaces were used to visualize the interactive effects of the independent variables. All the collected data were analyzed with Microsoft Excel v.2010 and SAS v.9. (SAS, 2012) The Proc GLM procedure was used to determine the treatment effects using a Type I error rate (α) of 0.05.

4.3.4 Measurement of Physical Properties

4.3.4.1 Pellet durability index

Approximately 100 g of extrudates from each blend were manually sieved (U.S.A. standard testing, ASTM E-11 specification, Daigger, Vernon Hills, IL) to remove initial fines, and then tumbled in a pellet durability tester (Model PDT-110, Seedburo Equipment Company, Chicago, IL) for 10 min. Afterwards, the samples were again sieved, and then weighed on an electronic balance (Explorer Pro, Model: EP4102, Ohaus, Pine Brook, NJ) (ASAE, 2004). PDI was calculated by Eq. (4.3):

$$PDI = \left(\frac{M_a}{M_b}\right) \times 100 \tag{4.3}$$

where, M_a was the mass (g) after tumbling and M_b was the sample mass (g) before tumbling.

4.3.4.2 Bulk density

Bulk density was determined as the ratio of the mass of extrudates that they filled up to a given bulk volume and measured using a standard bushel tester (Seedburo Equipment Company, Chicago, IL) following the method recommended by USDA (1999).

4.3.4.3 Water absorption index and water solubility index

Extrudates were ground to fine powders using a coffee grinder (Black & Decker ® Corporation, Towson, ML, USA). The ground extrudates (2.5g) was suspended in distilled water (30 mL) in a tarred 60 mL centrifuge tube. The suspension was stirred intermittently and centrifuged at 3000g for 10 min. The supernatant was decanted into a tarred aluminum cup and dried at 135°C for 2 h.(AACC, 2000) The weight of the gel remaining in the centrifuge tube was measured. The water absorption index and water solubility index were calculated by Eq. (4.4) and Eq. (4.5) respectively:

$$WAI(unitless) = \frac{W_g}{W_{ds}} \tag{4.4}$$

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

$$WSI(\%) = \left(\frac{W_{ss}}{W_{ds}}\right) \times 100 \tag{4.5}$$

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

4.3.4.4 Mass flow rate

Extrudate samples exiting the die were collected at 30s intervals and weighed using an electronic balance (PB 5001, Mettler Toledo, Switzerland) to quantify the mass flow rate.

4.4 Results and Discussion

4.4.1 Pellet durability index

Pellet durability indicates the mechanical strength of the extrudates (Rosentrater et al., 2005). The higher the pellet durability index, the more stable the extrudates will be during storage, feeding, and handling processes. The response surface plot presented in Figure 4.2 shows that at low temperature, with increase in soy white flakes content, pellet durability index initially decreased and after reaching a minimum it increased further. Similar findings were observed in our previous study (Singh and Muthukumarappan, 2014a). ANOVA showed that moisture content had no significant effect on pellet durability index and hence response surface plots of interaction effect involving moisture content are not shown. Lack of fit was not significant relative to the pure error, which meant the model was well fitted. The regression equation for pellet durability index in coded and actual form is shown in Table 4.6 and Table 4.7, respectively. The effect of changing the level of temperature from 110 to 140°C on pellet durability of extrudates was found to be significant ($P<0.05$) but no definite pattern was observed. The values of pellet durability index of extruded products under experimental conditions are presented in Table 4.8. Pellet durability index is found to be maximum at higher temperature and lower screw speed. It is believed that the extent of heat treatment, along with the level of starch transformation as well as water content influence the pellet durability index quality of the extrudates (Rosentrater et al., 2009a, b).

4.4.2 Bulk density

The targeted fish (Catla) is a surface feeder and a lower bulk density is required to obtain floating feed (Mjoun and Rosentrater, 2011). Changing the moisture content of ingredient mix had a significant effect on the bulk density (P = 0.0466). Also combined effect of moisture content and temperature had a significant effect on the bulk density (P = 0.0328) (Table 4.4). Response surface plot presented in Figure 4.3 shows that lower bulk density is achieved when moisture content is increased at higher barrel temperature. At higher barrel temperature and high moisture content the bubble growth is dominant factor for expansion of the extrudates (Padmanabhan and Bhattachayray, 1989). An increase in barrel temperature will increase the degree of superheating of water within the extruder encouraging bubble formation and also a decrease in melt viscosity leading to reduced density (Ding et al., 2006), which was observed in this work.

ANOVA showed that soy white flakes and screw speed had no significant effect on bulk density and hence response surface plots of interaction effect involving soy white flakes and screw speed are not shown. The regression equation for bulk density in coded and actual form is shown in Table 4.6 and Table 4.7, respectively. The treatment combination effects of soy white flakes, moisture content, temperature and screw speed on bulk density of extrudates are presented in Table 4.8.

4.4.3 Water absorption index and water solubility index

When percentage of soy white flakes in ingredient mix was increased from 20% to 50% the water absorption index initially decreases and then increases as shown in Figure 4.4(A). When screw speed was increased from 100 rpm to 200 rpm, water absorption index also decreased. As the moisture content of ingredient was increased from 20% to 30%, water absorption index increased (Figure 4.4). Water absorption index or WAI, is related to the extent of starch gelatinization followed by degradation and dextrinization, which are mainly responsible for water absorption (Bortone, 2004; Gomez and Aguilera, 1984). WAI is also indirectly related to water holding capacity, which thus affects storage stability of product (Chevanan et al., 2007a). From our result [Figure 4.4(B)], it can be seen that WAI decreased with an increase in screw speed and a decrease in moisture content which is in agreement with studies conducted by Anderson et al. (1970); Oikonomou and Krokida (2012). High shear rate (high screw speed) reduces the availability of undamaged polymer chains. As a result, the availability of hydrophilic groups which can bind water molecules are low which is responsible for lower values of WAI (Gomez and Aguilera, 1983).

The experimental results show that WSI of all examined extrudates decreased with increasing moisture levels from 20% to 30% (Figure 4.5). Water solubility index (WSI) is generally used as a measure for starch degradation (Einde et al., 2003) which means that at lower WSI there is minor degradation of starch and this leads to less numbers of soluble molecules in the extrudates (Hernandez-Diaz et al., 2007). Higher moisture content in extrusion process can diminish protein denaturation and starch degradation (Rodriguez-Miranda et al., 2012). Proteins can cross-link with starches (Fernández-Gutiérrez et al., 2004; Goel et al., 1999), preventing amylose solubilization and diminishing WSI. Similar findings where WSI decreased with increase in levels of moisture were also observed by Badrie and Mellowes (1991); Chinnaswamy and Hanna (1990); Williams et al. (1977). From Figure 4.4(A) and Figure 4.5, it can be inferred that WAI is inversely related to WSI.

4.4.4 Mass flow rate

One common way to estimate the productivity of an extrusion processing is to examine the mass flow rate of the output (Ayadi et al., 2013). Mass flow rate in a single screw extruder depends on the drag flow developed by screw rotation, die geometry, shear rate, diet formulation, moisture content of the feed, viscosity and the pressure flow generated due to the restriction of the die (Chevanan et al., 2007a; Kannadhason et al., 2010; Mercier et al., 1989b). The interaction effect of temperature and screw speed is found to be significant (Table 4.5). Response surface plots presented in Figure 4.6 showed that at higher screw speed, mass flow rate is increased with increase in temperature level from 110^{o}C to 140^{o}C. This may be due to decrease in the viscosity of the melt. Also, mass flow rate was found to increase with increase in the level of screw speed as shown in Figure 4.6. This may be due to the reason that as the screw speed increases, the ability to convey the material along the extruder barrel increases and as a result more mass of extrudates per hour is produced.

ANOVA showed (Table 4.5) that moisture content had no significant effect on mass flow rate and hence response surface plots of interaction effect involving moisture effect are not shown. The results were not in agreement with the findings of Chevanan et al. (2007a) who reported that increasing moisture content from 15% to 25% resulted in a 32.5% decrease in the mass flow rate. The regression equation for the relationship between mass flow rate and independent variables in terms of coded and actual form is shown in Table 4.6 and Table 4.7, respectively. The experimental values of mass flow rate of extrudates under different designed extrusion conditions are shown in Table 4.8.

4.4.5 Optimization and validation

Numerical optimization was carried out in Design Expert v8 considering all five dependent variables. The optimization resulted in 43 solutions and the top ten solutions are shown in Table 4.9. Validation of the predicted responses was carried out by extruding the blends at three different optimum conditions as shown in Table 4.9. Good agreement was found between the predicted and experimental values. The experimental values for solution #9 were very close to the predicted values. The optimum condition was: 39.56% SWF, 20% moisture content, 112^{o}C barrel temperature and 195 rpm screw speed.

4.5 Conclusions

Response surface methodology revealed the significant effects of soy white flakes, moisture content, barrel temperature and screw speed on the properties of extruded aquafeed. The effects of soy white flakes on most of the properties of the extrudates were found to be quadratic; the effects were linear for water solubility effects. Moisture content and temperature had quadratic effects on the pellet durability index, where increasing the moisture content and temperature initially decreased and then increased the pellet durability index. Moisture content, temperature and screw speed all had linear effect on the water absorption index. Interactive effects of temperature and screw speeds were found on pellet durability index and mass flow rate. Increase in barrel temperature produces less compacted extrudates. Increasing screw speed resulted in increase in pellet durability and mass flow rate but decrease in water absorption index of the extrudates. This study will be useful in identifying desirable operating conditions for the production of targeted extruded aqua feed.

Table 4.1 Ingredient composition of feed blends.

Feed ingredients	**Mass of ingredients (g kg^{-1} dry matter)**		
	Blend I	**Blend II**	**Blend III**
Soy white flakes	200	350	500
HP-DDG	400	250	100
CGM	20	20	2
Corn Flour	300	300	300
Fish meal	50	50	50
Soy bean oil	10	10	10
Vitamin & Mineral mix	20	20	20

†HP-DDG – High protein distiller's dried grains; CGM – Corn gluten meal

Table 4.2 Independent numerical and categorical variables and their levels.

Numerical variables	**Symbol**	**Coded variable levels**		
		-1	0	1
Soy white flakes (%)	X_1	20	35	50
Moisture content (% db)	X_2	20	25	30
Temperature (^oC)	X_3	110	125	140
Screw speed (rpm)	X_4	100	150	200

Table 4.3 Experimental design layout.

Run	**Coded variables**				**Actual variables**			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (%db)	X_3 (^{o}C)	X_4 (rpm)
1	0	-1	-1	0	35	20	110	150
2	0	1	1	0	35	30	140	150
3	0	0	1	1	35	25	140	200
4	-1	0	0	-1	20	25	125	100
5	0	0	-1	-1	35	25	110	100
6	0	-1	1	0	35	20	140	150
7	0	1	-1	0	35	30	110	150
8	0	0	0	0	35	25	125	150
9	1	0	1	0	50	25	140	150
10	0	0	0	0	35	25	125	150
11	-1	-1	0	0	20	20	125	150
12	-1	1	0	0	20	30	125	150
13	0	0	1	-1	35	25	140	100
14	1	0	0	1	50	25	125	200
15	0	-1	0	-1	35	20	125	100
16	0	1	0	1	35	30	125	200
17	0	0	0	0	35	25	125	150
18	1	1	0	0	50	30	125	150
19	0	0	-1	1	35	25	110	200
20	-1	0	0	1	20	25	125	200
21	0	0	0	0	35	25	125	150
22	-1	0	1	0	20	25	140	150
23	1	-1	0	0	50	20	125	150
24	1	0	0	-1	50	25	125	100
25	0	1	0	-1	35	30	125	100
26	-1	0	-1	0	20	25	110	150
27	0	0	0	0	35	25	125	150
28	1	0	-1	0	50	25	110	150
29	0	-1	0	1	35	20	125	200

Table 4.4 Analysis of Variance (ANOVA) for pellet durability index and bulk density.

Source	**df**	**Pellet durability index**				**Bulk density**			
		SS	**MS**	***F* value**	***P*-value**	**SS**	**MS**	***F* value**	***P*-value**
Model	14	395.79	28.27	3.95	0.0074	25298.81	1807.06	2.57	0.0444
X_1	1	2.86	2.86	0.40	0.5379	3104.08	3104.08	4.41	0.0544
X_2	1	0.11	0.11	0.01	0.9044	3355.57	3355.57	4.76	0.0466
X_3	1	27.61	27.61	3.86	0.0697	4.08	4.08	0.01	0.9404
X_4	1	17.76	17.76	2.48	0.1376	363.00	363.00	0.52	0.4846
X_1^2	1	112.60	112.60	15.73	0.0014	375.48	375.48	0.53	0.4773
X_2^2	1	124.56	124.56	17.40	0.0009	573.13	573.13	0.81	0.3823
X_3^2	1	140.31	140.31	19.60	0.0006	10565.17	10565.17	15.00	0.0017
X_4^2	1	32.77	32.77	4.58	0.0505	42.74	42.74	0.06	0.8090
X_1X_2	1	15.43	15.43	2.16	0.1642	342.23	342.23	0.49	0.4972
X_1X_3	1	3.19	3.19	0.45	0.5152	58.77	58.77	0.08	0.7769
X_1X_4	1	6.60	6.60	0.92	0.3534	25.01	25.01	0.04	0.8532
X_2X_3	1	2.77	2.77	0.39	0.5442	3948.05	3948.05	5.61	0.0328
X_2X_4	1	0.14	0.14	0.02	0.8896	361.00	361.00	0.51	0.4858
X_3X_4	1	43.76	43.76	6.11	0.0269	2952.13	2952.13	4.19	0.0599
Residual	14	100.23	7.16	-	-	9859.69	704.26	-	-
Lack of fit	10	87.92	8.79	2.86	0.1617	8968.43	896.84	4.03	0.0959
Pure error	4	12.31	3.08	-	-	891.26	222.81	-	-

[†]df – degree of freedom, SS – Sum of squares, MS – Mean square

Table 4.5 Analysis of Variance (ANOVA) for water absorption index, water solubility index and mass flow rate.

Source	df	Water absorption index				Water solubility index				Mass flow rate			
		SS	**MS**	***F* value**	***P*-value**	**SS**	**MS**	***F* value**	***P*-value**	**SS**	**MS**	***F* value**	***P*-value**
Model	14	4.35	0.31	5.47	0.0015	19.82	1.42	4.65	0.0034	88.37	6.31	9.36	< 0.0001
X_1	1	0.12	0.12	2.13	0.1661	4.65	4.65	15.28	0.0016	0.39	0.39	0.58	0.4576
X_2	1	0.65	0.65	11.36	0.0046	5.90	5.90	19.38	0.0006	0.04	0.04	0.07	0.8012
X_3	1	0.60	0.60	10.53	0.0059	0.55	0.55	1.81	0.1996	0.05	0.05	0.08	0.7844
X_4	1	0.54	0.54	9.53	0.0080	1.22	1.22	4.00	0.0652	69.33	69.33	102.80	< 0.0001
X_1^2	1	1.26	1.26	22.12	0.0003	5.04	5.04	16.55	0.0012	4.38	4.38	6.49	0.0232
X_2^2	1	0.21	0.21	3.69	0.0753	0.09	0.09	0.31	0.5881	1.68	1.68	2.50	0.1364
X_3^2	1	0.21	0.21	3.65	0.0767	0.46	0.46	1.50	0.2416	2.59	2.59	3.84	0.0703
X_4^2	1	0.04	0.04	0.76	0.3985	0.02	0.02	0.08	0.7812	0.53	0.53	0.78	0.3909
X_1X_2	1	0.01	0.01	0.09	0.7629	0.00	0.00	0.01	0.9314	0.10	0.10	0.15	0.7026
X_1X_3	1	0.08	0.08	1.36	0.2625	0.02	0.02	0.05	0.8194	0.11	0.11	0.17	0.6866
X_1X_4	1	0.00	0.00	0.04	0.8423	0.07	0.07	0.21	0.6510	1.38	1.38	2.05	0.1744
X_2X_3	1	0.14	0.14	2.54	0.1333	0.62	0.62	2.03	0.1758	2.17	2.17	3.21	0.0948
X_2X_4	1	0.21	0.21	3.75	0.0733	0.31	0.31	1.01	0.3315	0.45	0.45	0.67	0.4282
X_3X_4	1	0.15	0.15	2.56	0.1317	0.18	0.18	0.59	0.4539	3.18	3.18	4.72	0.0476
Residual	14	0.80	0.06	-	-	4.26	0.30	-	-	9.44	0.67	-	-
Lack of fit	10	0.70	0.07	2.85	0.1626	3.56	0.36	2.04	0.2569	8.53	0.85	3.73	0.1080
Pure error	4	0.10	0.02	-	-	0.70	0.17	-	-	0.91	0.23	-	-

†df – degree of freedom, SS – Sum of squares, MS – Mean square

Table 4.6 Final equation in terms of coded factors after excluding the insignificant terms for pellet durability index, bulk density, water absorption index, water solubility index and mass flow rate

Coded model equations	**R^2**	**Adj R^2**	**Pred R^2**	**Adeq precision**
$Y_{PDI} = +83.70 - 3.31x_3 x_4 + 4.17x_1^2 + 4.38x_2^2 + 4.65x_3^2$	0.80	0.60	-0.06	6.08
$Y_{BD} = +369.87 - 16.72x_2 - 31.42x_2x_3 + 40.36x_3^2$	0.72	0.44	-0.51	6.41
$Y_{WAI} = +3.84 + 0.23x_2 + 0.22x_3 - 0.21x_4 + 0.44x_1^2$	0.84	0.69	0.19	9.53
$Y_{WSI} = +14.76 + 0.62x_1 - 0.70x_2 - 0.88x_1^2$	0.82	0.65	0.10	9.58
$Y_{MFR} = +8.88 + 2.40x_4 + 0.89x_3 x_4 - 0.82x_1^2$	0.90	0.81	0.48	13.52

[†]PDI – Pellet durability index; BD – Bulk density; WAI – Water absorption index; WSI – Water solubility index; MFR – Mass flow rate.

Table 4.7 Best-Fit Response Surface Models for Extrudate Physical Properties.

Response Surface Model	R^2	**Std. Deviation**	***F* Statistic**	***P* value**
$Y_{PDI} = +473.86 - 1.68X_1 - 8.39X_2 - 4.19X_3 + 0.18X_4 + 0.03X_1X_2 - 3.97\times10^{-3}X_1X_3 + 1.71\times10^{-3}X_1X_4 - 0.01X_2X_3 + 7.57\times10^{-4}X_2X_4 - 4.41\times10^{-3}X_3X_4 + 0.02X_1^2 + 0.17X_2^2 + 0.02X_3^2 + 8.99\times10^{-4}X_4^2$	0.80	2.68	3.95	0.0074
$Y_{BD} = +1744.68 - 2.75X_1 + 20.20X_2 - 28.38X_3 + 3.04X_4 + 0.12X_1X_2 - 0.02X_1X_3 + 3.33\times10^{-3}X_1X_4 - 0.42X_2X_3 + 0.04X_2X_4 - 0.04X_3X_4 + 0.03X_1^2 + 0.36X_2^2 + 0.18X_3^2 + 1.03\times10^{-3}X_4^2$	0.72	26.54	2.57	0.0444
$Y_{WAI} = -14.90 - 0.07X_1 - 0.15X_2 + 0.34X_3 - 6.44\times10^{-3}X_4 + 4.89\times10^{-4}X_1X_2 - 6.19\times10^{-4}X_1X_3 + 3.22\times10^{-5}X_1X_4 - 2.53\times10^{-3}X_2X_3 + 9.23\times10^{-4}X_2X_4 - 2.54\times10^{-4}X_3X_4 + 1.96\times10^{-3}X_1^2 + 7.19\times10^{-3}X_2^2 - 7.95\times10^{-4}X_3^2 + 3.26\times10^{-5}X_4^2$	0.84	0.24	5.47	0.0015
$Y_{WSI} = +47.65 + 0.25X_1 - 0.40X_2 - 0.49X_3 + 1.16\times10^{-4}X_4 + 3.22\times10^{-4}X_1X_2 + 2.85\times10^{-4}X_1X_3 + 1.70\times10^{-4}X_1X_4 + 5.24\times10^{-3}X_2X_3 - 1.11\times10^{-3}X_2X_4 + 2.83\times10^{-4}X_3X_4 - 3.92\times10^{-3}X_1^2 - 4.80\times10^{-3}X_2^2 + 1.18\times10^{-3}X_3^2 - 2.45\times10^{-5}X_4^2$	0.82	0.55	4.65	0.0034
$Y_{MFR} = +91.74 + 0.17X_1 - 0.49X_2 - 1.09X_3 - 0.19X_4 + 2.13\times10^{-3}X_1X_2 - 7.52\times10^{-4}X_1X_3 + 7.83\times10^{-4}X_1X_4 + 9.81\times10^{-3}X_2X_3 + 1.34\times10^{-3}X_2X_4 + 1.19\times10^{-3}X_3X_4 - 3.65\times10^{-3}X_1^2 - 0.02X_2^2 + 2.81\times10^{-3}X_3^2 + 1.14\times10^{-4}X_4^2$	0.90	0.82	9.36	<0.0001

Table 4.8 Treatment combination effects for soy white flakes, moisture content of raw material, temperature and screw speed on extrudate physical properties.

Treatment	**PDI (%)**	**BD (kg m^{-3})**	**WAI (-)**	**WSI (%)**	**MFR (kg h^{-1})**
1	93.44±0.03^{d}	391.33±3.60^{k}	3.23±0.10^{o}	16.02±0.22^{b}	9.89±0.05^{d}
2	88.30±0.09^{k}	361.33±2.91^{o}	4.29±0.08^{de}	13.94±0.10^{lm}	10.15±0.51^{d}
3	81.05±0.25^{q}	343.00±3.54^{q}	3.49±0.16^{m}	15.29±0.09^{c}	13.23±0.35^{a}
4	89.78±0.12^{j}	339.67±4.80^{q}	4.48±0.12^{c}	12.60±0.04^{op}	8.04±0.03$^{i\text{-}k}$
5	92.37±0.12^{f}	415.67±1.71^{ef}	3.71±0.05^{l}	15.06±0.06^{de}	8.16±0.12^{ij}
6	90.83±0.26^{h}	453.00±0.84^{a}	4.41±0.08^{cd}	14.15±0.15$^{i\text{-}k}$	8.82±0.35^{fg}
7	94.24±0.17^{c}	425.33±2.17^{d}	3.86±0.10^{jk}	14.24±0.12$^{h\text{-}j}$	8.29±0.18$^{h\text{-}j}$
8	84.70±0.22^{n}	366.00±2.93^{n}	4.04±0.08$^{g\text{-}i}$	14.30±0.09^{hi}	8.67±0.37$^{f\text{-}h}$
9	93.58±0.08^{d}	444.67±0.77^{b}	3.98±0.04$^{h\text{-}j}$	15.06±0.09^{de}	8.31±0.31$^{h\text{-}j}$
10	81.28±0.43^{q}	350.00±1.58^{p}	3.93±0.02^{ij}	14.39±0.11^{h}	9.35±0.15^{e}
11	92.60±0.28^{ef}	398.33±2.02^{j}	4.15±0.05^{fg}	13.77±0.03^{mn}	7.86±0.56^{jk}
12	90.87±0.09^{h}	333.67±2.43^{r}	4.77±0.10^{b}	12.51±0.10^{p}	6.37±0.15^{l}
13	93.34±0.78^{d}	454.64±2.25^{a}	4.51±0.03^{c}	14.21±0.06^{ij}	5.88±0.25^{lm}
14	90.96±0.05^{h}	401.67±1.59^{ij}	4.50±0.11^{c}	14.65±0.07^{g}	10.39±0.31^{cd}
15	92.31±0.08^{fg}	413.33±1.38^{f}	4.14±0.11^{fg}	15.19±0.03^{cd}	5.78±0.33^{m}
16	91.97±0.18^{g}	405.67±2.14^{hi}	4.24±0.08^{ef}	14.07±0.02$^{j\text{-}l}$	11.62±0.27^{b}
17	85.78±0.27^{l}	372.33±1.90^{m}	3.86±0.03^{jk}	14.83±0.05^{fg}	9.13±0.28^{ef}
18	94.66±0.11^{b}	381.33±1.31^{l}	4.94±0.04^{a}	13.79±0.05^{mn}	7.56±0.50^{k}
19	93.31±0.05^{d}	412.67±1.89^{fg}	3.45±0.04^{mn}	15.29±0.12^{c}	11.94±0.40^{b}
20	85.17±0.15^{m}	341.33±2.61^{q}	4.14±0.04^{fg}	12.72±0.10^{o}	10.82±0.19^{c}
21	84.05±0.19^{o}	391.67±2.72^{k}	3.74±0.11^{kl}	15.30±0.04^{c}	9.10±0.21^{ef}
22	95.52±0.10^{a}	440.33±0.98^{c}	4.06±0.04^{gh}	14.10±0.13$^{j\text{-}l}$	9.00±0.43^{ef}
23	88.53±0.21^{k}	409.00±0.27^{gh}	4.17±0.10$^{e\text{-}g}$	14.95±0.16^{ef}	8.41±0.44$^{g\text{-}i}$
24	90.44±0.19^{i}	390.00±2.39^{k}	4.75±0.03^{b}	14.02±0.13^{kl}	5.26±0.24^{n}
25	91.17±0.05^{h}	369.00±4.81^{mn}	4.12±0.02^{fg}	13.74±0.21^{n}	6.05±0.08^{lm}
26	92.92±0.13^{e}	419.67±1.21^{e}	3.66±0.06^{l}	14.01±0.09^{kl}	8.16±0.30^{ij}
27	82.70±0.35^{p}	369.33±2.66^{mn}	3.64±0.12^{l}	15.00±0.14^{ef}	8.15±0.32^{ij}
28	94.55±0.09^{bc}	439.33±2.23^{c}	4.14±0.05^{fg}	14.71±0.16^{g}	8.15±0.18^{ij}
29	92.36±0.20^{f}	412.00±1.01^{fg}	3.34±0.07^{no}	16.63±0.08^{a}	10.01±0.11^{d}

[†]The values with the same superscript for a given property are not significantly different (P< 0.05). PDI – Pellet durability index, BD – Bulk density, WAI – Water absorption index, WSI – Water solubility index, MFR – Mass flow rate.

Table 4.9 Solutions for optimal conditions and validation.

Solution #	**SWF (%)**	**MC (%db)**	**Temp (^oC)**	**SS (rpm)**	**MFR (kg h^{-1})**	**PDI (%)**	**BD (kg m^{-3})**	**WAI (-)**	**WSI (%)**
1	36.15	20.00	114	200	11.00	94.59	404.90	3.18	16.38
2	36.03	20.00	114	200	11.00	94.59	404.86	3.17	16.37
3	36.01	20.00	114	200	10.99	94.45	404.15	3.18	16.36
4	37.10	20.00	114	200	10.99	94.34	404.09	3.21	16.38
5	38.33	20.00	113	200	11.06	95.30	409.43	3.20	16.47
6	38.88	20.02	114	200	10.99	94.32	404.63	3.26	16.40
7	35.07	20.43	113	200	11.07	94.37	406.52	3.16	16.28
8	34.55	20.34	112	200	11.10	95.28	410.47	3.11	16.33
9	39.56	20.00	112	195	10.88	95.52	412.76	3.23	16.49
10	40.47	20.00	110	176	10.37	95.52	417.93	3.23	16.50
Validation									
3	36.01	20.00	114	200	10.81	94.98	406.15	3.26	16.39
9	39.56	20.00	112	195	10.92	95.46	411.55	3.21	16.45
10	40.47	20.00	110	176	10.49	95.69	416.31	3.29	16.38

†SWF – Soy white flakes; MC – Moisture content of blends; Temp – Barrel temperature; SS - Screw speed; MFR – Mass flow rate; PDI – Pellet durability index; BD – Bulk density; WAI – Water absorption index; WSI – Water solubility index.

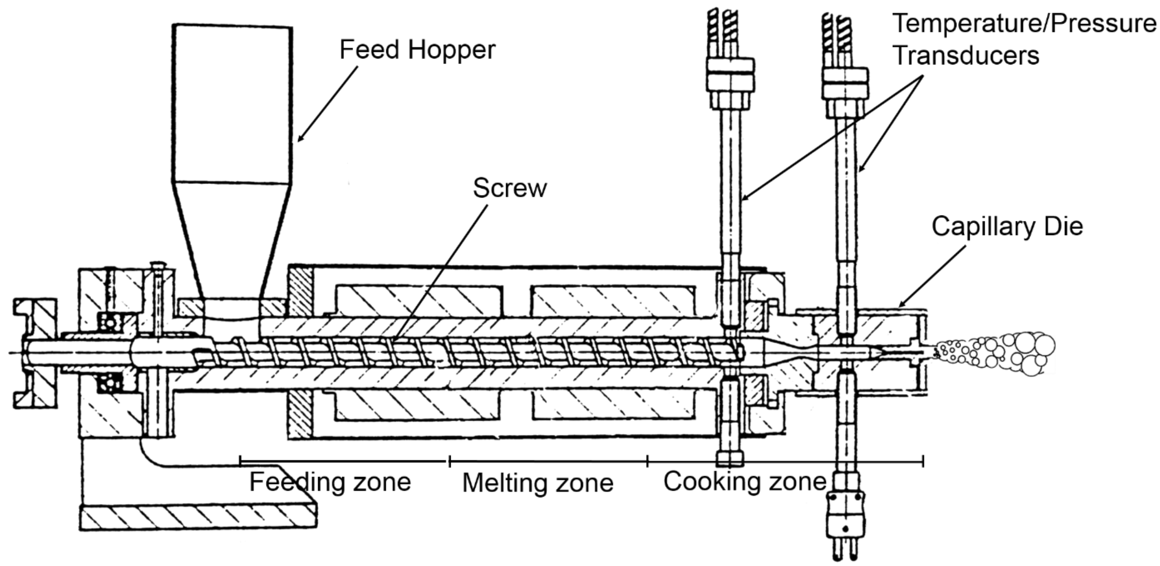

Figure 4.1 Schematic representation of a single screw extruder (Brabender Plasti-Corder, Model PL 2000, South Hackensack, NJ).

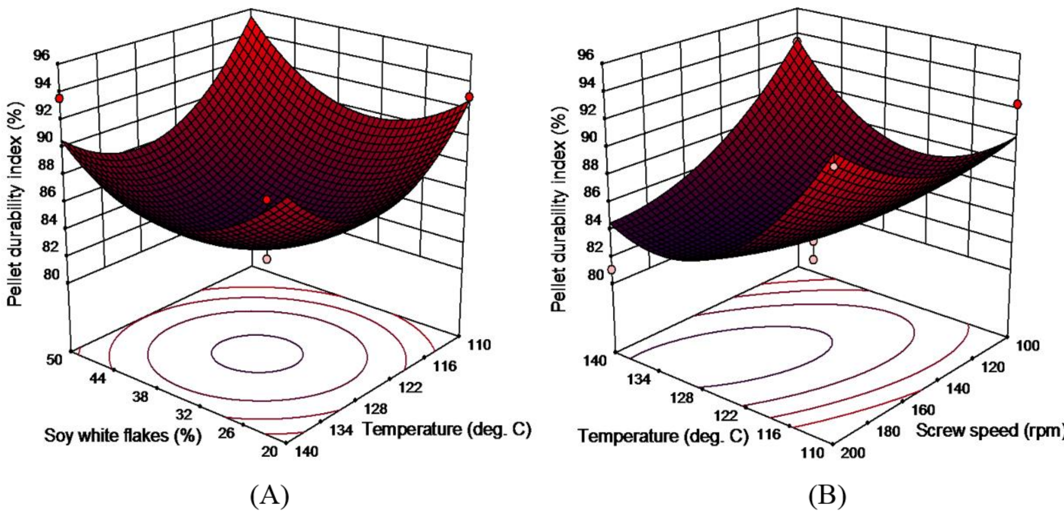

Figure 4.2 Response surface plots of pellet durability index for the effect of (A) soy white flakes content and temperature at 25% moisture content and 150 rpm, and (B) temperature and screw speed at 35% soy white flakes content and 25% moisture content.

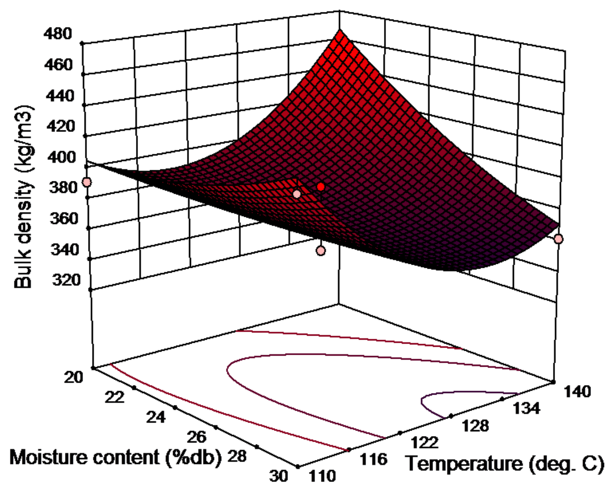

Figure 4.3 Response surface plots of bulk density for the effect of moisture content and temperature at 35% soy white flakes content and 150 rpm.

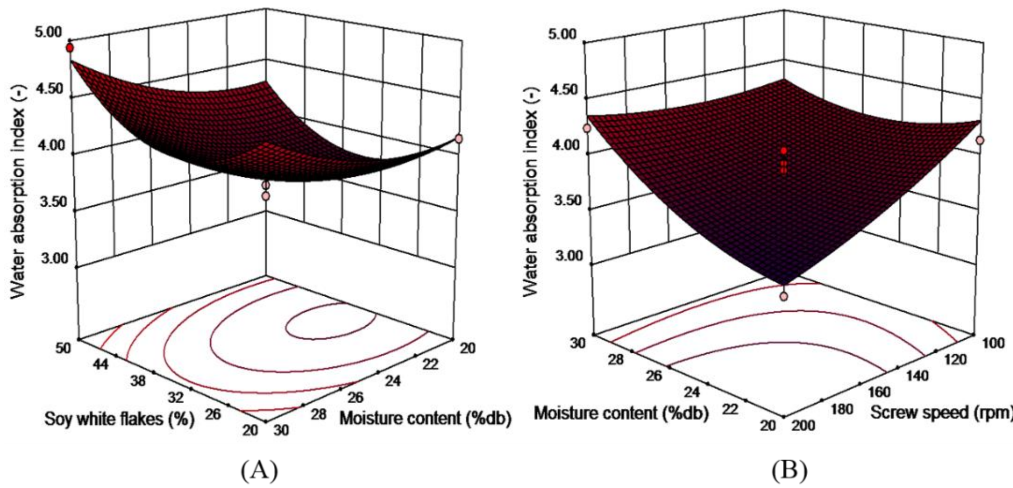

Figure 4.4 Response surface plots of water absorption index for the effect of (A) soy white flakes content and moisture content at 125°C temperature and 150 rpm screw speed, and (B) moisture content and screw speed at 35% level of soy white flakes and 125°C temperature.

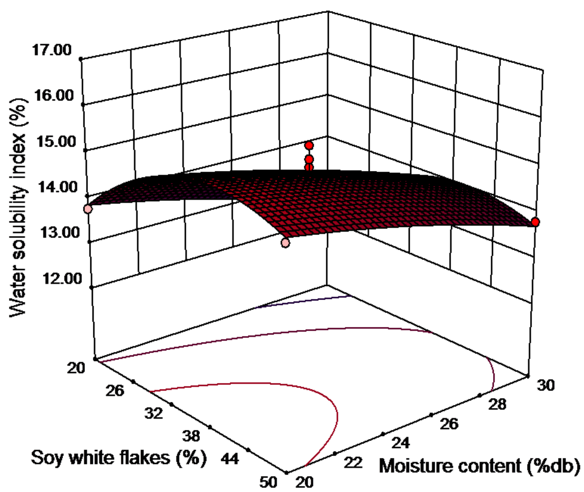

Figure 4.5 Response surface plots of water solubility index for the interaction effect of soy white flakes content and moisture content at 125^{o}C temperature and 150 rpm screw speed.

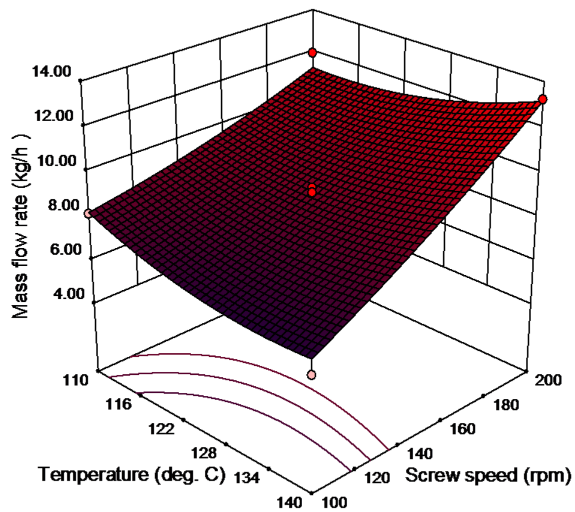

Figure 4.6 Response surface plots of mass flow rate for the interaction effect of temperature and screw speed at 35% soy white flakes content and 25% moisture content.

CHAPTER 5

Modeling and Optimizing the Effect of Extrusion Processing Parameters on Nutritional Properties of Soy White Flakes Based Aquafeed Using Response Surface Methodology

5.1 Abstract

Thermomechanical effects of extrusion cooking leads to physiochemical changes that may be either beneficial or detrimental. The effects of extrusion processing on the nutritional value of food constituents, specifically protein, as well as the ultimate product are ambiguous. During the process, peptide bonds are hydrolyzed and molecular structure of protein changes, which can reduce the nutritional value of the protein and amino acids. Very few studies have focused on the effect of extrusion processing conditions on nutritional value of aquaculture diets. Our objective was to study the effects of inclusion of soy white flakes (up to 50%) and other extrusion processing parameters such as moisture content, barrel temperature and screw speed on the nutritional properties of aquafeed extrudates using a single-screw extruder and to optimize the processing conditions. The extrusion was carried out at three level each of soy white flakes (SWF) content (20%, 35% and 50%), moisture content (20, 25 and 30 % db), barrel temperature (110, 125 and 140ºC) and screw speed (100, 150 and 200 rpm). Response surface regression models were established to correlate the nutritional properties of extrudates to the process variables. Using response surface methodology, extrusion processing was optimized for the maximum values of the analyzed nutritional properties and minimum values of trypsin inhibitor activity (TIA). Extrudate nutritional properties including crude protein, crude fat, crude fiber, ash, lysine content and trypsin inhibitor activity, were

significantly (*P*<0.05) affected by the process variables. SWF was the most significant variable with quadratic effects on most of the properties. As level of SWF content was increased from 20 to 50%, there was an increase in lysine content, TIA, protein content, ash content and decrease in fat and fiber content. Increasing temperature resulted in decrease in lysine content and trypsin inhibitor activity of extrudates. Our study shows that aquafeed with maximum lysine content and minimum trypsin inhibitor activity can be obtained by adding SWF up to 50%.

5.2 Introduction

Aquaculture is one of the fastest growing sector of agriculture and the most rapidly expanding markets for manufacturing fish feeds (Riaz, 1997). Aquaculture feed requires protein content of 26% to 50% (Lovell, 1989) and hence formulated feed contains high amounts of protein. The main protein sources used in aqua feed production is primarily fishmeal which is supplied through the consumption of wild fish stocks. Indubitably, with the increasing rate of farmed fish production (FAO, 2009) and consequent rise in the prices of fishmeal, (Hardy, 2010) the continued use of fishmeal as the main protein source of the feed will no longer be ecologically and economically sustainable in the long run. Soybean products and Distiller Dried Grains (DDGs) contains high amount of protein and are thus a possible alternative source of protein for aquaculture feeds (Kim et al., 1989; Singh and Muthukumarappan, 2014a, b; Wu et al., 1994; Wu et al., 1996). Distillers Dried Grains (DDG) and Distillers Dried Grains with Solubles (DDGS), a co-product from corn-based dry grind fuel ethanol manufacturing, is also a viable protein source. Many researchers have used DDGS as the base protein source in aquafeed formulations and evaluated its effect on the nutritional qualities of the aquafeeds (Coyle et al., 2004; Lim et al., 2007; Lim et al., 2009; Robinson and Li, 2008; Webster et al., 1992; Wu et al., 1996). Mjoun and Rosentrater (2011) used DDGS for the development of good quality extruded aquafeed. Research carried out by Wu et al. (1994); Wu et al. (1996) indicated that tilapia fish grown with DDGS can improve the economic viability of aquaculture farms. Use of soy products like full fatted soybean meal, defatted toasted soybean meal (SBM) and defatted untoasted soybean meal or white flakes (WF) is becoming common (Fallahi et al., 2012; Singh and Muthukumarappan, 2014a, b). Romarheim et al. (2005) found that extrusion of WF diet increased the digestibility of protein and all amino acids whereas extrusion of fishmeal and SBM had no significant effect on amino acid digestibility. Some researchers have explored SBM (Arndt et al., 1999; Arnesen et al., 1989; Fowler, 1980; Krogdahl et al., 1994; Refstie et al., 2000; Refstie et al., 2001; Reinitz, 1980; Vielma et al., 2000). They observed that inclusion of more than 25% SBM as the fish meal (FM) replacer in the fish feed resulted in a significant decrease in fish growth performance. In fact, the lack of some essential amino acids (EAA), such as lysine and methionine (NRC, 1993) and the presence of anti-nutritional factors (ANFs), such as trypsin inhibitors (Arndt et al., 1999; Bureau et al., 1998; Krogdahl et al., 1994) and non-starch polysaccharides (NSPs) (Arnesen et al., 1989) accounted for much of the reduction in growth performance and the low digestibility of the SBM-based diets (Dabrowski et al., 1989; Olli and Krogdahi, 1994).

Extrusion processing is the most common and economic technology used in the aqua-feed industry. It is a very complex process, involving numerous independent variables (such as temperature, moisture content, blend composition, screw speed, die size and configuration, screw configuration, etc.) and dependent variables (such as specific mechanical energy, residence time, torque, pressure, etc.) which provide thermal and shear energy to the extruding materials, inducing chemical and physical changes, and ultimately transferring the state of the materials at both macroscopic and microscopic scale. Quality of the food and feed products are related to both physical and nutritional characteristics of the product. The complexity of the process indicates that there is a strong interrelationship between the effect of extrusion processing on physical and nutritional properties of the feed/food. According to Harper (1981), extrusion cooking can minimize heat degradation of the food nutrients and enhance the digestibility of the major ingredients, mainly protein and starch.

Although extrusion cooking offers several advantages, its effect on nutrient retention in the food product is still a major concern. Protein denaturation, starch gelatinization, lipid oxidation, and Maillard reaction taking place among the ingredients' components are the main reasons for the change in the nutritional qualities of the extrudates (Guy, 2001; Tran et al., 2008). While protein denaturation during the extrusion cooking can boost the protein digestibility, reduce gossypol, and inactivate proteinase inhibitors (Camire, 1991), overheating causes degradation and reduces the bioavailability of the protein and essential amino acids like lysine due to Maillard reaction, and loss of heat-labile vitamins due to exposure to high temperatures (Tran et al., 2008). Since, the dough containing soy white flakes has been subjected to thermal treatment during the production; the overheating of soy white flakes during extrusion processing can be possible which may result in loss of nutrient quality.

In our previous studies, we have explored the effect of SWF inclusion on physical properties of aquafeeds produced by the extrusion cooking process (Singh and Muthukumarappan, 2014a, b). Very few studies have been conducted focusing on the effect of extrusion processing conditions on the nutritional value of aquaculture diets. Optimization of extrusion processing for production of nutritious food has been conducted by some researchers. Study has been conducted on the optimization of extrusion processing for physical properties of aquafeed (Singh and Muthukumarappan, 2015) but not on nutritional properties of aquafeeds. Response surface methodology (RSM) may be employed to optimize critical extrusion processing parameters by estimating the linear, interactive, and quadratic effects. Thus the objectives of this study were 1) to understand the effect of extrusion process parameters on nutritional properties of soy white flakes based extruded diets and 2) to model and optimize the extrusion process parameters for nutritionally high quality feed using response surface method.

5.3 Materials and Methods

Soy white flakes were donated by South Dakota Soybean Processors, Volga, SD. Corn flour was purchased from Cargill Dry Ingredients (Paris, IL). HP-DDG was obtained from the Dakota Ethanol LLC (Wentworth, SD). Corn gluten meal and fishmeal was purchased from Consumer Supply Distributing Co. (Sioux City, IA). Vitamin and mineral premix was obtained from Lortscher Agri Service, Inc. (Bern, Kansas, USA). Soybean oil was obtained from USDA (Brookings, SD).

5.3.1 Feed Blends Formulation

Three isocaloric (304 kcal/100 g) blends were formulated with a net protein content of 32.3% db. The different ingredients in the blends include HP-DDG, soy white flakes, corn gluten meal (CGM), corn flour, fish meal, soybean oil, and vitamin-mineral mix (Table 5.1). The ingredients were mixed in a laboratory scale Hobart mixer (Hobart Corporation, Troy, Ohio, USA) for 10 minutes and stored overnight at ambient temperature for moisture stabilization. The moisture balancing of the blends was done by adding required quantities of water during mixing. The analyzed proximate composition of each blends are given in Table 5.2.

5.3.2 Extrusion Processing

The extrusion processing was performed using a single screw extruder (Brabender Plasti-Corder, Model PL 2000, South Hackensack, NJ) which was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 210 rpm (0 to 22 rad/s). The extruder had a barrel with length-to-diameter ratio of 20:1 and a barrel diameter of 19 mm. A uniform 19.05 mm pitch screw with compression ratio of 3:1 was used in the experiments. The screw had a variable flute depth, with depths at the feed portion of 19.05 mm, and near the die of 3.81 mm. The center of the die assembly was conical, and tapered from an initial diameter of 6.0 mm to an exit diameter of 3 mm, respectively, at the discharge opening. The length and diameter of the die was 17.5 mm and 3 mm (l/d = 5.83), respectively. The extruder barrel was equipped with external band heaters with provisions to control the temperature of all three zones: feed zone, transition zone/melting zone, and die sections. The material gets gelatinized and plasticized under thermal and mechanical stresses generated by the rotation of screws in melting zone of the extruder. The gelatinized material then enters the cooking zone where the extruder barrel is fully filled due to pressure generated at die nozzle. When the process reached the steady state, samples were collected at the die. All samples were stored in a conditioned room prior to further analysis.

5.3.3 Experimental Design and Statistical Analysis

Experiments were conducted using Box-Behnken design which was developed using Design-Expert 8.0.7.1 (Statease, Minneapolis, MN), consisting four numerical independent variables namely soy white flakes (X_1), moisture content (X_2), temperature (X_3) and screw speed (X_4) each at three levels as shown in Table 5.3. Pellet durability index, bulk density, water absorption and solubility indices and mass flow rate of the extrudates were measured as the response/dependent variables. All measurements were done in triplicate. Using Equation (**5.1**, the numerical independent variables in actual form (X_1, X_2) were converted to their coded form (x_1, x_2).

$$x_i = \frac{(X_i - X_o)}{\Delta X} \tag{5.1}$$

where x_i is the dimensionless coded value of the ith independent variable, and Xi, X_0, and ΔX correspond to the actual value, actual value at the center point, and the step change of the ith variable, respectively.

Twenty-nine experiments were performed in randomized order including five replications at the design center to obtain an accurate estimation of the experimental error (Table 5.4). The lysine content (Y_{Lys}), trypsin inhibitor activity (Y_{TIA}), crude protein (Y_{CP}), crude fat (Y_{CF}) crude fiber (Y_{CFb}) and ash content (Y_{Ash}) were taken as the six responses of the designed experiments. A second order polynomial equation was used to describe the effect of the independent variables in terms of linear, quadratic and their interactions on the dependent variables as given by Equation (**5.2**.

$$Y_i = b_o + \sum_{i=1}^{4} b_i X_i + \sum_{i=1}^{4} b_{ii} X_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} X_i X_j + \varepsilon \quad (5.2)$$

where Y_i is the predicted response; b_0 is the interception coefficient; b_i, b_{ii}, and b_{ij} are coefficients of the linear, quadratic, and interaction terms; ε is the random error; and X_i is the independent variables studied. 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) presented in Table 5.5, Table 5.6 and Table 5.7. 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 F-test (Table 5.8 and Table 5.9). 3D response surfaces were used to visualize the interactive effects of the independent variables.

5.3.4 Measurement of Nutritional Properties

5.3.4.1 Nutrient Analysis

The extrudates were air dried and protein, fiber, fat, ash content were determined following AOAC official Methods 990.03, 978.10, 920.39, 920.48 respectively (AOAC International, 2012). Nitrogen free extracts (NFE) was determined as the percentage weight of sample after deducting moisture, protein, fat, fiber, and ash content. The protein, fat and ash content were measured in triplicate for all the treatments.

5.3.4.2 Lysine content

The lysine content of the un-extruded and extruded samples was determined according to the method described in AOAC Official Method 994.12 (AOAC International, 2012). The method includes 3 main steps of acid hydrolysis using HCl and separation of amino acid using High Performance Liquid Chromatography (Agilent

Technologies 1260) and protein post column derivatization of amino acids using ninhydrin reagent. A 5 µm, 4 .6 x 110 mm Na amino acid cation exchange column was used for separation of lysine. The operating conditions used for the separation were: a) injector volume: 10 µL, b) mobile phase flow rate: 0.6mL/min, c) run time: 40 min. The operating conditions used for the post column derivatization using ninhydrin reagent were a) flow rate: 0.30 mL/min, b) runtime: 40 min. The lysine content in the samples are detected by using multichannel wavelength scanning UV/vis detector at wavelength of 570 nm.

5.3.4.3 Trypsin Inhibitor Activity (TIA)

The trypsin inhibitor activity was determined according to the differential absorbance method described in (ISO, 2001).

5.3.4.3.1 Extraction of the sample

The extrudates were ground to fine powder with a grinder and sieved through 500µm. About 1±0.001g of the ground sample was extracted with 50 ml of sodium hydroxide solution of 0.01mol/l (the pH was adjusted to 9.5±0.1) for overnight (15 to 24h). The clear sample extract was diluted to appropriate dilutions range with water so that 1ml sample could inhibit 40-60% of the trypsin used as standard based on expected TIA.

5.3.4.3.2 Procedure

For each dilution of sample extract, a corresponding blank solution was prepared. 5 ml of L-BAPA was added in each tubes. 1ml of diluted sample extract followed by 2 ml of water was added in the blank sample and sample solution. 1 ml of acetic acid was added to the blank sample solution only and all the centrifuge tubes were vortexed to mix the contents thoroughly and thereafter placed in water bath at 37°C for 10min. Then 1ml of trypsin solution was added to all the tubes and placed in water bath at 37°C. The reaction was terminated exactly after 10min±5s by addition of 1ml acetic acid to centrifuge tubes containing standard and sample. A corresponding blank standard and standard was also prepared similar to blank sample and sample respectively. Diluted sample extract was not added in blank standard and standard solution and 3 ml of water was added instead of 2 ml. The absorbance of the clear solutions relative to water was measured at 410nm in a 10mm cuvette.

5.3.4.3.3 Calculation of TIA

The inhibition percentage of the sample extracts solutions by the equation:

$$i = \frac{(A_r - A_{br}) - (A_s - A_{bs})}{(A_r - A_{br})} \times 100\% \tag{5.3}$$

where i is the inhibition percentage, in percent, A_r is the absorbance of the solution with standard, A_{br} is the absorbance of the blank with standard, A_s is the absorbance of the solution with sample;

A_{bs} is the absorbance of the blank with sample. The TIA activity, expressed in mg of trypsin inhibited per g of sample was calculated as:

$$TIA = \frac{i \times m_1 f_1 f_2}{100\% \times m_o} \tag{5.4}$$

where TIA is the trypsin inhibitor activity, in milligrams per gram, i is the inhibition percentage, in percent, m_0 is the mass of the test sample, in grams, m_1 is the mass of trypsin, in milligrams, f_1 is the dilution of the sample extract in milliliters, f_2 is a conversion factor (2.8×10^{-4}) based on the purity and dilution of trypsin. Trypsin inhibitor activity in blends is given in Table 5.2.

5.4 Results and Discussion

5.4.1 Nutrient Content

The results for crude protein, fat, fiber, ash and NFE of extruded samples obtained from this experiment ranged from 28.08 to 36.00%, 0.83 to 2.59%, 2.01 to 5.40% and 5.33 6.55% respectively. The multiple regression models for predicting the protein, fat, fiber, ash values showed 92, 60, 85 and 98% and a non-significant *F*-value of 67.47, 8.99, 5.88 and 38.96 as lack of fit respectively (Table 5.9). The variable which showed significant influence on protein content and fat content is the levels of SWF content (P<0.05) (Table 5.5). The regression analysis results (Table 5.5) indicated that the linear terms of SWF content had a significant (P<0.05) positive effect on protein content and negative effect on fat content of extrudates. However, interaction between the independent variables and quadratics terms had no significant effect. The response surface plots for protein content in extrudates in shown in Figure 5.1

ANOVA showed that SWF content and screw speed had a significant effect (P<0.05) but moisture content and temperature as an independent variable had no significant effect on fiber content in all the extrudates (Table 5.6). However, the interaction effect of moisture and temperature had significant effect on fiber content. The response graph shows that fiber content decreased with increasing levels of SWF content and screw speed (Figure 5.2).

The response surface plot generated for ash content in extrudates is shown in Figure 5.3. The surface shows that with increase in levels of moisture content and SWF content, ash content in all the extrudates was increased. Table 5.6 shows that there is no significant effect of temperature and screw speed on ash content.

The regression equation for the relationship between nutrient content (crude protein, crude fat, crude fiber and ash) and independent variables in terms of coded and actual form is shown in Table 5.8 and Table 5.9 respectively.

5.4.2 Lysine Content

Lysine is one of the essential amino acids and its availability plays an important role in the quality of feed stuffs. Excessive thermal processing can destroy the amino acid chains of the protein molecules and thus modify the functionality. The most important lysine modification occurs during Maillard reaction. Loss of lysine availability due to Maillard reaction has been studied extensively (Asp and Björck, 1989; Björck and Asp, 1983; Cheftel, 1986). Loss of lysine during the extrusion processing can be related to extrusion temperature, screw speed, die diameter, feed moisture content, and feed ingredients such as starch and protein etc. (Asp and Björck, 1989; Camire et al., 1990).

The regression model for predicting lysine content could explain 86% of the observed variations and a non-significant *F*-value of 6.32 as lack-of-fit. The regression equation for the relationship between lysine content and independent variables in terms of coded and actual form is shown in Table 5.8 and Table 5.9 respectively. The ANOVA Table 5.7 shows that the linear and quadratic term of SWF content are significant (P<0.05). Also, moisture content and temperature as an independent variable had a significant effect (P<0.05) on lysine content, however, their interaction and quadratic terms had no significant effect (Table 5.7).

The lysine content of the raw blends was 5.6%, 6.00%, and 7.10% for blend I, blend II and blend III respectively (Table 5.2). The lysine content of the extrudates increased with increase in SWF content (Figure 5.4a). It appeared that for all the extrudates, as the processing temperature increased from 110°C to 140°C, lysine content decreased significantly (Figure 5.4a). A similar finding was observed by Skrede and Krogdahl (1985). Also, as moisture content is increased from 20 to 30%, there was a decrease in lysine content (Figure 5.4b). The results were not in agreement with the findings of Bjorck et al. (1983); Noguchi et al. (1982), who reported that increase in moisture content resulted in increase in lysine content.

5.4.3 Trypsin Inhibitor Activity

Soybean contains heat labile anti-nutrient which affects the activity of protease enzymes in animals. The inclusion of soy product in aquafeed poses challenge due to the presence of protease inhibitors (trypsin inhibitors). Soybean protease inhibitors cause reduced protein digestibility (Laskowski and Kato, 1980) and loss of energy and protein (Nitsan and Liener, 1976; Olli et al., 1994a; Skrede and Krogdahl, 1985). The protease inhibitors in soy may be readily inactivated or destroyed during feed processing that involves heat, although the efficiency of inhibition depends on temperature, moisture and residence time in the extruder (Björck and Asp, 1983; Clarke E and Wiseman, 1999; Zarkadas and Wiseman, 2005).

The trypsin inhibitor activity of the blends containing 20 to 50% soy white flakes extruded at different temperature was analyzed. The TIA was highest in the un-extruded diet. Extrusion reduced TIA in all diets and the maximum reduction achieved in the extrudates was approximately 40%. Changing the level of soy white flakes content and processing temperature from 110°C to 140°C had a significant effect on TIA (Table 5.7). The response surface plot presented in Figure 5.5 shows that lower TIA was achieved when temperature was increased. Also, TIA of the extrudates is decreased when levels of

SWF content was decreased from 50 to 20%. Soy products containing less than 5mg/g (mg bovine trypsin inhibited/g feed) are thought to have been sufficiently heated for rainbow trout and Atlantic salmon (Barrows et al., 2007; Olli et al., 1994a). All the extrudates analyzed had lower TIA than 5mg/g of sample which is indication of adequate heat processing of soy product included in the blend (Figure 5.5).

ANOVA showed that moisture content and screw speed had no significant effect on trypsin inhibitor activity and hence response surface plots of interaction effect involving soy white flakes, moisture content and screw speed are not shown. The regression equation for trypsin inhibitor activity in coded and actual form is shown in Table 5.8 and Table 5.9 respectively.

5.4.4 Optimization and validation

Numerical optimization was carried out in Design Expert v8 considering all seven dependent variables. The optimization resulted in 63 solutions and the top 12 solutions are shown in Table 5.10. Validation of the predicted responses was carried out by extruding the blends at three different optimum conditions as shown in Table 5.10. Good agreement was found between the predicted and experimental values. The experimental values for solution #11 were very close to the predicted values. The optimum condition was: 47.55% SWF, 20.01% moisture content, 140°C barrel temperature and 100 rpm screw speed.

5.5 Conclusions

Experiments were conducted in a single screw extruder to model and optimize the effect of extrusion processing parameters on nutritional properties of extrudates. Response surface methodology revealed the significant effects of soy white flakes, moisture content, barrel temperature on the nutritional properties of extruded aquafeed. The effects of soy white flakes on most of the properties of the extrudates were found to be quadratic; the effects were linear for crude fat and crude fiber content. As level of SWF content was increased from 20 to 50%, there was an increase in lysine content, TIA, protein content, ash content and decrease fat and fiber content. Moisture had a linear effect on lysine and ash content. Temperature had a linear effect on lysine content and trypsin inhibitor activity, where increasing the barrel temperature from 110°C to 140°C decreased the lysine content and TIA in the extrudates. Further reduction of TIA content, beyond the levels obtained in the present study, could probably be achieved by using more severe extrusion conditions, but the chemical composition and nutritive value could then have been negatively affected. Screw speed as an independent variable had no significant effect on any of the properties studied. However, interactive effects of screw speed, moisture and temperature had significant effect on fiber content. This study will be useful in identifying desirable operating conditions for the production of extruded aqua feed using soy white flakes as an alternative protein source.

Table 5.1 Ingredient composition of blends

Feed ingredients	**Mass of ingredients (g kg^{-1} dry matter)**		
	Blend I	**Blend II**	**Blend III**
Soy white flakes	200	350	500
HP-DDG	400	250	100
Corn flour	300	300	300
Fish meal	50	50	50
CGM	20	20	20
Vitamin & mineral mix	20	20	20
Soybean oil	10	10	10

†HP-DDG – High protein distiller's dried grains; CGM – Corn gluten meal

Table 5.2 Analyzed proximate composition, Lysine and TIA of feed blends

Chemical composition	**Blend I**	**Blend II**	**Blend III**
Crude protein (% DM)	33.15	34.45	35.74
Crude Fat (% DM)	4.94	4.31	3.69
Crude Fiber (% DM)	3.75	3.24	2.74
Ash (% DM)	5.54	5.80	6.07
NFE (% DM)	52.63	52.19	51.76
Lysine (% protein)	5.60	6.00	7.10
TIA (mg/g)	4.20	4.40	4.50

†DM – Dry matter; NFE – Nitrogen free extract; TIA – Trypsin inhibitor activity

Table 5.3 Independent numerical variables and their levels

Numerical variable	**Symbol**	**Coded variable levels**		
		-1	0	1
Soy white flakes (%)	X_1	20	35	50
Moisture content (%db)	X_2	20	25	30
Temperature (°C)	X_3	110	125	140
Screw speed (rpm)	X_4	100	150	200

Table 5.4 Experimental design layout

Run	**Coded variable**				**Actual Variable**			
	x_1	x_2	x_3	x_4	X_1 (%)	X_2 (%db)	X_3 (°C)	X_4 (rpm)
1	0	0	0	0	35	25	125	150
2	-1	0	0	-1	20	25	125	100
3	-1	-1	0	0	20	20	125	150
4	1	0	-1	0	50	25	110	150
5	0	0	1	1	35	25	140	200
6	0	0	-1	1	35	25	110	200
7	1	0	1	0	50	25	140	150
8	0	-1	0	1	35	20	125	200
9	0	1	1	0	35	30	140	150
10	1	1	0	0	50	30	125	150
11	0	1	0	-1	35	30	125	100
13	-1	0	0	1	20	25	125	200
14	0	0	0	0	35	25	125	150
15	0	-1	1	0	35	20	140	150
16	0	0	0	0	35	25	125	150
17	0	0	-1	-1	35	25	110	100
18	1	0	0	-1	50	25	125	100
19	-1	0	1	0	20	25	140	150
20	0	-1	-1	0	35	20	110	150
21	0	0	1	-1	35	25	140	100
22	-1	0	-1	0	20	25	110	150
23	0	0	0	0	35	25	125	150
24	1	0	0	1	50	25	125	200
25	0	-1	0	-1	35	20	125	100
26	-1	1	0	0	20	30	125	150
27	0	1	0	1	35	30	125	200
28	1	-1	0	0	50	20	125	150
29	0	1	-1	0	35	30	110	150

Table 5.5 Analysis of variance (ANOVA) for Crude Protein, Crude Fat

		Crude Protein				**Crude Fat**			
Source	df	SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value
Model	4	124.80	31.20	67.47	< 0.0001	4.13	1.03	8.99	0.0001
X_1	1	124.72	124.72	269.71	< 0.0001	3.59	3.59	31.19	< 0.0001
X_2	1	0.03	0.03	0.06	0.8162	0.12	0.12	1.06	0.3127
X_3	1	0.03	0.03	0.05	0.8180	0.02	0.02	0.17	0.6802
X_4	1	0.02	0.02	0.05	0.8289	0.40	0.40	3.51	0.0732
Residual	24	11.10	0.46	-	-	2.76	0.12	-	-
Lack of Fit	20	7.30	0.36	0.38	0.9331	1.77	0.09	0.36	0.9453
Pure Error	4	3.80	0.95	-	-	0.99	0.25	-	-

[†]df , degrees of freedom; SS, sum of squares; MS, mean square.

Table 5.6 Analysis of variance (ANOVA) for Crude Fiber and Ash

		Crude Fiber				**Ash**			
Source	df	SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value
Model	14	12.97	0.93	5.88	0.0010	2.43	0.17	38.96	< 0.0001
X_1	1	3.17	3.17	20.14	0.0005	2.16	2.16	485.13	< 0.0001
X_2	1	0.06	0.06	0.37	0.5526	0.05	0.05	11.80	0.0040
X_3	1	0.02	0.02	0.14	0.7138	0.00	0.00	0.29	0.6003
X_4	1	1.59	1.59	10.10	0.0067	0.00	0.00	0.17	0.6843
X_1^2	1	0.62	0.62	3.93	0.0674	0.12	0.12	26.36	0.0002
X_2^2	1	0.00	0.00	0.02	0.8820	0.00	0.00	0.59	0.4568
X_3^2	1	2.29	2.29	14.54	0.0019	0.00	0.00	0.06	0.8136
X_4^2	1	0.00	0.00	0.03	0.8747	0.00	0.00	0.38	0.5473
X_1X_2	1	0.14	0.14	0.89	0.3625	0.07	0.07	16.16	0.0013
X_1X_3	1	0.81	0.81	5.11	0.0402	0.00	0.00	0.02	0.8848
X_1X_4	1	0.89	0.89	5.66	0.0321	0.00	0.00	0.01	0.9314
X_2X_3	1	1.21	1.21	7.66	0.0151	0.01	0.01	1.45	0.2491
X_2X_4	1	0.45	0.45	2.86	0.1129	0.00	0.00	0.00	0.9980
X_3X_4	1	1.91	1.91	12.10	0.0037	0.00	0.00	0.41	0.5306
Residual	14	2.21	0.16	-	-	0.06	0.00	-	-
Lack of Fit	10	1.54	0.15	0.91	0.5888	0.06	0.01	12.96	0.0124
Pure Error	4	0.67	0.17	-	-	0.00	0.00	-	-

[†]df , degrees of freedom; SS, sum of squares; MS, mean square.

Table 5.7 Analysis of variance (ANOVA) for Lysine content and TIA

		Lysine				**TIA**			
Source	df	SS	MS	*F*-value	*P*-value	SS	MS	*F*-value	*P*-value
Model	14	4.63	0.33	6.32	0.0007	3.03	0.22	5.15	0.0021
X_1	1	2.54	2.54	48.62	< 0.0001	1.29	1.29	30.63	< 0.0001
X_2	1	0.43	0.43	8.18	0.0126	0.00	0.00	0.10	0.7600
X_3	1	0.26	0.26	5.03	0.0417	0.63	0.63	14.92	0.0017
X_4	1	0.05	0.05	0.86	0.3701	0.16	0.16	3.90	0.0684
X_1^2	1	1.17	1.17	22.32	0.0003	0.72	0.72	17.11	0.0010
X_2^2	1	0.08	0.08	1.55	0.2340	0.03	0.03	0.66	0.4310
X_3^2	1	0.04	0.04	0.76	0.3976	0.00	0.00	0.11	0.7490
X_4^2	1	0.12	0.12	2.26	0.1549	0.05	0.05	1.21	0.2908
X_1X_2	1	0.01	0.01	0.17	0.6821	0.00	0.00	0.09	0.7668
X_1X_3	1	0.01	0.01	0.16	0.6977	0.02	0.02	0.52	0.4817
X_1X_4	1	0.07	0.07	1.35	0.2652	0.00	0.00	0.02	0.9032
X_2X_3	1	0.00	0.00	0.08	0.7778	0.06	0.06	1.38	0.2598
X_2X_4	1	0.05	0.05	0.88	0.3648	0.01	0.01	0.25	0.6258
X_3X_4	1	0.02	0.02	0.32	0.5789	0.12	0.12	2.77	0.1183
Residual	14	0.73	0.05	-	-	0.59	0.04	-	-
Lack of Fit	10	0.64	0.06	2.80	0.1664	0.50	0.05	2.16	0.2379
Pure Error	4	0.09	0.02	-	-	0.09	0.02	-	-

[†]df , degrees of freedom; SS, sum of squares; MS, mean square, TIA – Trypsin Inhibitor activity

Table 5.8 Final equations in terms of coded factors after excluding the insignificant terms for Lysine, TIA, crude protein, crude fat, crude fiber and ash content

Coded model equation	R²	Adj R²	Pred R²	Adeq precision
$Y_{Lys} = 4.98 + 0.46x_1 - 0.19x_2 - 0.15x_3 + 0.42x_1^2$	0.86	0.73	0.28	8.01
$Y_{TIA} = 3.79 + 0.33x_1 - 0.23x_3 - 0.33x_1^2$	0.84	0.67	0.17	8.58
$Y_{CP} = 32.22 + 3.22x_1$	0.92	0.90	0.89	23.16
$Y_{CF} = 1.75 - 0.55x_1$	0.60	0.53	0.44	10.37
$Y_{CFb} = 4.26 - 0.51x_1 - 0.36x_4 - 0.45x_1x_3 + 0.47x_1x_4 - 0.55x_2x_3 + 0.69x_3x_4 - 0.59x_3^2$	0.85	0.71	0.35	10.25
$Y_{Ash} = 5.64 + 0.42x_1 + 0.07x_2 + 0.13x_1x_2 + 0.13x_1^2$	0.98	0.95	0.86	23.27

Table 5.9 Best-fit response surface models for extrudate nutritional properties

Response surface model	**R²**	**Std. deviation**	***F*-statistic**	***P*-value**
$Y_{Lys} = 13.575 - 0.116X_1 - 0.164X_2 - 0.080X_3 + 0.0018X_1^2$	0.86	0.23	6.32	0.0007
$Y_{TIA} = 8.841 + 0.076X_1 - 0.072X_3 - 0.0015X_1^2$	0.84	0.21	5.15	0.0021
$Y_{CP} = 24.220 + 0.215X_1$	0.92	0.68	67.47	< 0.0001
$Y_{CF} = 1.628 - 0.036X_1$	0.60	0.34	8.99	0.0001
$Y_{CFb} = -45.39 + 0.28X_1 - 0.17X_4 - 0.002X_1X_3 + 0.0006X_1X_4 - 0.0073X_2X_3 + 0.0009X_3X_4 - 0.003X_3^2$	0.85	0.40	5.88	0.0010
$Y_{Ash} = 4.362 - 0.056X_1 - 0.022X_2 + 0.0017X_1X_2 + 0.0006X_1^2$	0.98	0.07	38.96	< 0.0001

Table 5.10 Solutions for optimal conditions and validation

Solution #	SWF (%)	MC (%db)	T (°C)	SS (rpm)	Lysine (% protein)	TIA (mg/g)	Crude Protein (%db)	Crude Fat (%db)	Crude Fiber (%db)	Ash (% db)
1	50.00	20.00	140	100	6.30	3.0	35.49	0.96	2.51	6.05
2	50.00	20.10	140	100	6.29	3.0	35.49	0.96	2.49	6.05
3	50.00	20.00	140	101	6.29	3.0	35.49	0.96	2.52	6.05
4	50.00	20.00	140	103	6.28	3.0	35.49	0.97	2.54	6.05
5	50.00	20.46	140	100	6.27	3.0	35.50	0.97	2.42	6.06
6	49.79	20.00	139	100	6.27	3.0	35.44	0.96	2.66	6.04
7	50.00	20.00	140	105	6.27	3.0	35.49	0.98	2.56	6.05
8	48.50	20.00	140	100	6.15	3.0	35.17	1.01	2.73	6.00
9	50.00	20.00	140	115	6.22	3.1	35.48	1.01	2.66	6.05
10	50.00	20.00	135	100	6.25	3.2	35.48	0.94	3.09	6.04
11	47.55	20.01	140	100	6.07	3.0	34.96	1.05	2.81	5.96
12	50.00	24.29	140	100	6.13	3.2	35.53	1.04	1.67	6.17
Validation										
9	50.00	20.00	140	115	6.15	3.2	34.40	0.95	3.10	6.25
10	50.00	20.00	135	100	6.30	3.3	35.00	0.94	2.91	5.89
11	47.55	20.01	140	100	6.10	2.9	34.91	1.10	2.85	6.00
12	50.00	24.29	140	100	6.08	3.1	34.20	0.92	2.60	6.01

[†]SWF, soy white flakes; MC, moisture content of blends; T, temperature; SS, screw speed; TIA, trypsin inhibitor activity; NFE, nitrogen free extract

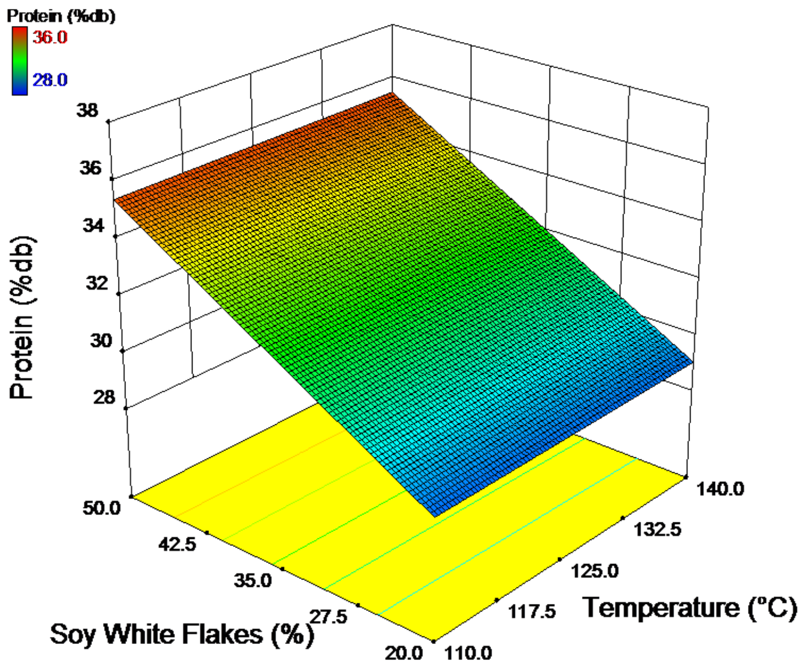

Figure 5.1 Response surface plot of crude protein content in extrudates for the interaction effect of soy white flakes content and temperature at 25% moisture content and 150 rpm screw speed.

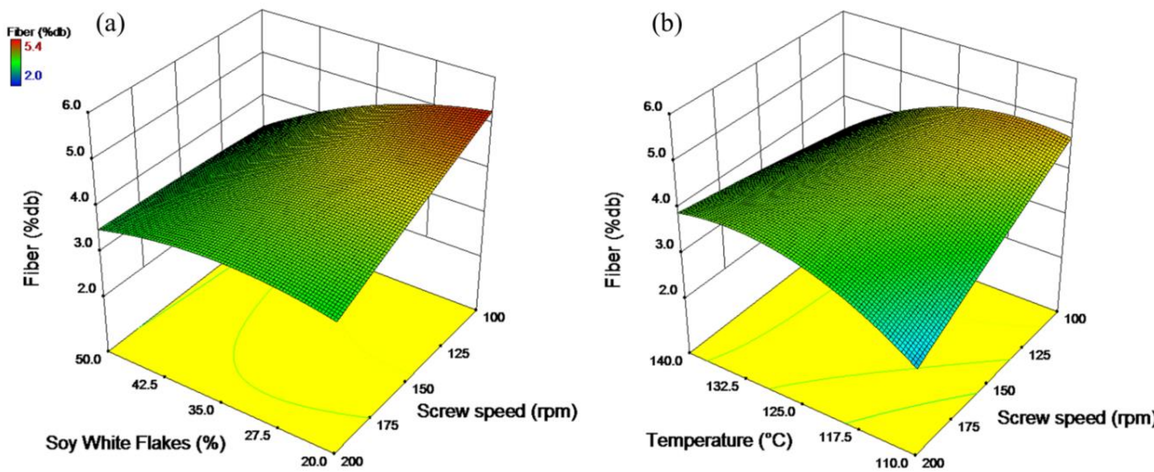

Figure 5.2 Response surface plots of crude fiber content in extrudates for the effect of (a) soy white flakes content and screw speed at 25% moisture content and 125°C, and (b) temperature and screw speed at 35% soy white flakes content and 25% moisture content.

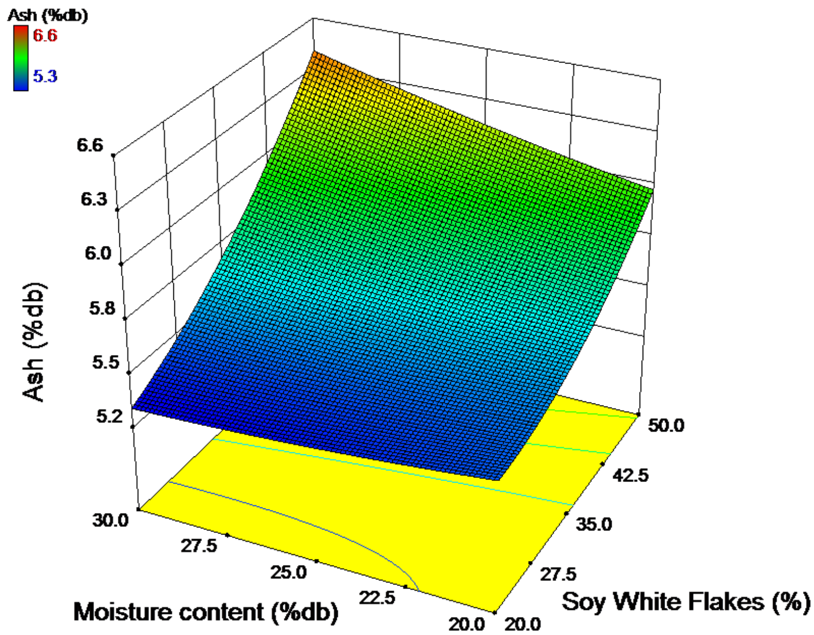

Figure 5.3 Response surface plot of ash content in extrudates for the interaction effect of moisture content and soy white flakes content 125°C temperature and 150 rpm screw speed.

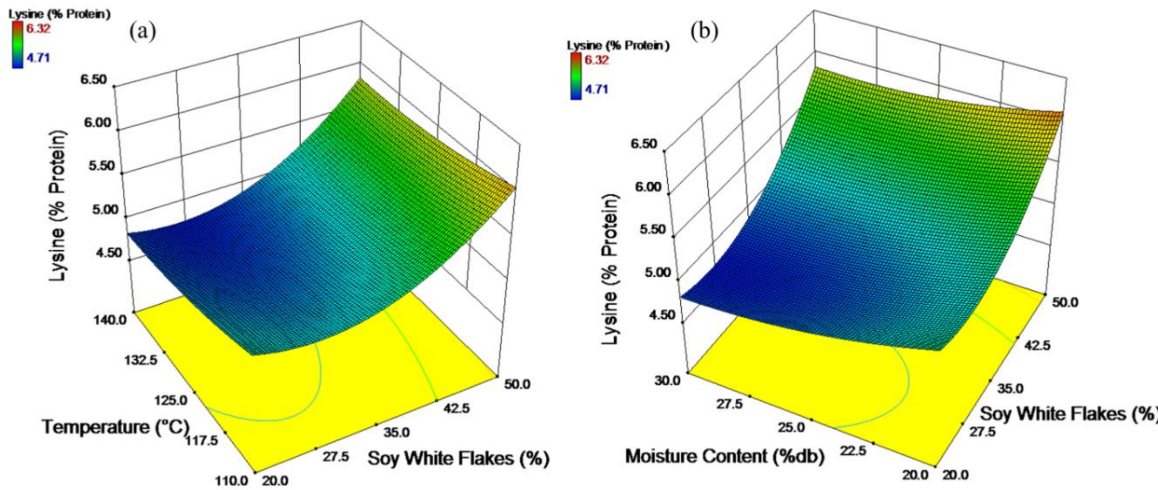

Figure 5.4 Response surface plots of lysine content in extrudates for the effect of (a) temperature and soy white flakes content at 25% moisture content and 150 rpm and (b) moisture content and soy white flakes content at 125°C and 150 rpm.

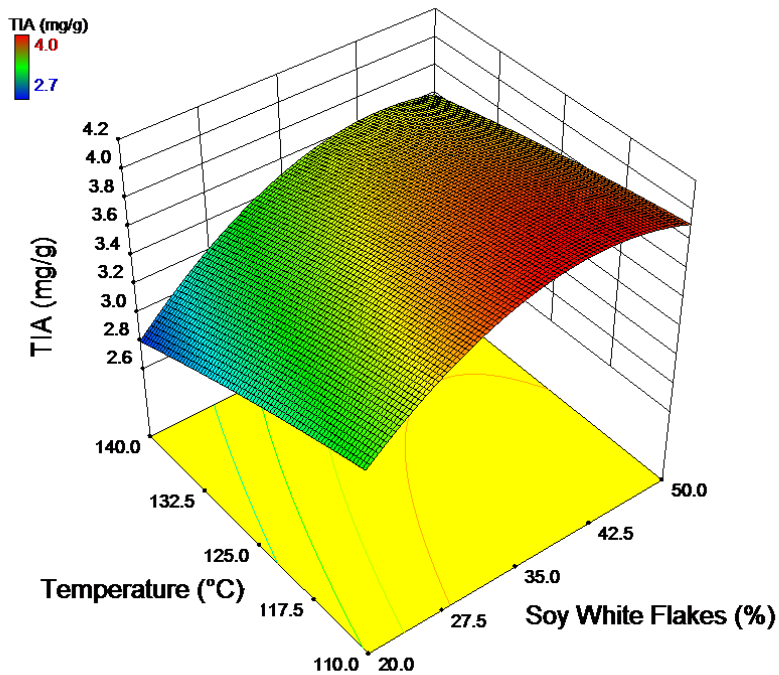

Figure 5.5 Response surface plot of trypsin inhibitor activity for the interaction effect of temperature and soy white flakes content at 25% moisture content and 150 rpm screw speed.

CHAPTER 6 [†]

A Viscosity Model for Soy White Flakes Based Aquafeed Dough in a Single Screw Extruder

6.1 Abstract

A viscosity model was developed to study the influence of soy white flakes during extrusion of fish feed and the effect of extrusion processing parameters on mass flow rate, torque, specific mechanical energy and viscosity of dough inside a single screw extruder were evaluated. Full factorial design was used for three variables each at three levels which generated total 27 experimental runs. Round capillary die rheometer was used to determine the rheological data of the dough within the extruder. It was observed that increase in the soy white flakes content resulted in a higher mass flow rate, torque and specific mechanical energy and a decrease in the apparent viscosity. The specific mechanical energy, mass flow rate increased and viscosity decreased with increase in screw speed. Higher barrel and die temperature led to decrease in the apparent viscosity of the dough, torque and specific mechanical energy. The viscosity model developed in this study can be applied to develop large-scale extrusion models to determine the effect of soy white flakes on the feed material extrudates.

6.2 Introduction

Feed and food extrusion cooking is a unit operation in which various products are produced using cereals, oilseeds or other carbohydrate mixtures. Fish feed is one of these extruded products. Extrusion cooking is a high-temperature short-time process in which final product is obtained by a combination of several unit operations such as heating,

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[†] Singh, S.K. and Muthukumarappan, K., 2016. A viscosity model for soy white flakes based aquafeed dough in a single screw extruder. *Journal of Food Process Engineering.* DOI: 10.1111/jfpe.12357

mixing, shearing and forcing the material through a die in a single step. Soy white flakes (SWF) contains high quantity of protein (42%) and previous laboratory experiments with soy white flakes have yielded extrudates with physical properties that were found to be suitable for aquaculture feeds (Singh and Muthukumarappan, 2014a, b; Singh and Muthukumarappan, 2015). However, for consistent large scale production of aquaculture feeds using soy white flakes as a protein source, a thorough understanding regarding the changes occurring inside the extruder is necessary.

During extrusion, the rate and extent of cooking, mixing, shearing and compressing processes are strongly related to the rheological properties of the raw materials. Viscosity is one of the most important rheological properties of non-Newtonian biological materials and is often used for continuous online control of product characteristics (Chen et al., 1978; Lam and Flores, 2003). The viscosity of the dough inside extruder barrels has been studied by many authors. For example, the viscosity of dough of different grains including wheat, corn, and soybean were measured using straight tube viscometer (Harper et al., 1971), cylindrical dies of different lengths (Harmann and Harper, 1974), capillary die (Jao et al., 1978), viscoamylograph (Remsen and Clark, 1978), and capillary rheometer (Luxenburg et al., 1985) attached to food extruders, and viscosity models have been developed. Even though a number of models exists, no single model has won universal acceptance. Harper et al. (1971) developed a viscosity model equation for biological materials as:

$$\eta = K\dot{\gamma}^{n-1}\exp(b_1 / T) \tag{6.1}$$

where η is the apparent viscosity of the dough inside the barrel (Pa·s), *K* is a consistency factor (Pa.s^n), $\dot{\gamma}$ is the shear rate (1/s), n is the flow behavior index (dimensionless), T is the product temperature at the barrel (K), b_1 is an empirical constant for temperature (K). Many researchers used this same model to study the viscosity of dough in extruders (Altomare et al., 1992; Bhattacharya and Hanna, 1986; Harmann and Harper, 1974; Jao et al., 1978; Luxenburg et al., 1985; Remsen and Clark, 1978). The previous researchers mainly focused on the effects of shear rate, temperature, moisture content, time-temperature history, strain history, and starch gelatinization on viscosity. Not many models show the effect of ingredient levels on viscosity, although the influence of ingredient levels on the final properties of extrudate has been recognized. Lam and Flores (2003) introduced a correction factor for the particle size distribution of the raw ingredients in the viscosity model of dough inside the barrel of a single screw extruder. To date, however, no study has been attempted to quantify the viscosity of dough containing relatively high concentrations of soy white flakes.

The objectives of this study were (a) to develop a viscosity model as a function of shear rate, temperature and level of SWF, and (b) to study the effect of SWF level, screw speed, and temperature profile on extrusion processing parameters including mass flow rate, torque, specific mechanical energy and viscosity of the dough.

6.3 Materials and Methods

6.3.1 Raw materials and feed formulation

Soy white flakes were donated by South Dakota Soybean Processors (Volga, SD). Corn flour was purchased from Cargill Dry Ingredients (Paris, IL). Dried distiller's grains with solubles (DDGS) were obtained from POET (Sioux Falls, SD). Corn gluten meal and fishmeal was purchased from Consumer Supply Distributing Co. (Sioux City, IA). Vitamin and mineral premix was obtained from Lortscher Agri Service, Inc. (Bern, Kansas, USA). Soybean oil was obtained from USDA (Brookings, SD). Three iso-nitrogenous blends (hereafter referred to as “dough”) were formulated with a net protein content of 32.7% db. The different ingredients in the dough include soy white flakes, DDGS, corn gluten meal (CGM), corn flour, fish meal, soybean oil, and vitamin-mineral mix (Table 6.1). The ingredients were mixed in a laboratory scale Hobart mixer (Hobart Corporation, Troy, Ohio, USA) for 10 minutes and stored overnight at ambient temperature for moisture stabilization. The moisture balancing of the doughs was kept constant at 25% wb and was done by adding required quantities of water during mixing. Before extrusion, moisture content of doughs were determined using the method 44-15A (AACC, 2000). The proximate composition of the feed blends is represented in Table 6.2.

6.3.2 Experimental design

Experiments were conducted using a full factorial design with three variables. The three independent variables were SWF content, barrel temperature profile and screw speed of the extruder. Extrusion trials were conducted using three levels of each variable (Table 6.3) keeping moisture content constant at 25% db for a total of 27 treatments.

6.3.3 Extrusion

To monitor the data necessary for the viscosity measurements, extrusion processing was performed using a single screw extruder (Brabender Intelli-Torque Plasti-Corder®, South Hackensack, NJ) at Industrial Agricultural Products Center, University of Nebraska, Lincoln, NE which was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 225 rpm. The extruder had a barrel with length to diameter ratio of 20:1 and a barrel diameter of 19 mm. A uniform 19 mm pitch screw with compression ratio of 3:1 was used in the experiments. The extrusion experiments were performed varying screw speed from 80 rpm to 160 rpm. The extruder barrel was equipped with external band heaters with provisions to control the temperature of all three zones: feeding zone, metering zone and die zone. The material was fed through the feed hopper to the feeding zone and conveyed to the metering zone where it gets gelatinized and plasticized under thermal and mechanical stresses generated by the rotation of screws. The gelatinized material then enters the die zone where the die section is fully filled due to pressure generated at die nozzle. The levels of temperature gradient in the barrel are 45-100-100^{o}C, 45-120-120^{o}C, 45-140-140^{o}C hereafter referred to as temperature of 100, 120, and 140^{o}C. The temperature of the melt was kept same at the metering zone and die zone of the extruder for each experimental condition. When the process reached the steady state, samples were collected at the die.

6.3.4 Viscosity Measurement

Rheological data of the dough within the extruder were determined online by a round capillary die rheometer mounted to the head of the extruder. Figure 6.1(a) presents the schematic of the round capillary die used in this study. An illustration of the experimental set–up can be seen in Figure 6.2(a). Material enters the die’s orifice, converging at a 30^{o} angle to pressure restriction neck [Figure 6.2(b)]. After the material has reached the main channel, it flows past the flush mounted temperature and pressure monitoring ports, and is then forced into a capillary nozzle insert [Figure 6.2(c)]. A high pressure region is generated at the entrance of the nozzle insert and is recorded by the pressure transducer which was used in the determination of the pressure drop across the nozzle insert with respect to atmospheric pressure. Three capillary die nozzle inserts, all 2 mm in diameter, were used to determine entrance pressure drops and to perform Bagley corrections. Length/Diameter (*L/D*) ratios were 10:1, 15:1, and 20:1 [Figure 6.1(b)]. The rheological properties of the dough were calculated from the pressure drop of the flow in die. Various shear rates necessary to obtain a viscosity curve were generated by increasing the screw speed of the extruder from 80 to 160 rpm. Die temperatures were measured with a thermocouple in melt contact prior to the capillary entrance: pressures were monitored with a Dynisco pressure transducer at the same position. The temperature of the rheometer was kept constant as the exit temperature of the dough (i.e. temperature at the end of the extruder) using a heating jacket. Measurements of pressure drops and temperatures were made when steady state was reached. The mass flow rate was measured by weighing the product at the die exit, at given time intervals (30 sec). For each extrusion condition, i.e. with a given melt temperature and SWF content level, ten pressure readings were taken. The measurements were repeated for each *L/D* ratio (*L/D* = 10, 15 and 20). The shear stress τ (Pa) can be calculated by Eq. (6.2)

$$\tau = \frac{\Delta PR}{2L} \tag{6.2}$$

where ΔP is the pressure drop (Pa) measured across the capillary nozzle insert with respect to atmospheric pressure having capillary length *L* (mm) and radius *R* (mm) = *D*/2. By varying the *L/D* ratios of die nozzle inserts, the appropriate Bagley correction factor was applied in the calculation of shear stress at the wall of the die to offset the effects of entrance and exit pressure. The apparent shear rate $\dot{\gamma}_{app}$ (s^{-1}) was calculated by Eq. (6.3).

$$\dot{\gamma}_{app} = \frac{4Q}{\pi R^3} \tag{6.3}$$

where Q is the volumetric flow rate (mm^3/s), R is the capillary radius (mm). The Weissenberg–Rabinowitsch correction was applied to obtain the true shear rate $\dot{\gamma}_t$ (s^{-1}) to compensate for the non-laminar flow of a viscoelastic biopolymer as follows:

$$\dot{\gamma}_t = \left[\frac{1}{4}\left(3 + \frac{\partial \ln \dot{\gamma}_{app}}{\partial \ln \tau}\right)\right]\dot{\gamma}_{app} \tag{6.4}$$

The true viscosity η_t (Pa.s) was calculated using Eq. (6.5).

$$\eta_t = \frac{\tau}{\dot{\gamma}_t} \tag{6.5}$$

6.3.5 Statistical Analysis

The statistical analysis was performed with the Statistical Analysis System v9.3 (SAS Institute, Cary, NC). Multiple regression analysis (SAS) was used to determine the parameters of the viscosity equations. Second-order polynomial regression equations were generated using Design Expert v8 and response surface plots were produced.

6.4 Results and Discussion

6.4.1 Viscosity Model

The experimental data were compared with the data obtained from Eq. (6.1). It was found that Eq. (6.1) did not closely fit the experimental data (R^2 = 0.824), because it did not consider the soy white flakes influence. When exponential models based on Eq. (6.1) with a multiplying SWF term were used to describe the data, the R^2 decreased and when exponential models with the reciprocal SWF term were used to describe the data, the R^2 increased. Thus, the viscosity of dough had an inverse relationship with the SWF level. The empirical model is presented as Eq. (6.6) and its parameters are shown in Table 6.4. This model includes the effects of SWF, shear rate and temperature on viscosity. Modeling the viscosity comprises estimating the parameters n, b_1, and b_2 by multiple linear regression analysis. This was achieved by linearizing the above equation by taking the natural logarithm on both sides. The results show a shear thinning behavior (n = 0.95) of proteinaceous dough (~ 32.8 %db), as also reported in (Cervone and Harper, 1978; Harmann and Harper, 1973, 1974; Remsen and Clark, 1978).

$$\eta = K\dot{\gamma}^{n-1}\exp(b_1 / T)\exp(b_2 / SWF) \quad (6.6)$$

The reciprocal temperature shows that increase in viscosity was caused by decreasing product temperature over the range of temperatures studied. These results are consistent with the results of other studies (Bhattacharya and Hanna, 1986; Chen et al., 1978; Harper et al., 1971; Kokini et al., 1992). The response surface plot in Figure 6.3 shows the change in viscosity with different screw speed and temperature at 40% SWF level.

6.4.2 Effect of Temperature Profile

Temperature profile had a significant effect ($p<0.05$) on the studied extrusion processing parameters (Table 6.5). The apparent viscosity of the dough decreased as temperature increased, and was lowest at the highest temperature profile (Figure 6.3). Similar trends were observed by (Lam and Flores, 2003) during extrusion of fish feed blends in a single screw extruder. Moreover, the mass flow rate at higher temperatures was found to be higher, while the torque required to rotate the screw decreased, probably due to the higher degree of melting at the higher temperatures, which thus resulted in decreased viscosity of the ingredient melt inside the barrel (Figure 6.5 and Figure 6.6). In general, viscosity of fluid materials decreases with increasing temperature in an Arrhenius fashion, so results of this type would be expected in the fully melted feed material and the resulting dough. Because the reduced viscosity facilitated a reduced torque requirement, as the temperature was increased, the specific mechanical energy also decreased.

6.4.3 Effect of Screw speed

Screw speed also had a significant effect ($p<0.05$) on the extrusion parameters studied in the experiment (Table 6.5). As screw speed increased from 80 to 160 rpm, the mass flow rate increased (Figure 6.6). This behavior was expected, because drag flow in extruders has been shown to be directly proportional to screw speed (Harper, 1981), and higher screw speeds generally result in higher mass flow rates. The apparent viscosity decreased on increasing the screw speed from 80 rpm to 160 rpm (Figure 6.3), indicating that the molten dough inside the barrel exhibited shear thinning behavior. Increasing screw speed reduces viscosity due to increased shear rates and molecular degradation. Specific mechanical energy was also found to be significant which increased with increase in screw speed (Figure 6.4).

6.4.4 Effect of SWF Level

The level of SWF content in the ingredient mix had a significant effect ($p<0.05$) on all extrusion parameters studied (Table 6.5). Increasing SWF content from 30 to 50% resulted in a decrease in viscosity. The average protein content of the feed blends was 32.8% (db) and there was no significant difference in protein content between them. But, the blend containing 30% SWF did have 19.0% higher fiber content, and 18.1% higher ash content compared to the blend containing 50% SWF (Table 6.2). Increase in SWF in the blend resulted in an increase in carbohydrates or Nitrogen Free Extract (Table 6.2), which led to an increased level of gelatinized starch in the ingredient melt inside the barrel during processing. It also led to lower fiber content. These differences in chemical constituents in itself may have contributed to the decreased apparent viscosity when the SWF content in the ingredient blend was increased from 30% to 50% (Jin et al., 1994), and may have also contributed to the competing effects between the various extrusion processing parameters. Increasing the SWF content from 30% to 50% resulted in a significant increase in the torque requirements.

6.5 Conclusions

We examined the processing behavior of SWF blends and investigated the viscosity of developed doughs in the laboratory extruder. Aquaculture feed mixes used in this study exhibited shear thinning behavior for blends containing SWF up to 50%. By power law modeling of the observed apparent viscosity during processing, it was determined that the coefficient for temperature was much higher than the coefficient for SWF, which indicated that the viscosity of the blends is greatly affected by the level of temperature. Increasing the SWF content resulted in a higher mass flow rate, torque and specific mechanical energy. The specific mechanical energy and mass flow rate increased and viscosity decreased as screw speed increased. At higher temperatures in the barrel and the die, the viscosity of the dough decreased, which led to a decreased torque requirement and specific mechanical energy.

Table 6.1 Ingredient composition of feed blends.

Feed Ingredients	**Mass of ingredients (g/100g dry matter)**		
	Blend I	**Blend II**	**Blend III**
Soy white flakes	30	40	50
DDGS	29	15	4
Corn Flour	25	30	33
Fish meal	5	3	0
Corn gluten meal	6	6	6
Vitamin & Mineral mix	2	2	2
Soy bean oil	4	4	5

†DDGS - Distiller's dried grains with solubles

Table 6.2 Proximate composition of the feed blends (% dry matter).

	Crude protein (%)	**Crude fat (%)**	**Ash (%)**	**Crude fiber (%)**	**NFE (%)**
Blend I	32.91	6.03	5.74	3.89	51.42
Blend II	32.72	6.00	5.28	3.55	52.45
Blend III	32.70	5.94	4.70	3.25	53.41

†NFE – Nitrogen Free Extract

Table 6.3 Experimental levels of independent variables.

Independent variable	**1**	**2**	**3**
Soy white flakes (%)	30	40	50
Barrel temperature (^{o}C) †	45-100-100	45-120-120	45-140-140
Screw speed (rpm)	80	120	160

†Feeding zone-metering zone-die zone

Table 6.4 Coefficients for the viscosity equation.

Equation Parameters	**Parameter Estimate**	**Standard Error**	**t for H_o Parameter = 0**	**Prob > |t|**
ln(*K*)	-290.393	27.4428	-10.58	< 0.0001
(*n*-1)	-0.04113	0.00595	-6.92	< 0.0001
b_1	196424	13261	14.81	< 0.0001
b_2	-1071.70	214.011	-5.01	< 0.0001

†*K* – Consistency factor; *n* – flow behavior index; b_1 – empirical constant for temperature; b_2 – empirical constant for soy white flakes

Table 6.5 Interaction results for soy white flakes content (SWF), temperature (T) and screw speed (SS) on process parameters (*P* values).

Variable	**Viscosity (Pa.s)**	**SME (W-h/kg)**	**Torque (N-m)**	**Mass flow rate (kg/h)**
SWF	< 0.0001	< 0.0001	< 0.0001	< 0.0001
T	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SS	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SWF×T	0.7666	0.3536	0.0340	0.7252
SWF×SS	0.2109	0.0389	0.3193	0.4747
T×SS	< 0.0001	< 0.0001	0.0655	< 0.0001

[†]SME – Specific mechanical energy

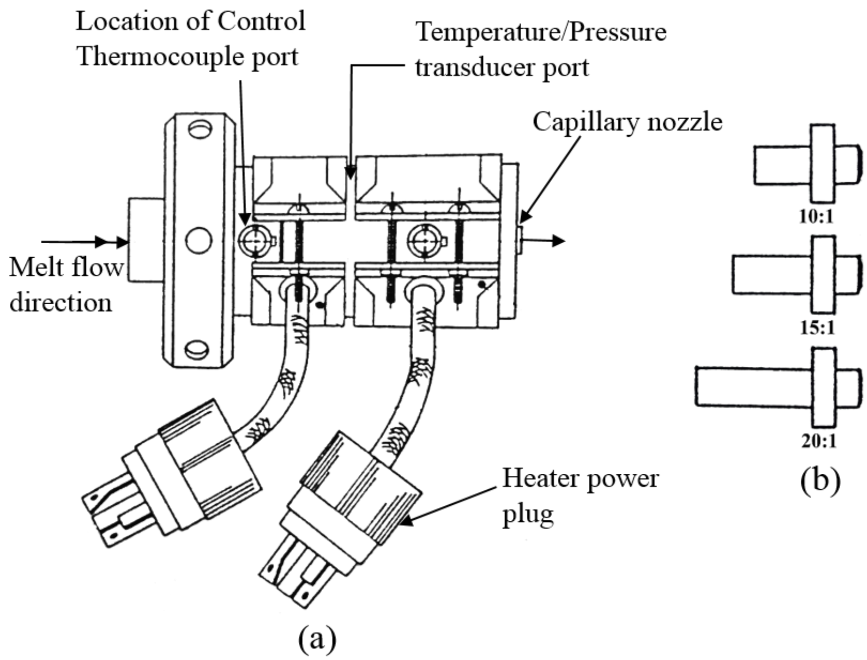


Figure 6.1 (a) Round capillary die and (b) Capillary nozzles having different L/D ratio

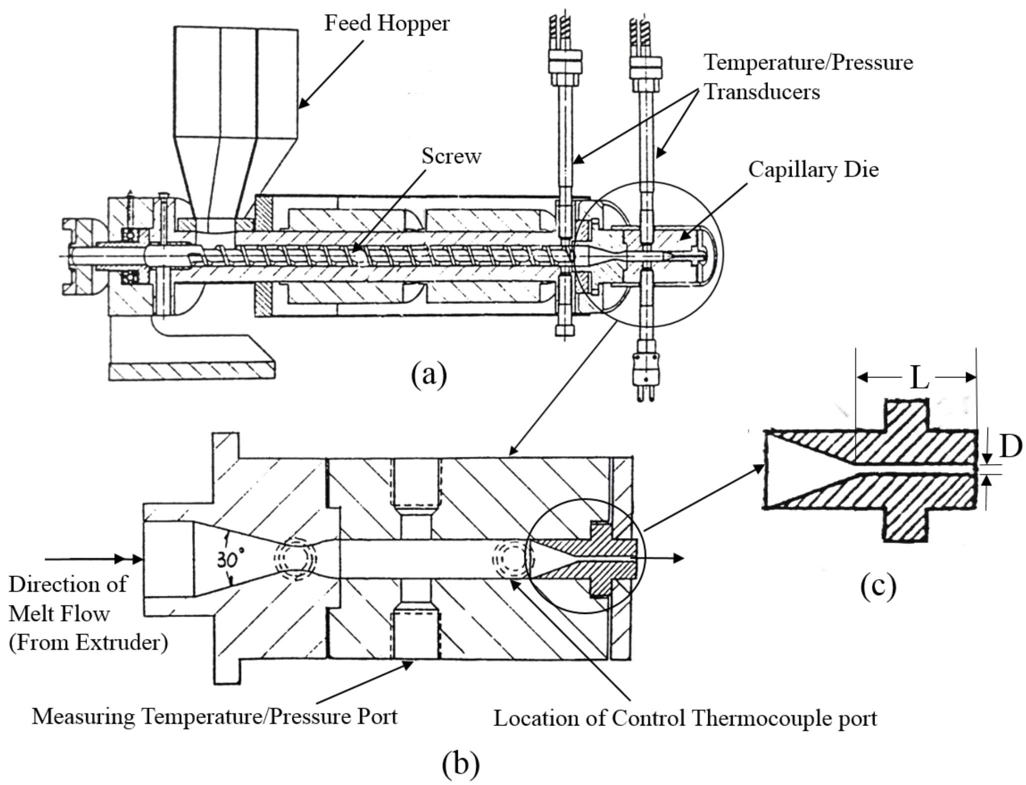


Figure 6.2 (a) Schematic representation of an extruder/die configuration and position of temperature /pressure transducers (b) cross sectional view of a round capillary die and (c) capillary nozzle

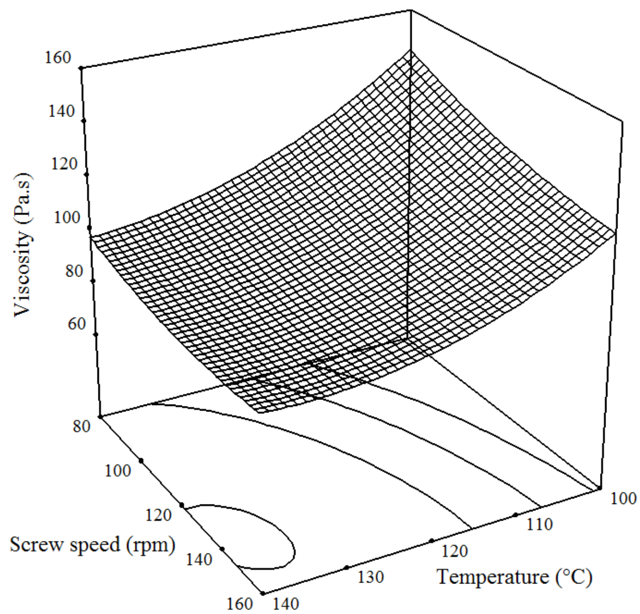


Figure 6.3 Response surface plot of viscosity for the effect of screw speed and temperature at 40% soy white flakes

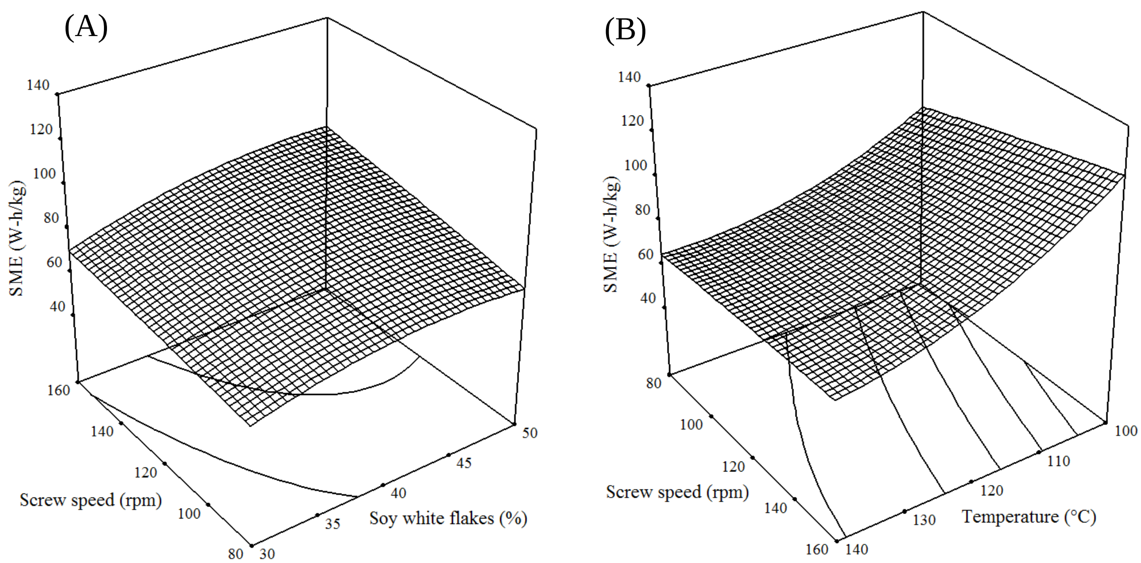


Figure 6.4 Response surface plots of specific mechanical energy for the effect of (A) Screw speed and soy white flakes at 120 °C, (B) Screw speed and temperature at 40% soy white flakes

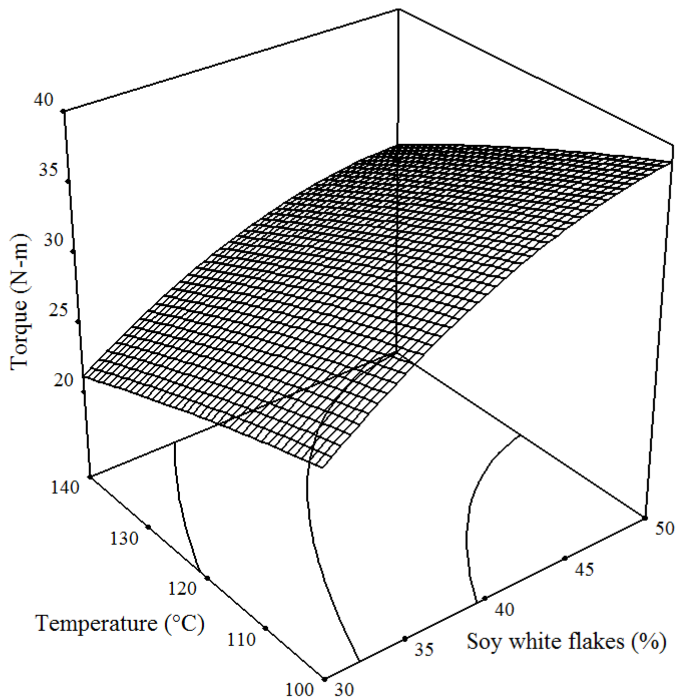


Figure 6.5 Response surface plot of torque for the effect of temperature and soy white flakes at 120 rpm

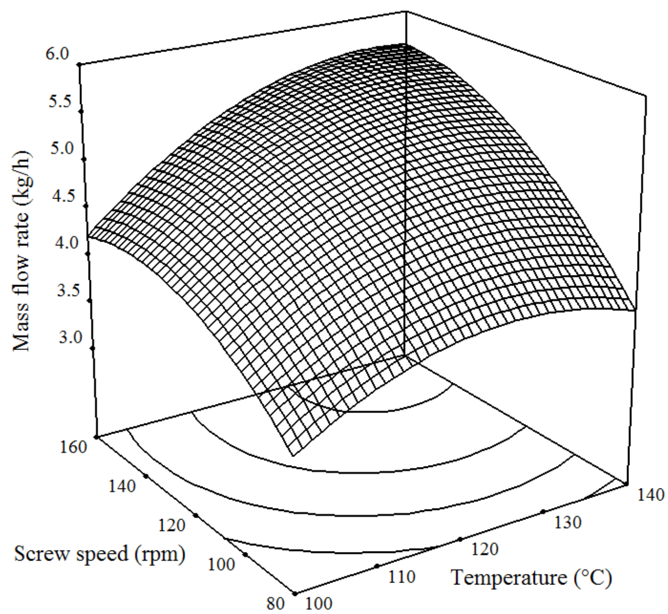


Figure 6.6 Response surface plot of mass flow rate for the effect of screw speed and temperature at 40% soy white flakes

CHAPTER 7 [†]

Rheological Characterization and CFD Simulation of Soy White Flakes Based Dough in a Single Screw Extruder

7.1 Abstract

Understanding the rheological properties such as shear profile, viscosity of dough inside the extruder is very important for product development, process control, final product quality, and scaling up operations. This study aims to characterize the rheological properties of high protein dough and perform isothermal flow simulation in a complex geometry for generalized Newtonian fluids. The flow of soy white flakes based dough in a single screw extruder was simulated by using computational fluid dynamics (CFD). Process conditions considered were screw speeds (40, 80, 120, 160, 200 rpm), barrel temperature (100, 120, 140^{o}C) and soy white flakes (SWF) contents (30, 40, 50%db). A Mesh Superposition Technique was used to reduce the geometrical complexity. Simulation results were validated quantitatively by experimental data. The results showed good agreement between experimental and computational results. Moreover, the flow profiles in the extruder were analyzed by using the influence of screw speed and level of soy white flakes content. Qualitative behavior of local shear rate and viscosity along the screw are analyzed and comparisons of different process conditions are presented. The results of these simulations can be used to optimize processing conditions and also to improve the product quality of extrudates.

[†] Singh, S.K. and Muthukumarappan, K., 2016. Rheological characterization and CFD simulation of soy white flakes based dough in a single screw extruder. *Journal of Food Process Engineering*. DOI: 10.1111/jfpe.12368

7.2 Introduction

Feed and food extrusion cooking is a unit operation in which various products are produced using cereals, oilseeds or other carbohydrate mixtures. Fish feed is one of these extruded products. Extrusion cooking is a high-temperature short-time process in which final product is obtained by a combination of several unit operations such as heating, mixing, shearing and forcing the material through a die in a single step. Previous laboratory experiments with soy white flakes have yielded extrudates with physical properties that may be suitable for aquaculture feeds (Singh and Muthukumarappan, 2014a, b; Singh and Muthukumarappan, 2015). However, for consistent large scale production of aquaculture feeds using soy white flakes as protein source, a thorough understanding regarding the changes occurring inside the extruder is necessary. During extrusion, the rate and extent of cooking, mixing, shearing and compressing processes are strongly related to the rheological properties of the raw materials. The knowledge of temperature and shear profiles within the extruder helps in controlling the quality of the final product (Emin et al., 2011). Different raw materials have different rheological properties. Hence, understanding the rheology of a melt/dough inside the extruder can help to optimize product development, process control, product quality, and scale up processes (Lam and Flores, 2003).

Viscosity is one of the most important rheological properties of non-Newtonian biological materials. The viscosity of the dough inside extruder barrels has been studied by many authors. For example, the viscosity of dough of different grains including wheat, corn, and soybean were measured using straight tube viscometer (Harper et al., 1971), cylindrical dies of different lengths (Harmann and Harper, 1974), capillary die (Jao et al., 1978), viscoamylograph (Remsen and Clark, 1978), and capillary rheometer (Luxenburg et al., 1985) attached to food extruders, and viscosity models have been developed. Harper et al. (1971) developed a viscosity model where they incorporated moisture and temperature effects. Many researchers used this same model to study the viscosity of dough in extruders (Altomare et al., 1992; Bhattacharya and Hanna, 1986; Harmann and Harper, 1974; Jao et al., 1978; Luxenburg et al., 1985; Remsen and Clark, 1978). Lam and Flores (2003) introduced a correction factor for the particle size distribution of the raw ingredients in the viscosity model of dough inside the barrel of a single screw extruder. Singh and Muthukumarappan (2016) incorporated the effect of soy white flakes in the viscosity model of dough in a single screw extruder. Meuser et al. (1987) suggested that viscosity (or shear stress) can be used as a variable for continuous on-line control. However, the rheologically complex flow behavior of melt due to geometrical nature of single screw extruder having grooved barrel presents many challenges, particularly with respect to inhomogeneous distribution of the stresses in the flow field. Therefore, measurement of the exposed stresses in extruders is a very difficult task and remains challenging.

Computational fluid dynamics (CFD) simulation of flow characteristics inside the extruder may be used to improve the understanding of underlying mechanisms and provide an insight in to the effects of process parameters. Many researchers have designed a single screw extruder using Finite Element Meshing (FEM) and CFD. Dhanasekharan and Kokini (2003) analyzed a computational method based on numerical simulations to obtain simultaneous scale-up of mixing and heat transfer for single screw extrusion of wheat dough. Connelly and Kokini (2007) used CFD to study and compare the mixing ability of single and co-rotating twin screw mixers. El-Sadi and Esmail (2005) did numerical simulation of flow to investigate the performance of micro pump using complex liquid. The dispersive mixing efficiency of the plasticized starch in a twin screw extruder was studied and simulated by Emin and Schuchmann (2013). Computer simulation of moistened defatted soy flour in a single screw extruder has been performed by Ghoshdastidar et al. (2000) to predict the dough behavior in an extrusion process. Siregar et al. (2014a) designed and analyzed single screw extruder for processing of Jatropha seeds using FEM and CFD. To date, however, no study has been attempted to simulate the flow of dough containing relatively high concentrations of soy white flakes. Therefore, the approach of this study is to simulate the non–Newtonian flow of dough in a single screw extruder by using CFD.

The objectives of this study were to (a) characterize the rheological properties of soy white flakes based dough in a single screw extruder online by capillary die rheometer, and (b) simulate the rheological complex fluid flow inside the extruder.

7.3 Materials and Methods

7.3.1 Materials and dough formulation

Soy white flakes were donated by South Dakota Soybean Processors (Volga, SD). Corn flour was purchased from Cargill Dry Ingredients (Paris, IL). Dried distiller's grain with solubles (DDGS) was obtained from the POET (Sioux Falls, SD). Corn gluten meal and fishmeal was purchased from Consumer Supply Distributing Co. (Sioux City, IA). Vitamin and mineral premix was obtained from Lortscher Agri Service, Inc. (Bern, Kansas, USA). Soybean oil was obtained from USDA (Brookings, SD). Three iso-nitrogenous blends (hereafter referred to as "dough") were formulated with a net protein content of 32.7% db. The different ingredients in the dough include soy white flakes, DDGS, corn gluten meal (CGM), corn flour, fish meal, soybean oil, and vitamin-mineral mix (Table 7.1). The ingredients were mixed in a laboratory scale Hobart mixer (Hobart Corporation, Troy, Ohio, USA) for 10 min and stored overnight at ambient temperature for moisture stabilization. The moisture balancing of the doughs was done by adding required quantities of water during mixing.

7.3.2 Extrusion Processing

To monitor the data necessary for the numerical simulation, extrusion processing was performed using a single screw extruder (Brabender Intelli-Torque Plasti-Corder®, South Hackensack, NJ) at Industrial Agricultural Products Center, University of Nebraska, Lincoln, NE. The extruder was powered by a 7.5-HP motor with an operating range of screw speeds from 0 to 225 rpm. It had a barrel with length to diameter ratio of 20:1 and a barrel diameter of 19 mm. A uniform 19 mm pitch screw with compression ratio of 3:1 was used in the experiments. The extrusion experiments were performed varying screw speed from 40 rpm to 200 rpm. The extruder barrel was equipped with external band heaters with provisions to control the temperature of all three zones: feed zone, transition zone/melting zone, and die sections. The material was fed through the feed hopper to the feeding zone and gets conveyed to the melting zone where it gets gelatinized and plasticized under thermal and mechanical stresses generated by the rotation of screws. The gelatinized material then enters the die section. Experiments were conducted using three levels of soy white flakes (30, 40 and 50%db), three levels of temperature gradient in the barrel (45-100-100^{o}C, 45-120-120^{o}C, 45-140-140^{o}C) hereafter referred to as temperature of 100, 120, and 140^{o}C keeping moisture content constant at 25% db. The temperature of the melt was kept same at the melting zone and die section of the extruder for each experimental condition. When the process reached the steady state, samples were collected at the die.

7.3.3 Rheological Measurements

Rheological data of the dough within the extruder were determined online by a round capillary die rheometer mounted to the head of an extruder during all the experiments. Figure 7.1(a) presents the schematic of the round capillary die. An illustration of the experimental set–up can be seen in Figure 7.2(a). Material enters the die's orifice, converging at a 30^{o} angle to a pressure restriction neck [Figure 7.2(b)]. After the material has reached the main channel, it flows past the flush mounted temperature and pressure monitoring ports, and is then forced into a capillary nozzle insert [Figure 7.2(c)]. A high pressure region is generated at the entrance of the nozzle insert and is recorded by the pressure transducer which was used in the determination of the pressure drop across the nozzle insert with respect to atmospheric pressure. Three capillary die nozzle inserts, all 2 mm in diameter, were used to determine entrance pressure drops. Length/Diameter (*L/D*) ratios were 10:1, 15:1, and 20:1 [Figure 7.1(b)]. The rheological properties of the dough can be calculated from the pressure drop of the flow in die. Various shear rates necessary to obtain a viscosity curve were generated by increasing the screw speed of the extruder. Screw speeds ranged from 40 to 200 rpm. Die temperatures were measured with a thermocouple in melt contact prior to the capillary entrance: pressures were monitored with a Dynisco pressure transducer at the same position. The temperature of the rheometer was kept constant as the exit temperature of the dough (i.e. temperature at the end of the extruder) using a heating jacket.

Measurements of pressure drops and temperatures were made when steady state was reached. The mass flow rate was measured by weighing the product at the die exit, at given time intervals (30 sec). For each extrusion condition, i.e. with a given melt temperature, SWF content level approximately ten pressure readings were taken. The measurements were repeated for each *L/D* ratio (*L/D* = 10, 15 and 20). The shear stress τ (Pa) can be calculated by Eq. (7.1).

$$\tau = \frac{\Delta PR}{2L} \tag{7.1}$$

where ΔP is the pressure drop (Pa) measured across the capillary nozzle insert with respect to atmospheric pressure having capillary length L (mm) and radius R (mm) = D/2. By varying the *L/D* ratios of die nozzle inserts, the appropriate Bagley correction factor was applied in the calculation of shear stress at the wall of the die to offset the effects of entrance and exit pressure. The apparent shear rate $\dot{\gamma}_{app}$ (s^{-1}) was calculated by Eq. (7.2).

$$\dot{\gamma}_{app} = \frac{4Q}{\pi R^3} \tag{7.2}$$

where Q is the volumetric flow rate (mm^3/s), R is the capillary radius (mm). The Weissenberg–Rabinowitsch correction was applied to obtain the true shear rate $\dot{\gamma}_t$ (s^{-1}) to compensate for the non-laminar flow of a viscoelastic biopolymer as follows:

$$\dot{\gamma}_t = \left[\frac{1}{4}\left(3 + \frac{\partial \ln \dot{\gamma}_{app}}{\partial \ln \tau}\right)\right]\dot{\gamma}_{app} \tag{7.3}$$

The true viscosity η_t (Pa.s) was calculated using Eq. (7.4)

$$\eta_t = \frac{\tau}{\dot{\gamma}_t} \tag{7.4}$$

Measured viscosity data were fitted to a power law model, which has been reported by other authors for extrusion processing of oat, corn, plasticized starch and some protein doughs (Cervone and Harper, 1978; Harmann and Harper, 1973, 1974; Padmanabhan, 1991; Remsen and Clark, 1978; Vergnes et al., 1993)

$$\eta = K\dot{\gamma}^{n-1} \tag{7.5}$$

where η is the viscosity (Pa.s), K is the consistency factor (Pa.s^n) and n is the power law index. Values of n are between 0 and 1 for pseudoplastics. For $n < 1$, the viscosity decreases with increasing shear rate; such materials are called shear thinning. Values for the power law index n and the consistency K were obtained by curve fitting using ANSYS POLYMAT as shown in Table 7.1.

7.3.4 CFD Simulations

The goal of this study was to analyze the flow behavior in the high shear rate zone extruder as shown in Figure 7.3. The flow through this section is assumed to be isothermal in accordance with negligible temperature change of the material at this region. The rheological data of dough inside the extruder measured with the online rheometric capillary die is fitted to the Power law viscosity model.

The governing equations regarding a time-dependent, isothermal and incompressible flow of a generalized Newtonian fluid is

$$\rho \frac{DV}{Dt} = -\nabla p + \nabla T + \rho g \tag{7.6}$$

$$\nabla . V = 0 \tag{7.7}$$

where *D/Dt* is the material derivative, V (ms^{-1}) is the velocity, *p* is the pressure, ρ (kgm^{3}) is the density, g (ms^{-2}) is the gravity and T (Nm^{-2}) is the extra stress tensor defined by:

$$T = 2\eta d \tag{7.8}$$

with the strain rate tensor *d* (s^{-1}) and the viscosity η (Pa.s). The shear rate $\dot{\gamma}$ (s^{-1}) is defined as the square root of the second invariant of the strain rate tensor:

$$\dot{\gamma} = \sqrt{2d : d} \tag{7.9}$$

The CFD code of ANSYS POLYFLOW® 15.0 which is a finite element code primarily for highly viscous flows was used to calculate the governing equations for determining the flow patterns in the single screw extruder. The flow in a single screw extruder is three dimensional and unsteady due to moving part i.e. screw. In this study, to reduce the complexity of the setup of such a simulation and to avoid the use of a remeshing algorithm, the mesh superposition technique (Avalosse, 1996) was used. A detailed study on the requirements for the meshing and the mixed interpolations can be found elsewhere (Alsteens et al., 2004). The meshing of the screw and the fluid region were conducted independently in ANSYS ICEM CFD®. The fluid mesh and screw mesh is made up of 148,600 and 87,780 volume elements respectively. The created meshes are then merged using the tool POLYFUSE. Some portions of the fluid overlap the screw cells in the resulting mesh (Figure 7.4). The meshes in the clearance between the tip of screws and the barrel wall had three bricks in order to capture high shear rates. To set boundary conditions, a fully developed velocity profile (Inflow) was imposed at the inlet section and zero normal velocity and tangential force were imposed at the outlet section. A non-slip condition was imposed on the surface of screw and inner surface of the barrel. The geometry of the computational domain was created using SolidWorks® 14.0 and its geometrical dimensions are represented in Figure 7.5 and listed in Table 7.2. For the time dependent simulations, a steady state simulation was performed at first. Then, the result of the steady state simulation was used as an initial velocity field in a time dependent simulation to obtain a physically relevant initial step. Due to symmetrical geometry, only half a turn of the screw was simulated. To obtain accurate evolution of flow pattern in time, 40 time steps were simulated for half a turn of screws.

7.4 Results and Discussion

7.4.1 Viscosity Model

Viscosity model describes the rheological characteristics of the melt inside the extruder and directly affects the flow behavior. Therefore, the selection of the viscosity model plays an important role on the accuracy of the simulation results. The dough inside the extruder is a non-Newtonian fluid and its rheological behavior is very complex, depending on different parameter such as shear rates, temperature and moisture content (Morgan et al., 1989). In the present study, a round capillary die rheometer mounted on the end of the extruder was used to measure the viscosity as described in material and methods section. Obtained results are given in Figure 7.6 and Figure 7.7 for different experimental conditions. The symbols represent the measured true viscosities of dough at varying shear rates. The results show a shear thinning behavior of proteinaceous dough, as also reported in (Cervone and Harper, 1978; Harmann and Harper, 1973, 1974; Remsen and Clark, 1978). The Power law model can satisfactorily describe the rheological behavior of dough at the shear rate range measured (i.e. 700 to 2000 s^{-1}). As expected, it can be seen that increasing temperature led to decrease in viscosity. This may be due to the higher degree of melting at the higher temperatures, which thus resulted in decreased viscosity of the dough inside the extruder.

Furthermore, increase in level of SWF content led to decrease in viscosity as shown in Figure 7.7. SWF contains less starch as compared to the corn flour and DDGS in the ingredient mix. Hence increasing the SWF content changed the nutrient composition, and thus the potential functionality of the ingredients in the dough, and may have resulted in the lower viscosity.

7.4.2 Experimental validation

For validating the computational simulation of the flow within an extruder, the torque experienced by the rotating screw was measured. In the present study, torque was measured by the intelli-torque drive system. The capillary die rheometer was mounted on the head of the extruder to monitor simultaneously the rheological behavior of dough. Obtained rheological data were used to perform the computational simulations of the flow in extruder. Torque experienced by the screw in the flow domain of the simulation was compared to the experimental values measured by the drive system. The results obtained at different experimental conditions are given in Table 7.3 showing good agreement between experimental and computational values. The deviations in the experimental values resulted from torque fluctuations during the experiments, whereas the deviations in computational values show the differences at varying time steps of simulations.

7.4.3 Flow profile

In this section, the flow profile in the extruder is discussed by using the influence of SWF content and screw speed. For the viscosity model, the data measured by the capillary die rheometer were fitted to the Power law model as discussed earlier. Figure 7.8 shows the distribution of the local shear rate on XY plane and XZ plane for varying process conditions. Although the distribution of the local shear rate are similar for all the process conditions studied, increasing screw speed led to higher shear rates as expected. Maximum shear rates are generated at the tip of the screws. Figure 7.9 shows the distributions of the viscosity on XY plane and XZ plane for varying process conditions. In the experiments performed at constant temperature (140^{o}C), difference in viscosities were measured when SWF content was varied as depicted in Figure 7.9(c, d, g, h) and (k, l, o, p). As representative of the flow field on the XY and XZ plane, images at two different screw speeds were depicted in Figure 7.9. Regardless of minor differences, all the images show similar distribution of the viscosities. Lower viscosity was observed at higher screw speed. When the viscosity and shear rate distributions are compared, it can be seen that at the positions where the viscosity is maximum, shear rates are relatively low. In contrast, at the positions where the shear rate is very high, the flow is mainly dominated by less viscous fluid.

7.5 Conclusions

It is highly challenging to analyze flow behavior within an extruder since the knowledge of various parameters such as the rheological behavior of dough, nature and strength of the flow with the time of exposure, are required. These challenges can be overcome by CFD simulation which provides access to local data on flow velocities and resulting shear stresses. Experiments were performed on a laboratory scale single screw extruder and capillary die rheometer was used to measure the rheological characteristics of the dough at the extrusion conditions. The flow of dough in a single screw extruder was simulated by using CFD. In order to validate the simulation, torque experienced by the rotating screws was compared to the experimental values measured by flush mounted transducer. The experimental and computational results obtained at different process conditions were in good agreement. Additionally, the flow profile in the extruder was analyzed by using the influence of screw speed and SWF content. The results suggest that high shear zones are generated at the clearances between screws and barrel at which the flow is dominated by less viscous fluid. Increase in screw speed from 40 to 200 rpm led to lower shear rate. Moreover, to investigate the influence of SWF content, three levels of SWF content were simulated. The results show that the increase in SWF content from 30 to 50% led to decrease in viscosity. The results of the simulation can be used to optimize the extrusion process influenced by complex flows of relatively high concentration of SWF based aquafeed dough in an extruder and also to improve the product quality.

Table 7.1 Fit parameters used for the model in simulation.

Conditions		***K***	***n***
SWF (%db)	**Temperature (^{o}C)**	**(Pa.s^{n})**	**(-)**
30	140	925	0.659
40	140	361	0.809
50	140	312	0.830
50	120	8044	0.410
50	100	36372	0.251

[†]SWF – Soy white flakes; *K* – Consistency factor; *n* – Flow behavior index

Table 7.2 Dimensions of screw and barrel represented in Figure 7.5.

Dimensions (mm)			
A-A'	3.18	R_i	8.22
B-B'	3.18	R_o	9.35
Z-Z'	50.8	R_b	9.5
X-X'	19.5	D	19.5

Table 7.3 Comparison of experimental and computational torque (N-m) experienced by the screw in the extruder for various process conditions.

SWF (% db)	**Temp. (^{o}C)**	**Screw speed (rpm)**			
		40		**200**	
		T experimental	**T computational**	**T experimental**	**T computational**
30	140	25.29 ± 0.27	25.63 ± 0.68	12.50 ± 0.32	12.90 ± 0.55
40	140	29.23 ± 0.13	28.81 ± 1.05	16.34 ± 0.11	16.63 ± 0.62
50	140	32.93 ± 0.16	33.35 ± 0.87	18.74 ± 0.19	18.12 ± 0.97
50	120	37.26 ± 0.31	36.60 ± 1.15	21.11 ± 0.30	21.67 ± 0.83
50	100	42.54 ± 0.22	41.87 ± 0.74	23.87 ± 0.24	23.51 ± 1.11

[†]SWF – Soy white flakes; Temp. – Barrel temperature; T - Torque

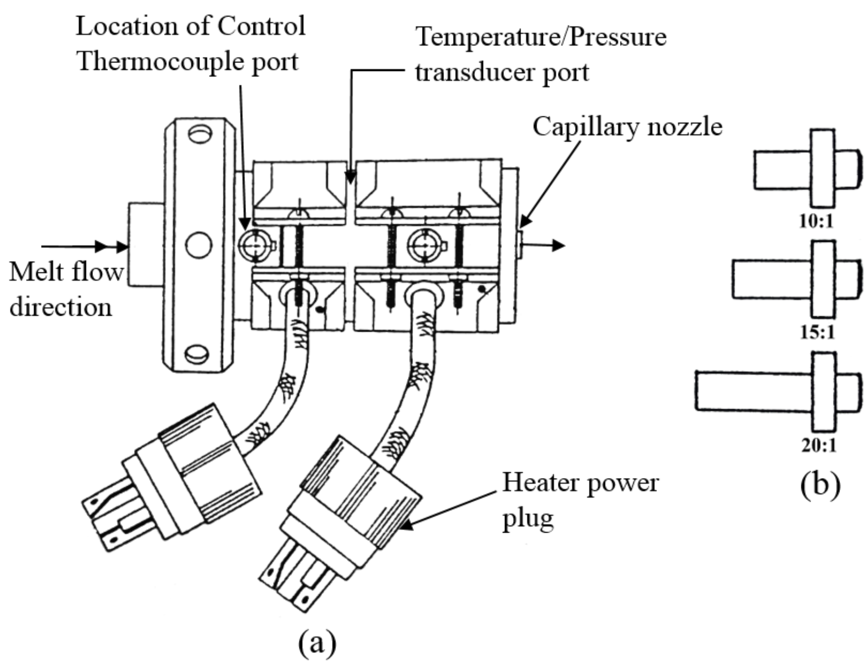


Figure 7.1 (a) Round capillary die (b) Capillary nozzles having different L/D ratio

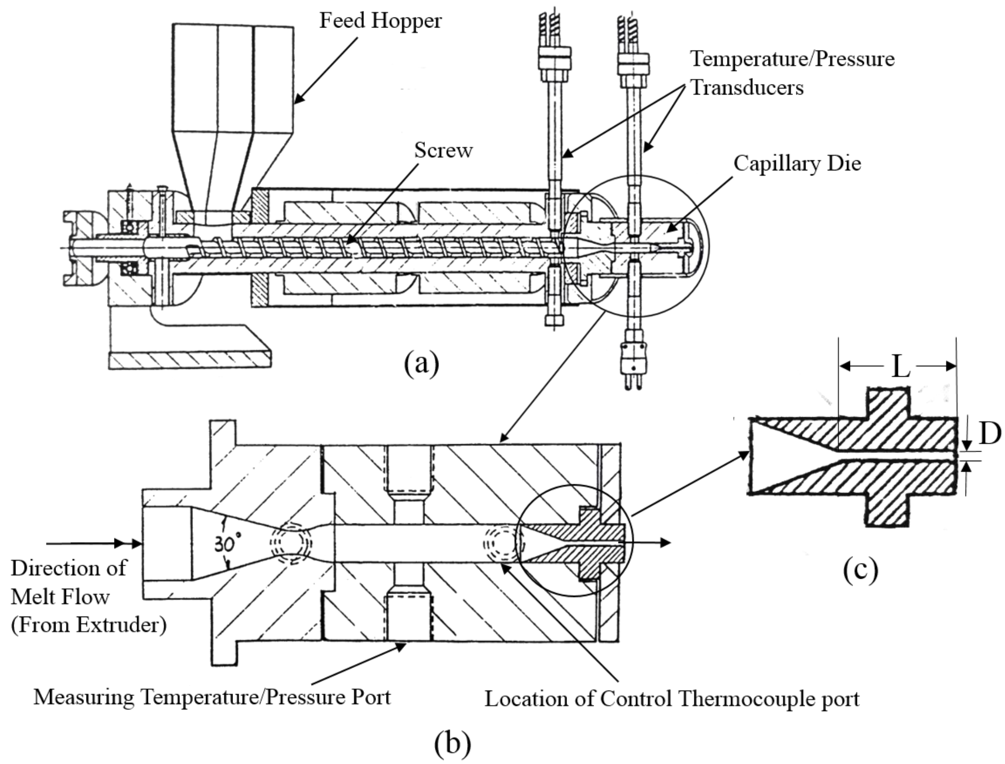


Figure 7.2 (a) Schematic representation of an extruder/die configuration and position of temperature/pressure transducers (b) X- sectional view of a round capillary die and (c) Capillary nozzle

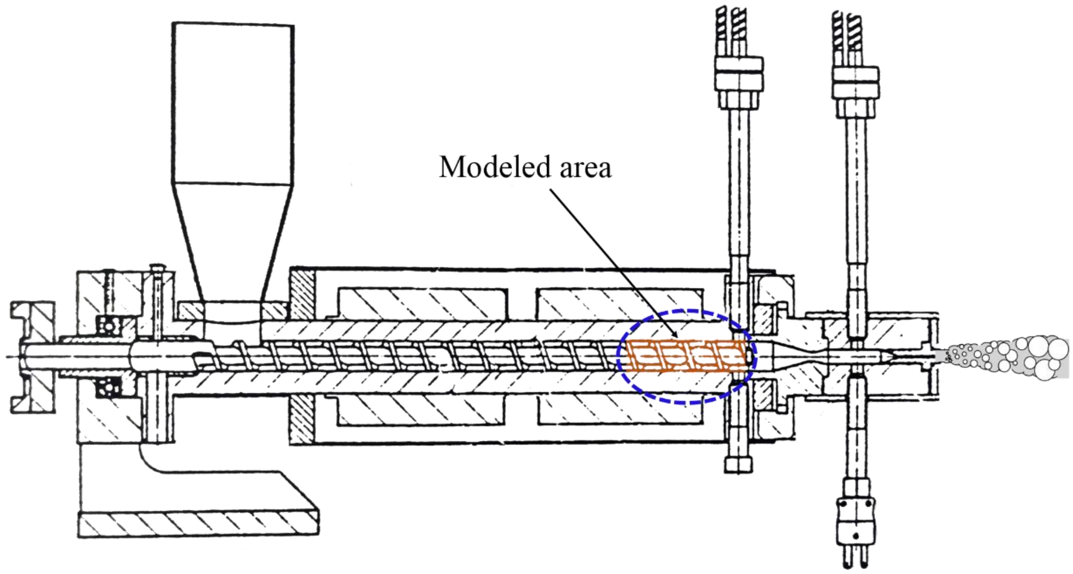


Figure 7.3 Schematic draw of the extruder and location of the modeled area

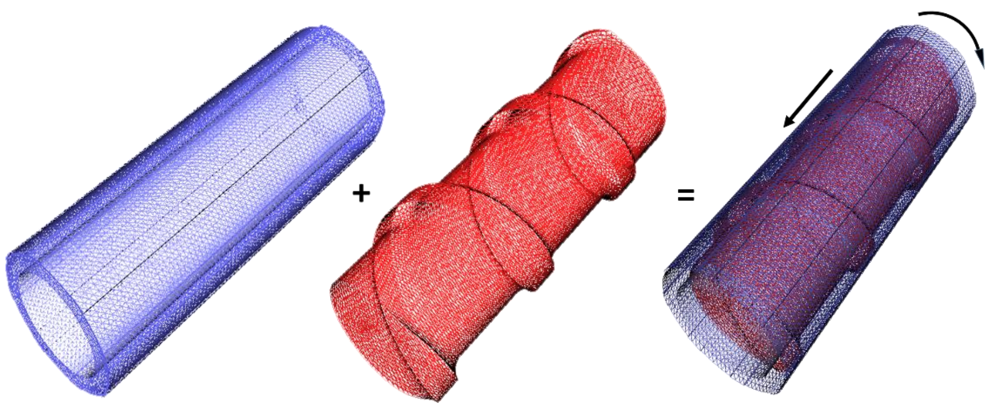


Figure 7.4 Domain discretization and mesh superposition

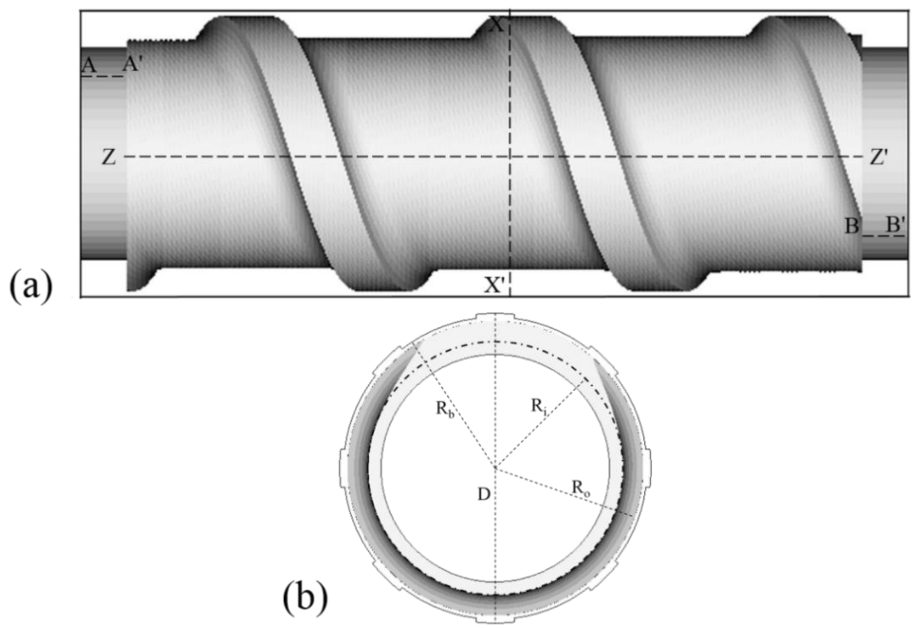


Figure 7.5 Geometrical dimensions of the screws and the barrel. (a) Top view, (b) Front view

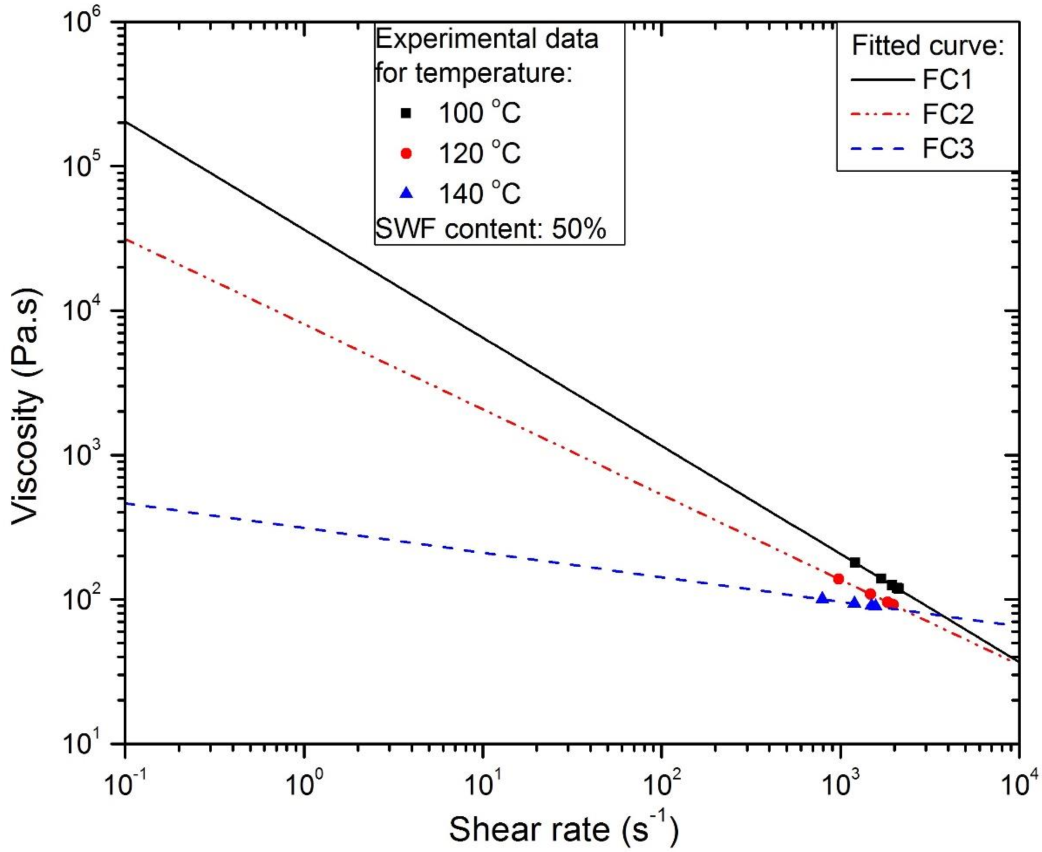


Figure 7.6 Viscosity curves of the dough fitted to power law model. Experimental data and fitted curves (FC) are represented as symbols and lines respectively

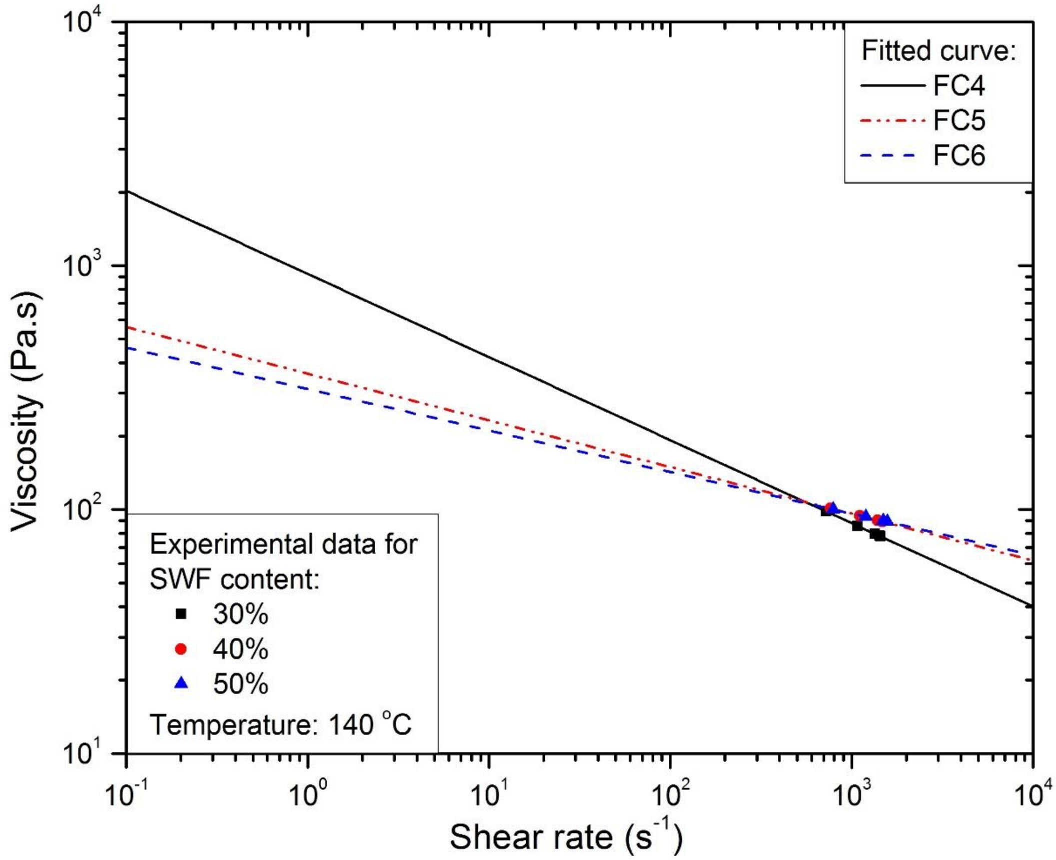
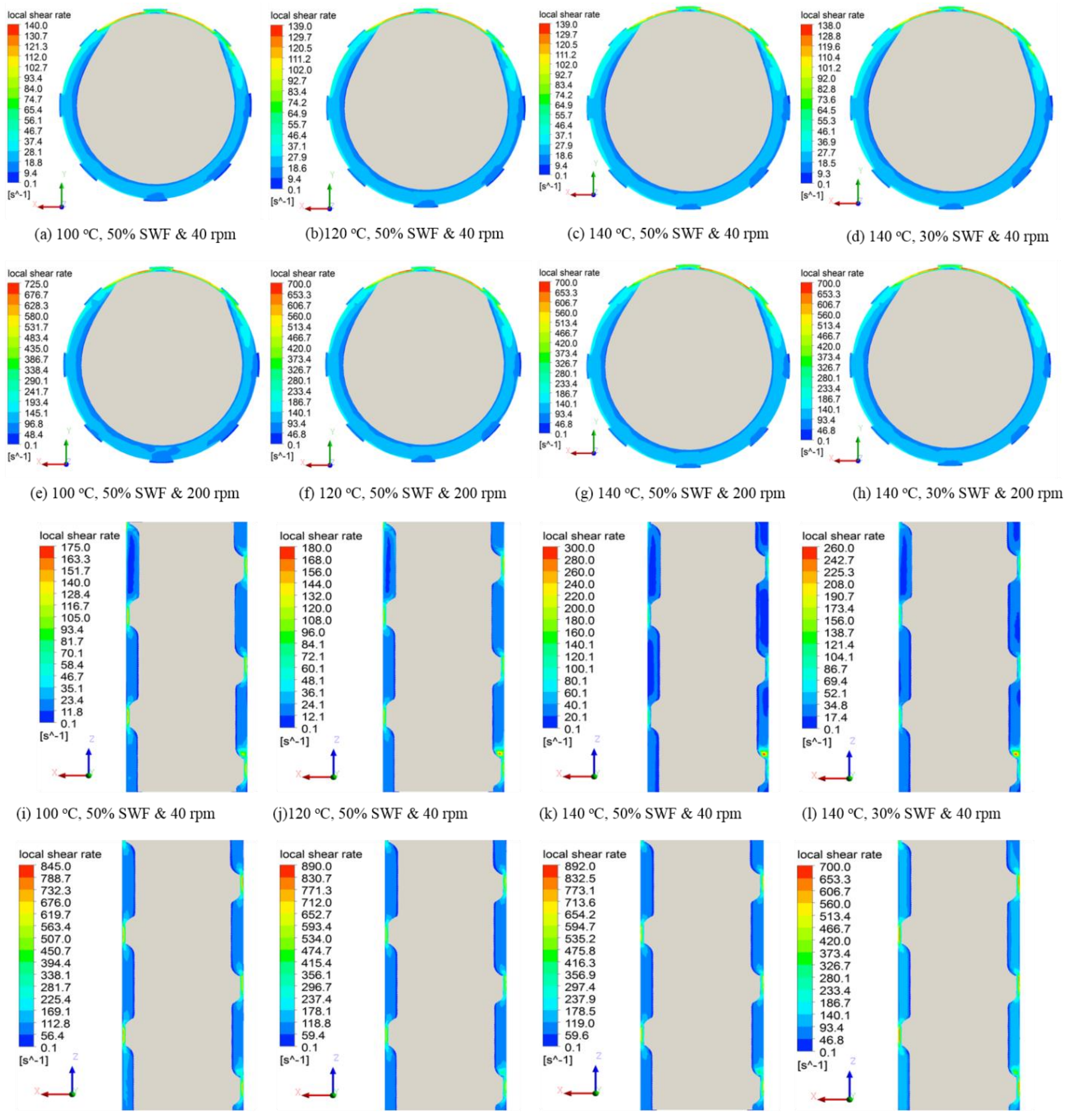


Figure 7.7 Viscosity curves of the dough fitted to power law model. Experimental data and fitted curves (FC) are represented as symbols and lines respectively



(a) 100 ºC, 50% SWF & 40 rpm (b)120 ºC, 50% SWF & 40 rpm (c) 140 ºC, 50% SWF & 40 rpm (d) 140 ºC, 30% SWF & 40 rpm

(e) 100 ºC, 50% SWF & 200 rpm (f) 120 ºC, 50% SWF & 200 rpm (g) 140 ºC, 50% SWF & 200 rpm (h) 140 ºC, 30% SWF & 200 rpm

(i) 100 ºC, 50% SWF & 40 rpm (j)120 ºC, 50% SWF & 40 rpm (k) 140 ºC, 50% SWF & 40 rpm (l) 140 ºC, 30% SWF & 40 rpm

(m) 100 ºC, 50% SWF & 200 rpm (n) 120 ºC, 50% SWF & 200 rpm (o) 140 ºC, 50% SWF & 200 rpm (p) 140 ºC, 30% SWF & 200 rpm

Figure 7.8 XY and XZ plane distribution of the shear rate for different process conditions. Grey zones represent the location of screws. Images were taken at time step 40.

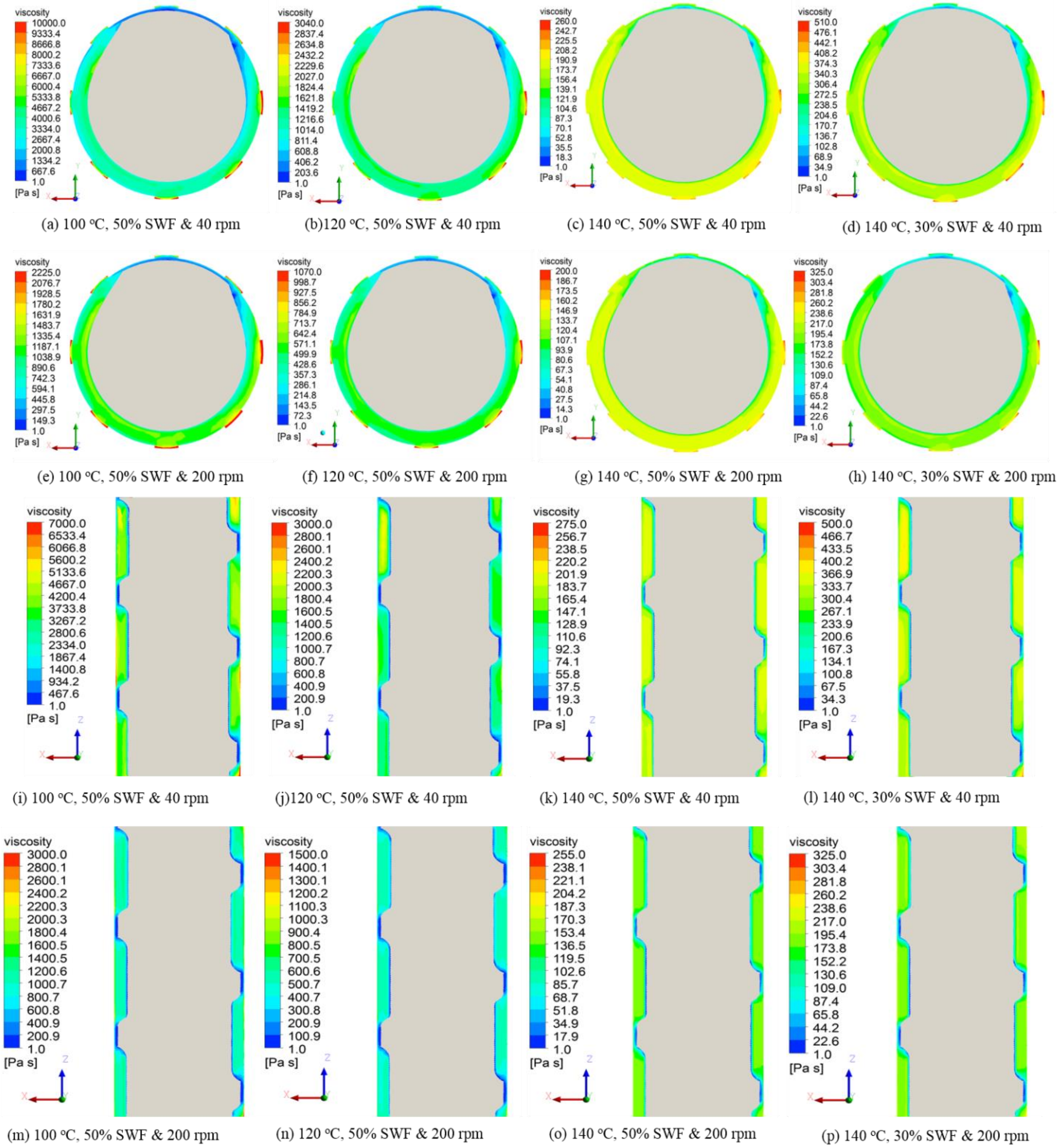


Figure 7.9 XY and XZ plane distribution of the viscosity for different process conditions. Grey zones represent the location of screws. Images were taken at time step 40.

CHAPTER 8

Conclusions

The main goal of this research was to maximize the amount of soy white flakes (SWF) incorporation as an alternative source of protein in the production of aquaculture feed and to understand the significant effects of extrusion processing such as ingredient composition, initial feed moisture content and processing temperature on physical, nutritional and rheological properties of extrudates. From our studies, we can conclude that 47% SWF can be used as a potential replacement to fish meal for the production of aquaculture feeds using a single screw extruder. Desired physical and nutritional properties of the feed can be achieved with optimum moisture content of 20%(db), barrel and die temperature of 139°C and screw speed of 198 rpm.

The specific conclusions of the investigation are as follows:

1. In the primary investigation of inclusion of SWF, increasing the level of SWF from 10% to 30%, significantly increased the value of WAI from 3.98 to 4.26 and UD from 0.89 g/cm^3 to 0.92 g/cm^3 but decreased the value of ER from 1.17 to 1.13 (α=0.05). In second study, further increase in the level of SWF content up to 50% resulted in significant increase in pellet durability and water absorption index.
2. Increasing moisture content from 15% to 35% resulted in a 37.5% increase in WAI and 17% and 14% decrease in bulk density and WSI, respectively.
3. As barrel temperature increased from 110 to 170°C, WAI and WSI increased by 12.3% and 4.3%, respectively. But there was a decrease in BD by 12% and UD by 28%.

4. Increasing L/D ratio from 3.33 to 7.25 resulted in increase in pellet durability index, expansion ratio, but a decrease in bulk density of the extrudates.
5. Increasing screw speed resulted in increase in pellet durability and mass flow rate but decrease in water absorption index of the extrudates.
6. SWF, moisture content, barrel temperature had significant effects on the nutritional properties of extruded aquafeed. As level of SWF content was increased from 20 to 50%, there was an increase in lysine content, TIA, protein content, ash content and decrease fat and fiber content. Increasing the barrel temperature from 110°C to 140°C decreased the lysine content and TIA in the extrudates.
7. Aquaculture feed mixes used in this study exhibited shear thinning behavior for dough containing SWF up to 50%. By power law modeling of the observed apparent viscosity during processing, it was observed, the viscosity of the dough is greatly affected by the level of temperature. Increasing the SWF content resulted in a higher mass flow rate, torque and specific mechanical energy. The specific mechanical energy and mass flow rate increased and viscosity decreased as screw speed increased. At higher temperatures in the barrel and the die, the viscosity of the dough decreased, which led to a decreased torque requirement and specific mechanical energy.
8. The flow of dough in a single screw extruder was simulated by using CFD and validation of simulation was done by comparing torque values. The results shows that the experimental and computational values obtained at different process conditions were in good agreement. Additionally, the flow profile in the extruder was analyzed by using the influence of screw speed and SWF content. The results

suggest that high shear zones are generated at the clearances between screws and barrel at which the flow is dominated by less viscous fluid. Increase in screw speed from 40 to 200 rpm led to lower shear rate. Moreover, to investigate the influence of SWF content, three levels of SWF content were simulated. The results show that the increase in SWF content from 30 to 50% led to decrease in viscosity. The results of the simulation can be used to optimize the extrusion process influenced by complex flows of relatively high concentration of SWF based aquafeed dough in an extruder and also to improve the product quality.

Overall SWF is a promising protein alternative for the aquaculture industry. Yet, more research needs to be done to examine nutritional efficacy by conducting feeding trials. The utilization of SWF in aquafeed will have value addition for soybean processing industry.

CHAPTER 9

Recommendations for Future Work

This research was conducted in order to maximize the amount of soy white flakes (SWF) incorporation into aquaculture feed particularly for Catla species and to understand the effects of extrusion processing such as ingredient composition, initial feed moisture content and processing temperature on physical, nutritional and rheological properties of extrudates. The results demonstrated that SWF has greater potential to be used as an alternative source of protein in aquaculture feed production. However, further studies are required in coordination with the fisheries scientists on feeding trials etc., so that the benefit of utilizing SWF for extruded aquaculture feed production can be understood in totality. For this purpose, some of our suggestions for future research include:

1. The formulations tried in our experiments were aimed at the protein requirement of Catla fish species. Extrusion trials could be conducted using ingredient mixes with different levels of SWF and net protein content adjusted to protein requirement of other fish species.
2. Further studies need to be done in coordination with the fisheries scientists on feeding trials to examine nutritional efficacy.
3. Further investigations need to be carried out on enrobing of oils on the extrudates in order to determine the oil absorption capacity and to increase the net energy content of extrudates containing SWF.

4. Experiments need to be conducted to study the effect of extrusion processing on lectin activity that would help to enhance the inclusion of soy-protein based in aqua diets.
5. Effect of extrusion on bioavailability of methionine, an essential amino acid for aquaculture diets, is also recommended.
6. Developing a model to predict the loss of nutrient based on thermal properties of the aqua diets as a function of extrusion processing parameters would be desirable.
7. Experiments need to be conducted to study the effect of processing parameters on physical, nutritional and rheological properties of SWF based aquaculture feed in a twin screw extruder.
8. Studying the effect of screw compression ratio on properties of extrudates in a single and twin screw extruder would be desirable.

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