Impact of Storage and Different Variety on Proximate Composition and Functionality of Lentils

BY

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THESIS ACCEPTANCE PAGE
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This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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Table of Contents

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

IMPACT OF STORAGE AND DIFFERENT VARIETY ON PROXIMATE COMPOSITION AND FUNCTIONALITY OF LENTILS

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Lentil (*Lens culinaris*) is an edible pulse and is a nutritious legume. The nutrient value and the application of lentils in other food resources have been well documented. However, there exists a lack of studies that focus on the storage impact of pulses on lentil composition and functionality. The focus of most studies has been on the composition of lentils while the composition relative to and functionality of stored lentils has not been sufficiently evaluated. Therefore, the objective of this research was to determine the effect of storage conditions on the nutrient composition and the functional properties of lentils. Four varieties of lentil (i.e., Avondale, CDC Richlea, CDC Maxim, and Pardina) were stored at room temperature (21-22 °C) and 40 °C and different relative humidity (40% and 55%) in sealed containers for 360 days. Statistical analysis using multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA) showed that lentils were impacted by different storage conditions. Over 360 days, there was higher starch content (%) of lentil flours stored at high temperature HT, (40°C) while lower protein content was observed in the samples stored under diverse storage conditions. Increasing days of storage resulted in water-holding capacity of lentil flour stored at (HT, 40 °C) and high relative humidity (HRH, 55 %). With increasing days of storage, lentils stored under

40 % and 55% RHs at 40 °C had significant color change, where all varieties tended to have a darker color after 360 days of storage suggesting enzymatic browning of the lentil seeds. Storage impacted the starch functionality (i.e., lower final viscosity, setback viscosity, gel strength; higher peak viscosity compared to the control sample). Therefore, increasing days of storage, and high temperature and relative humidity were observed to be the harshest storage conditions. The outcome of this study shows that storage conditions can substantially impact the nutrient profile and functionality of lentil flours and provided producers with knowledge of how quality is affected by storage. Based on this study, storage of lentils with low relative humidity (40 %) and low temperature (21 °C) is suggested for long-term storage.

1. INTRODUCTION

1.1. General Introduction

Pulses are the edible seeds of plants in the legume family. Pulses grow in pods and come in a variety of shapes, sizes, and colors. Lentil is one of the highest nutrient-dense food resources that have been consume in many countries. Canada and India are the largest producers of lentils (Kumar et al., 2013). Lentil (*Lens culinaris*) is an edible pulse and is a particularly nutritious legume with ample amounts of carbohydrates, protein, minerals, vitamins, phytochemicals, and fiber (Matina Joshi et al., 2017). Lentils are considered suitable ingredients to develop innovative products such as pasta, noodles, snacks, plant-based meat alternatives, and baked goods due to their functional properties. Lentil is one of the highest nutrient-dense food resources that have been consumed in many countries.

The storage conditions impact the proximate composition and functionality of pulses and legumes. Storage causes significant changes in the technological, sensory, and nutritional quality properties of beans (C. D. Ferreira et al., 2017). Garruti & Bourne (1985) reported that legumes stored at elevated temperatures (>25 °C) and relative humidity (>65%), which are common in tropical weather conditions, require more time to cook. This results in high processing and cooking costs due to high energy consumption, inferior nutritional quality, and negative changes in texture and palatability of pulses. Nutrient value of pulses and the application of pulses in other food resources have been the focus of many studies. For example, lentil protein was added to doughnuts to make the doughnut healthier and high in protein (Eckert et al., 2018). Additionally, studies on functional characteristics are absent. However, there exists a lack of information that focuses on the storage impact of pulses on lentil composition and functionality. The focus

of most studies has been on the composition of lentils while the composition relative to and functionality of stored lentils has not been sufficiently evaluated. As a result, there is a need for a study that can provide specific details on the significant impact of storage on functional and compositional changes of pulses. For this reason, this study has been conducted to determine the effect of storage conditions on the nutrient composition and the functional properties of lentils and to provide producers with knowledge of how quality is affected by storage and how long lentil can be retained before quality is affected.

1.2. Research Objectives

I hypothesis that different storage conditions will affect the nutrient composition and functional properties of lentils differently based on storage condition. The effects of various storage conditions on lentils are comparatively limited, according to the available literature. As a result, the first focus of this study was to establish the effect of storage on lentil functionality and composition. Lentil samples were stored under a range of environmental conditions. The changes in functional and composition profiles were then measured. The chemical composition and functional properties of stored lentils were determined using analytical methods such as water absorption index, water solubility index, pasting properties, and foaming and emulsion properties. Therefore, the hypothesis was tested by performing the following objectives on lentils:

1) To determine the proximate composition of stored lentils samples.
2) To characterize the techno-functionality of stored lentils samples.
3) To determine the impacts of storage on different cultivars of lentils.

1.3. General Experimental Approach

Four different lentils cultivars (i.e., Avondale, CDC Richlea, CDC Maxim, and Pardina) provided by seed handlers were stored at two different temperatures (21 ℃ and 40 ℃), and two different relative humidity (40% and 55%) in sealed containers. Then, whole lentil seeds of each variety were subjected to proximate analysis (i.e., moisture content) and physical analysis (i.e., cook firmness and color) while milled lentils of each variety were subjected to proximate composition (i.e., moisture, ash, lipid, protein, and starch) and functional properties (i.e., pasting properties, gel strength, water absorption index, water solubility index, water holding capacity, oil holding capacity, and foaming and emulsion properties). The storage plan targeted 360 days and sampling days were 0, 30, 60, 90, 180, 270, and 360 days. The changes during storage were evaluated.

2. LITERATURE REVIEW

2.1. Introduction to Pulses and Lentils

Pulses, including lentils, are edible seeds of legumes and are eaten globally as whole grains or as decorticated and separated kernels (Joshi et al., 2017). In the Middle East and South Asia, lentils are a staple food and are mostly consumed with cereal grains such as rice (Matina Joshi et al., 2017). There are 11 types of pulses: dry beans, dry broad beans, dry peas, chickpeas, cowpea, Pigeon pea, lentil, Bambara ground nut, vetch, lupins, and minor pulses are grown worldwide and are recognized by the United Nations Food and Agriculture Organization (FAO). Dry peas, lentils, beans, and chickpeas are the four major types of pulses.

Generally, in the spring, lentil crops are sown and harvested at the end of the season, which is normally hot and dry. Lentil seeds are also susceptible to mechanical damage, especially at low moisture levels during harvesting and handling operations (Kumar et al., 2013). During 2009-2013, the average annual global production of lentils was recorded at 4.45 million metric tons and Canada, Turkey, the USA, Australia, and India are the largest lentil-producing countries, contributing more than three-fourths of the overall lentil production worldwide. The largest producer of lentils in the world in 2017 was Canada with a share of 3.73 million metric tons (MMT), followed by India (1.22 MMT), Turkey (430 thousand metric tons, TMT), the United States (339 TMT), Kazakhstan (313 TMT), Nepal (254 TMT), Australia (221 TMT), Russian Federation (197 TMT), China (171 TMT), and Bangladesh (168 TMT) (Statista, 2017). Total lentil production was recorded at 6,537,581 metric tons in 2020. It has increased 13% compared with previous year in 2019 (FAO STAT, 2020).

Lentils have significant impacts on health and the environment. In 2019-20, cooked lentil puree was included in research designed to assess the environmental and nutritional impacts of reformulating beef burgers (Chaudhary & Tremorin, 2020). The authors showed that the application of blended beef/lentil decreases the environmental footprint (i.e., greenhouse gas, water, and land-use footprints) by 33% and increases the nutrient density of the reformulated lean beef burgers by around 20% (Chaudhary & Tremorin, 2020). Consequently, lentil is a nutritious legume with ample amounts of carbohydrates, protein, dietary fiber, vitamins, and minerals.

2.2. Lentil Seed Structure

Joshi et al. (2017) described lentil seeds as tiny lens-shaped seeds that come in a variety of colors, from yellow to red orange to green, brown, or black. The seed coat, cotyledons, and embryo, which include the radical, plumule, and embryonic axis, are the primary anatomical elements of a lentil seed. The seed coat, cotyledons, and embryo make up around 8%, 90%, and 2%, respectively, of the dry seed weight. Cotyledons are made up of parenchymatous cells with starch granules scattered throughout the protein bodies. Each cotyledon cell is attached to the central lamella, and there is a visible intercellular gap between adjacent cells (Tang and Sokhansanj, 1993). The parts of the lentils lead to the diverse nutrient composition.

2.3. Nutritional Composition of Lentil

Lentils (*Lens culinaris*) are one of the largest significant pulse crops in the world due to their high nutritional quality. Lentils are high in complex carbohydrates (e.g., starch; 35.9 – 45.6 %), protein (18.7- 28.8 %), dietary fiber (15 – 22 %), vitamins, minerals, and high energetic value (N. Wang et al., 2009; Hall, 2018). Lentil protein is rich in endogenous amino acids (e.g., arginine 7.8 %, leucine 7.2 %, lysine 6.7 %, alanine 4.2 %, glycine 4.1 % isoleucine 4.1 %, and histidine 2.4 %) (Khazaei et al., 2019). Lentils are an important source of potassium, phosphorus, magnesium, and calcium (Hall, 2018). In Canadian, Wang and Daun (2006) found that potassium (K) was the most abundant element in lentils, with a mean value of 1055.1 mg/100 g. Phosphorus (P), magnesium (Mg), and calcium (Ca) were the next most abundant and ranged from 344.7 to 725.8, 121.5 to 167.1, and 48.4 to 107.7 mg/100 g, respectively. Copper (Cu) and iron (Fe) varied between 0.8 and 1.3, and 6.6 and 9.8 mg/100 g, respectively, from 1.2 to 2.9 mg/100 g

for manganese (Mn), and from 2.9 to 5.9 mg/100 g for zinc (Zn) (Wang & Daun, 2006). Based on the reported data, the intake of 260 g of lentils per day provided enough Mg to meet the recommended daily allowance of 350 mg per person, and 160 g provided the recommended daily allowance of P (800 mg) and Fe (10 mg) for adults. Sufficient K to meet the recommended daily allowance of 99 mg per person is achieved by the consumption of 10 g of lentils per day (Wang & Daun, 2006). As a result, assessing the impacts of storage on lentils is warranted to determine if the nutrient is affected.

2.4. Health Benefits of Pulses

Pulses are high in protein and fiber, as well as vitamins and minerals like iron, zinc, folate, and magnesium, and eating half a cup of beans or peas per day can improve diet quality by increasing intakes of these nutrients (Table1). Furthermore, the phytochemicals, saponins, and tannins found in pulses have antioxidant and anti-carcinogenic properties, indicating that pulses may have anti-cancer properties. Consumption of pulses also improves serum lipid profiles and has a positive effect on several other cardiovascular disease risk factors, including blood pressure, platelet activity, and inflammation. Pulses are high in fiber and have a low glycemic index, making them especially beneficial to diabetics by aiding in the maintenance of healthy blood glucose and insulin levels (Mudryj et al. 2014). Therefore, lentil nutritional properties have been linked to cholesterol and lipid-lowering effects in humans, as well as a reduction in the incidence of colon cancer and type 2 diabetes (Roy et al. 2010).

Table 1.Health improving effect of lentil components (Shahwar et al., 2017)

Health effect	Responsible component
Antioxidant	Vitamin E, β-carotene, polyphenolics
Anticancer	Flavonoids, BBI, phytic acid, polyaterols, defensin, Lectins, RS, saponins, β-carotene
Antibiotic	Defensin
Anti-inflammatory	Phytosterols, BBI
Hypolipidemic	Phytosterols
Reduction of glycemic load	Resistant starches (RS) when replacing digestible starch
Blood pressure-lowering effect	K, proteins
Laxative	Insoluble dietary fiber, RS
Bifidogenic	Raffinose family oligosaccharides, RS

2.4.1. Lentils and Cardiovascular Health

Pulse grain consumption has been shown to lower serum cholesterol while increasing cholesterol saturation in the bile. Total serum cholesterol was reduced by 7%, LDL cholesterol by 6%, and serum triacylglycerols by more than 17%, with no change in HDL cholesterol (Anderson & Major, 2002). Consuming legumes has been linked to a lower risk of coronary heart disease (CHD) and cardiovascular disease (CVD). Consuming legumes four times or more per week versus less than once per week was associated with a 22% lower risk of CHD and an 11% lower risk of CVD (Shahwar et al. 2017). Many

systemic diseases, including cardiovascular disease, have been linked to chronic arsenic exposure. Selenium has been shown to aid in the elimination of arsenic as from the body and was studied to determine if a high-selenium lentil diet affected blood pressure and plasma lipid levels in an arsenic-exposed population. The control group incorporated low-selenium lentils into their diet, while the treatment group consumed high-selenium lentils. It was discovered women in the treatment group had lower cholesterol levels than the control group at 3 months when compliance was highest, and men in the treatment group had higher HDL than the control group (Krohn et al., 2022). No significant differences were observed in blood pressure; thus, increased dietary selenium may have a beneficial effect on cholesterol status in the human body (Krohn et al. 2022). The findings of this clinical trial indicate that high-Se lentils have a beneficial effect on the cholesterol profile of As-exposed people, as evidenced by a decrease in total cholesterol in women and an increase in HDL in men after 3 months. The improved lipid profile observed in both the control and treatment groups highlights the health benefits of increasing lentil consumption (Krohn et al. 2022).

2.4.2. Lentils and Diabetes

Pulses are crucial in the prevention and management of diabetes. As a result, consuming a diverse range of carbohydrate foods derived from pulses and other high-carbohydrate sources would be beneficial for both the general population and people with diabetes. Lentil fibers were found to prevent metabolic control impairment in diabetic rats when total carbohydrate intake was increased, implying that lentil carbohydrates, including their dietary fibers, may have promising implications for diabetic patients (Calle-Pascual et al., 1986), and lentils also have a low glycemic index (GI), which allows glucose to

enter the bloodstream slowly and creates a constant insulin response. Pulses have a low GI because they contain a high amount of non-starch polysaccharides, resistant starch, and oligosaccharides (Mark, 1999). Regular consumption of cooked lentils (50 g) by diabetic patients results in significant reductions in fasting blood sugar levels (FBS), glycemic load, and glycemic index in diabetic animals induced by streptozotocin (STZ), thus; the presence of polyphenols in lentils has been linked to health-promoting effects on metabolic disorders such as diabetes, obesity, coronary heart disease, and CVD, resulting in glycemic index reductions from the diet (Ganesan & Xu, 2017). Furthermore, in vitro and in vivo studies have shown that including lentils in the diet regulates starch digestibility, glycemic load, and glycemic index, all of which reduce diabetes complications (Ganesan & Xu, 2017). Therefore, a lentil-rich diet was found to be an effective treatment and management strategy for diabetes prevention and some other diseases.

2.4.3. Lentils and Cancer

Inverse correlations between pulse consumption and colon cancer mortality, as well as risks of prostate cancer, gastric cancer, and pancreatic cancer, have been reported in epidemiological studies (Jain et al., 2009). It has also been reported that bean or lentil consumption is associated with a lower risk of breast cancer (Mudryj et al. 2014). Bioactive proteins, including lectins, may play an important role in lowering the risk of cancer. According to epidemiological studies, eating a plant-based diet is strongly associated with a lower risk of developing certain types of cancer, and this could be because plants contain a variety of physiologically active components or phytochemicals that can alter the biochemical pathways involved in cancer initiation, promotion, or

progression (González et al. 2005). Food bioactive compounds are molecules that have a biological effect on the body that goes beyond basic nutrition. Pulses such as lentils contain both nutrients and antinutritional factors that can have positive bioactive properties (Singh et al., 2003). Bioactive peptides derived from pulse proteins, for example, may have antioxidant, antihypertensive, and anticancer properties; polyphenols have been shown to have antioxidant properties; and oligosaccharides and polysaccharides produce short-chain fatty acids, which have a variety of physiological benefits, including promoting intestinal health (Sarojini et al. 2021). Lentil is an excellent source of the B-vitamin folate, folate may play a protective role against colorectal, cervical, breast, and pharyngeal cancers, and therefore, significant evidence links plant-based diets, including pulses, to a lower risk of a variety of cancers (Patterson et al. 2009).

2.4.4. Lentil and Obesity

Obesity is often considered the root cause of other illnesses such as heart disease, cancer, and diabetes (Durstine et al., 2013). Lentil is high in protein, low in fat, and high in micronutrients, making it an excellent whole food for combating obesity-related non-communicable diseases. Lentils have been shown to increase satiation (meal completion) when compared to a meal of pasta and sauce, and lentils and yellow peas have been shown to reduce appetite and energy intake at a subsequent meal when compared to a meal of macaroni and cheese (Rebello et al. 2014). Moreover, the high fiber content and low glycemic response of lentils have been investigated as a means of increasing satiety, decreasing food intake, and thus controlling body weight. Lentils have the highest satiating properties of the four different pulses, resulting in lower food intake when

compared to other dietary meals (Mollard et al., 2012). Some observational studies show a consistent inverse relationship between pulse consumption and BMI or obesity risk (Shahwar et al. 2017). Prebiotic-rich meals alter microbial colonies in the human gut, resulting in improved satiety, intestinal motility modulation, generation of short-chain fatty acids, diarrhea, constipation avoidance, and pathogen colonization reduction (Dumas et al., 2006 and Mollard et al., 2012). Furthermore, eating a prebiotic-rich diet may help to boost the immune system, improve mineral absorption (particularly iron and selenium), and reduce obesity and metabolic syndrome risk factors (Siva et al. 2018). As a result, pulse consumption may affect satiety, which can assist consumers in overcoming environmental cues to eat or adhering to calorie restrictions. Studying the lentil seed structure is important to better understand the benefits of consuming lentils.

2.5. Lentil Protein

Metabolic and storage proteins are the two main forms of protein contained in lentil seeds. While storage proteins make up to 80% of the total protein in seeds, metabolic proteins, such as enzymes and structural proteins, are minor protein constituents (Joshi et al., 2017). Around 70 % are globulins, 16 % of lentil proteins are albumins, 11 % are glutelin, and 3 % are prolamins (Boye et al., 2010a). Legumin- and vicilin-like proteins are both globulins. The first group consists of six pairs of polypeptides with noncovalent interactions while the second group of proteins is generally isolated from seed extracts as trimers of glycosylated subunits (Jarpa-Parra, 2018).

2.5.1. Physicochemical and Functional Properties of Lentil Protein

The functional characteristics of proteins, such as solubility, water absorption, gelation, foaming, and emulsification, as well as their capacity to enhance the look, texture, and

mouthfeel of processed meals, are what determine how lentils are used by industry. Protein physical, chemical, and structural characteristics are connected to their functional characteristics. The intricate interactions between proteins, other food components, and the environment in which the proteins are present give rise to functional qualities. Therefore, a greater understanding of the physicochemical characteristics of proteins aids in the application of these proteins appropriately and offers better insights into their hydrothermal behavior (Kumar et al., 2013).

2.5.2. Solubility

Since most of the other functional qualities of proteins rely on solubility, it is one of the most crucial functional features of proteins. The solubility of proteins is significantly influenced by preparation techniques, particularly the temperature stress to which the protein is exposed. Proteins become less solubilized when heated; the degree of this reduction depends on the intensity and length of the heat treatment, therefore solubility is a sign that the protein is becoming denatured (Joshi et al., 2017). Alrosan et al. (2021) stated that the low solubility of lentil proteins (LPs) is one of the significant factors that limit their use in food applications. However, whey protein isolates (WPIs), which have high solubility and are used in various food industries, were mixed with LPs at pH 12 to create LP-WPI protein complexes with improved water solubility properties using the pH-recycling approach. The pH-recycling method has been successfully used to produce novel protein complexes with enhanced functional characteristics (Alrosan et al., 2021). Lentils are a significant source of plant-based protein, but their use in the food industry is limited since their proteins are poorly soluble in water (LPs). However, the LP-WPI protein complex was created by combining lentil proteins with whey protein isolates

(WPIs). The LP-WPI protein complex had a water solubility that was up to 92 % higher than that of LPs. Additionally, this could improve the functionality of lentils and be applied to food products. As a result, high food solubility indicates high digestibility, which may indicate an excellent use for new formulas and food; however, insolubility, is the inability of food to dissolve in a liquid, gaseous, or solid solvent (David et al., 2015).

2.5.3. Water Absorption Capacity

The availability of polar amino acids in a protein's chemical structure determines its water absorption capacity (WAC). Polar amino acids are the primary sites for water interaction (Kumar et al., 2013). The term "water absorption capacity" (WAC), also known as "water hydration capacity," "water holding capacity," and "water binding capacity," describes how much water is absorbed by flour or food per gram of protein or the ability of proteins to retain water in the face of gravity separation to produce the desired consistency (Godswill et al., 2019). When water is introduced to flour, the hydration process starts when starch and protein molecules interact hydrophobically and form hydrogen bonds with water molecules (Lam et al., 2018). Water holding capacity is the ability to physically hold water and is a very important functional property required in flours for many food applications (Ma et al., 2011a). Pathiratne et al. (2015) reported that in comparison to the raw and other treatments, the lentil flours made from 16 and 23 % tempered seeds micronized to 150 and 165 °C had significantly ($p < 0.05$) higher WHC values, demonstrating the pronounced effect of higher temperatures as well as at high tempering moisture levels on the water holding components of lentil flour. Also, these lentil flours may have had higher WHC because of the interaction between moisture and

heat. High WHC flours may be useful in baking applications since they can enhance the dough's handling properties (Godswill et al., 2019).

2.5.4. Emulsifying Properties

When a protein is used as an emulsifier or stabilizer, its emulsifying and interfacial qualities are crucial functional characteristics. The velocity of adsorption at the oil-water interface, the amount of protein adsorbed, the conformational rearrangement of the protein at the interface, the degree of interfacial tension reduction, and the development of a cohesive film all have an impact on a protein's ability to emulsify (Joshi et al., 2017). Due to the density differential and the energetically unfavorable contact between oil and water, emulsions are thermodynamically unstable systems. Emulsion preparation is critically dependent on the addition of emulsifiers and/or thickening agents. Stabilizers are a term that can be used to describe both emulsifiers and thickening agents. An emulsifier and a thickener differ from one another in the characteristics they give emulsion systems. When oil and water droplets in an emulsion are purposefully disrupted to produce a continuous phase during homogenization, emulsifiers are used to stop the droplets from separating and consolidating (Ma, 2012). Although soy is the most well-known plant protein source, interest in proteins from other legumes, such as lentils, is expanding. Amphiphilic proteins, which produce relatively thick interfacial layers around oil droplets to improve emulsion formation and stability, are present in pulse proteins. Accordingly, it has been demonstrated that lentil proteins are highly efficient natural emulsifiers for oil-in-water emulsion systems because they are remarkably resistant to environmental and compositional challenges like heat, pH, and the addition of salts (Alonso-Miravalles et al., 2020).

2.5.5. Gelation Properties

One of the most crucial functions of globular proteins is gelation, which is utilized to alter the texture of food. A protein gel is described as a three-dimensional, well-defined network made up of protein molecules dissolved in water (Ikeda & Nishinari, 2001). The conformational shift or partial denaturation of protein molecules, followed by progressive association or aggregation into a three-dimensional matrix structure that traps water, fat, and other dietary ingredients, are the two steps of the globular protein gelation mechanism. Moreover, heat treatment, salts, pressure or shearing, and the presence of different solvents can all cause protein gelation. The bulk of food protein gels is created by heating the ingredients. Numerous researchers have examined the effects of several variables on the heat-induced gelation of pea proteins, including the cultivar, the extraction method, the protein's variability, the solvent parameters, and the heating protocol (Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015). Proteins and carbohydrates, particularly starch, are responsible for good gelling properties. Additionally, the presence of carbohydrates increases the degree of connections between protein molecules and decreases the thermodynamic affinity of the protein for aqueous solution, boosting the gelling capacity (Godswill et al., 2019). The gelation properties of lentil protein isolate obtained through three distinct drying procedures were compared by (Joshi et al., 2011). In comparison to vacuum-dried powder, the authors discovered that spray and freeze-dried powders have better gelation ability and higher gel strength. Both spray- and freeze-dried gels displayed the expected properties of viscoelastic gels, with G' predominating over G" and a smaller value of loss tangent. In comparison to spray-

and freeze-dried powders, the vacuum-dried powder had a longer holding time at 90°C before gelation occurred.

2.6. Lentil Starch

The amount of carbohydrates in lentil seeds ranges from 35 to 53 % depending on the cultivars, with the majority of those being in the form of starch. In the seed cotyledons, starch is present as starch granules scattered throughout the protein matrix. Starch serves a variety of purposes in the food industry, including thickening, coating, gelling, adhesion, and encapsulation. It is also used as a minor ingredient (Kumar et al., 2013). Lentil starch composition is mostly combined with amylose and amylopectin, and also, the ratio of amylose to amylopectin changes depending on the botanical source of the starch, and it has a significant impact on the functionality and granular structure of the starch (Joshi et al., 2017). Because of its film-forming properties, high amylose starch is preferred for fried food coating batter, which provides a crispy texture in deep-fried products. High amylose starch is also well-known for its excellent film-forming properties. Lentil starch has a high amylose content, ranging from 29 to 45.5 % (Joshi et al., 2013).

2.6.1. Granule Structure of Lentil Starch

The structural periodicity of semi-crystalline native starch granules is hierarchical (Donald, 2001). Starch granules have a distinct layered structure when examined under a light microscope. This feature is caused by multiple concentric shells of increasing diameter extending from the hilum to the granule surface. These are called 'growth rings,' and they represent the periodic deposition of starch (Kumar et al., 2013). Joshi et al. (2017) explained that these layers alternate between high and low density, refractive

index, crystallinity, and acid and enzymatic hydrolysis resistance. The low-density amorphous rings are made up of disordered amylose and amylopectin, whereas the dense semi-crystalline rings are made up of a lamellar structure with alternating crystalline and amorphous regions (Cameron & Donald, 1992). The lamellae's crystalline regions are primarily formed by double helices of amylopectin side chains packed laterally into a crystalline lattice, whereas the amorphous regions contain amylose and amylopectin branching points (Joshi et al., 2017).

2.6.2. Gelatinization Characteristics of Lentil Starch

When starch is heated in the excess of water, at a characteristic temperature range it swells irreversibly and undergoes an order-to-disorder phase transition (Cooke & Gidley, 1992). This phenomenon is known as gelatinization and the characteristic temperature range at which this transition occurs is called gelatinization temperature. Gelatinization temperature is unique and characteristic of certain starch and is dependent on the botanic source and its composition. Crucial functional properties of starch such as pasting, and gelation are derived once the starch is gelatinized. The gelatinization causes the starch granules to enlarge, lose their crystallinity, uncoil and separate from their double helices (crystal melting), and lastly, become disrupted. At high concentrations, starch creates a three-dimensional gel network, delays phase separation, and gives foods like bread, cakes, and puddings their basic structure (Chen et al., 1997). To determine the gelatinization characteristics, such as the onset, peak, and conclusion point temperatures and the enthalpy of gelatinization, two typical instruments are used and include the rapid visco analyzer (RVA) and differential scanning calorimeter (DSC). The loss of double helical organization is what causes the heat of enthalpy recorded by (Gidley & Cooke,

1991). These gelatinization characteristics are not affected by the amylose (AM) to amylopectin (AP) ratio, but rather by the crystalline region's molecular architecture, which correlates to the distribution of amylopectin short chains (Joshi et al., 2017).

2.6.3. Swelling Behavior of Lentil Starch

Schoch & Maywald (1968) stated that in comparison to tuber starches, legume starches exhibit a single and restricted swelling behavior and a low amount of amylose leaching. This limited swelling is due to their high amylose content and close packing within the amorphous domains of the pulse starches, with strong interaction via hydrogen bonding between adjacent amylose chains. The swelling of starch granules should account for both intergranular and intragranular water. Most starches begin to swell at temperatures above 60°C, with a significant increase in swelling when temperatures exceed 70°C. The water hydration capacity of all lentils ranged from 42 to 190%, the swelling capacity ranged from 60% to 183% (Hall, 2020). These parameters varied depending on the different market classes and variety.

2.6.4. Pasting and Gelation Properties

Bemiller (2011) demonstrated the formulation of pasting and gelation when starch is heated in the presence of excess water, it causes granule swelling to continue, additional amylose leaching, and disruption of the fragile swollen granules. Pasting occurs after the gelatinization of starch granules. This produces a viscoelastic mass (called a paste) composed of a continuous phase of water and a molecular dispersion of swollen granules, granule ghosts, and granule fragments of a non-dissolved starch polymer (Bemiller, 2011). During pasting, granule swelling, and starch molecular leaching continue, and a peak viscosity, primarily due to swollen granules, is reached. The fragile swollen

granules disintegrate during the hold at elevated temperature, and the viscosity drops to a trough viscosity (a process called breakdown). The phase volume of swollen granules and their deformability dominate the behavior of starch paste systems. As hot pastes, particularly those containing amylose, cool, they develop distinct solid properties, i.e., gels. The transition from a viscous fluid (paste) to an elastic gel is determined by the RVA setback value (Atwell et al., 1988). Retrogradation is the molecular process that causes a setback. In the presence of excess water, starch exhibits a unique variation in viscosity with temperature. Pasting is the change in viscosity of starch caused by heating in excess of water after gelatinization. The starch granules swell to several times their initial size during gelatinization. They eventually rupture, allowing the amylose to enter the aqueous phase. RVA is a widely used instrumental method for determining the pasting characteristics of starches (Joshi et al., 2017). Therefore, the most crucial functional characteristics of starch are gel and paste formation. Determining if the starch is appropriate for a specific gelling or pasting application requires knowledge of the pasting and gel-forming behaviors.

2.7. Food Application of Lentils

By 2050, there is predicted to be a significant increase in food demand. The world's population expansion is a major contributor to this increase (Hofstrand., 2014). Consequently, there is a need for making more food products to meet the world's growing demand for food. Moreover, the current global plant-based food market is $44.2 billion and is expected to reach $77.8 billion by 2025, and double growth by 2030 (Statista, 2021). According to the current data, an increasing number of consumers are incorporating plant-based foods into their diets, as they not only have a positive effect on

health but also the environment. Puthalpet, (2022) highlights that shifting diets from meat and other animal products to plant-based foods has a high potential for reducing carbon footprint, mitigating climate change, and improving human health. Nowadays, the lentil has significant attention in food application due to their nutritional value and their positive impact on the environment. Eckert et al. (2018) found that the lentil protein-enriched doughnuts had better cooking characteristics with reduced cooking loss and diameter reduction compared to the egg-based doughnut. Also, the sensory scores for appearance, flavor, and overall liking were rated more favorable than the animal-based doughnut. Ma (2012) found that thermally processed pulse flours may be suitable as value-added ingredients in salad dressing applications. The addition of 1-3 % lentil flour to yogurt increased acid generation during fermentation, implying a prebiotic impact. Moreover, At 1-2 % supplementation, syneresis increased, but the 1-2 % lentil flour-supplemented yogurt had similar sensory qualities to yogurt made with 1-2 % skim milk powder (Agil et al. 2013). As a result, pulse-derived ingredients can be included in novel food products such as milk substitutes, meat products, extruded products, and baked goods due to their different variety of functions.

2.8. Processing of Lentils

Lentils are generally canned or dry packaged, whole or split, for retail sale, or processed into flour since antinutrients such as phytic acid and tannins prevent lentils from being eaten fresh (Dame, 2008). Lentils are used in soups, stews, salads, snack meals, and vegetarian dishes. However, lentil flour, lentil starch, and protein concentrates have become popular ingredients in a variety of meals recently. Joshi et al. (2017) divided the processing of lentils into three levels: primary, secondary, and tertiary processing.

Cleaning, grading, and packaging are all procedures in primary processing that supply the complete seeds for direct sale to customers or downstream processing. The basic premise of primary processing is to utilize a succession of mechanical separation processes to produce complete lentil seeds in their purest form, as well as the desired quality characterized by size, shape, density, and color. Unwanted organic and inorganic contaminants such as immature seeds, stalks, metals, and stones are removed using various mesh sizes and pneumatic processes. Following this, lentil seeds are sorted and graded according to color, shape, size, and density, so that clean, uniform seeds can be used for secondary processing or packaged for direct sale to customers. Lentil secondary processing includes decortication, splitting, sorting, and polishing of whole or split seeds. Thermal processing of whole seeds into cans and jars is also done in some cases. Similarly, tertiary level lentil processing entails grinding/milling of whole or decorticated seeds as well as fractionation of protein and starch-rich components for use in a variety of food products. Although this level of lentil processing is still in its infancy, it has a promising future due to the excellent nutritional and functional properties of lentil protein and starch. The relevant sections shed light on various aspects of lentil protein and starch, such as extraction, characterization, and functional application.

2.9. Storage Impact on Chemical Composition of Pulses

A lack of reported research exists on the changes in stored pulses. However, some researches have shown that pulse nutritional content, seed consistency, sensory properties, and volatile flavor compounds are influenced by storage (Sopiwnyk et al., 2020). Chapman et al. (2010) reported that as the age of the sample (pea-packed in cans and stored indoors at no abusive temperatures) increased, there was a substantial decline

in hedonic scores for taste, texture, appearance, and overall liking for pea soup made from the stored peas. Overall, the smallest change in the appearance of the samples was observed while the largest change was observed in texture. In addition, a significant decrease in thiamin concentration occurred over time.

Azarnia et al. (2011) reported that the flavor profile of pea cultivars was greatly affected by the conditions of storage, and compared to higher temperatures, the total area of volatile compounds was lower in peas processed at 4 °C. Changes in the physical properties were found. Sopiwnyk et al. (2020) reported significant changes in the color of some different types of flour: whole yellow pea flour, split yellow pea flour, whole navy bean flour, and decorticated red lentil flour, that were stored for 0–24 months under ambient warehouse conditions. Accordingly, L* values were slightly lower for whole yellow pea flour at 18 and 24 months of storage, and b* values were lower at 12 and 24 months than for most other storage periods and no consistent trend was observed for a* values. A higher L* value was found for split yellow pea flour at 12 months of storage, but lower values were found at 18 and 24 months of storage, and no clear patterns were observed. In contrast, changes in all three-color values were observed for whole navy bean flour. Starting at 3 months of storage, whole navy bean flour had decreased L* values compared to time zero values and continued to decrease during storage. In contrast, a* continued to increase with storage, and relative to all other storage periods, higher b* values were found at 18 and 24 months of storage. No consistent trends were observed for L*, a*, or b* values in stored decorticated red lentil flour (Sopiwnyk et al., 2020).

Bragança et al. (2020) reported that the lipid content of the grains stored from 60 to 360 days was noticeably reduced from the beginning to the end of the storage. Sravanthi et al. (2013) reported that a slight change occurred in the protein content (%) of red lentils at the end of 16-week storage at 10, 20, 30, and 40 ℃ with moisture contents between 10 – 20 %. As a result, based on the current literature review, several storage studies exist for lentils, but the information related to storage conditions of different varieties of lentils is still limited. Therefore, the focus of this study was to investigate the impacts of diverse storage conditions on the composition and functionality of different lentil varieties.

3. MATERIALS AND METHODOLOGY

3.1. Materials

3.1.1. Lentil Samples

Different cultivars of lentils having different colors were obtained in duplicate from a seed handler and stored in a pool (Table 2). These duplicates were continued throughout the storage and analytical phases of the study (Figure 1). In this research, four lentil cultivars were used (Table 2). To maintain relative humidity, storage was carried out by placing samples in jars containing two-way humidity packs as described by Vatansever et al. (2021).

Table 2: Different cultivars of lentils will be used in this study.

Cultivar	Market Class	1000 seed wt.(g)	L*	a*	b*
Avondale	Medium size green lentil	49	59.76	0.75	15.39
CDC Richlea	Medium size green lentil	51	60.68	0.75	15.47
CDC Maxim	Small size red lentil	35	53.79	4.11	7.79
Pardina	Small size brown lentil	42	51.97	0.66	8.60

Sourced from Hall (2020). Color scale, L* represents lightness, a* red-green, and b* yellow-blue.

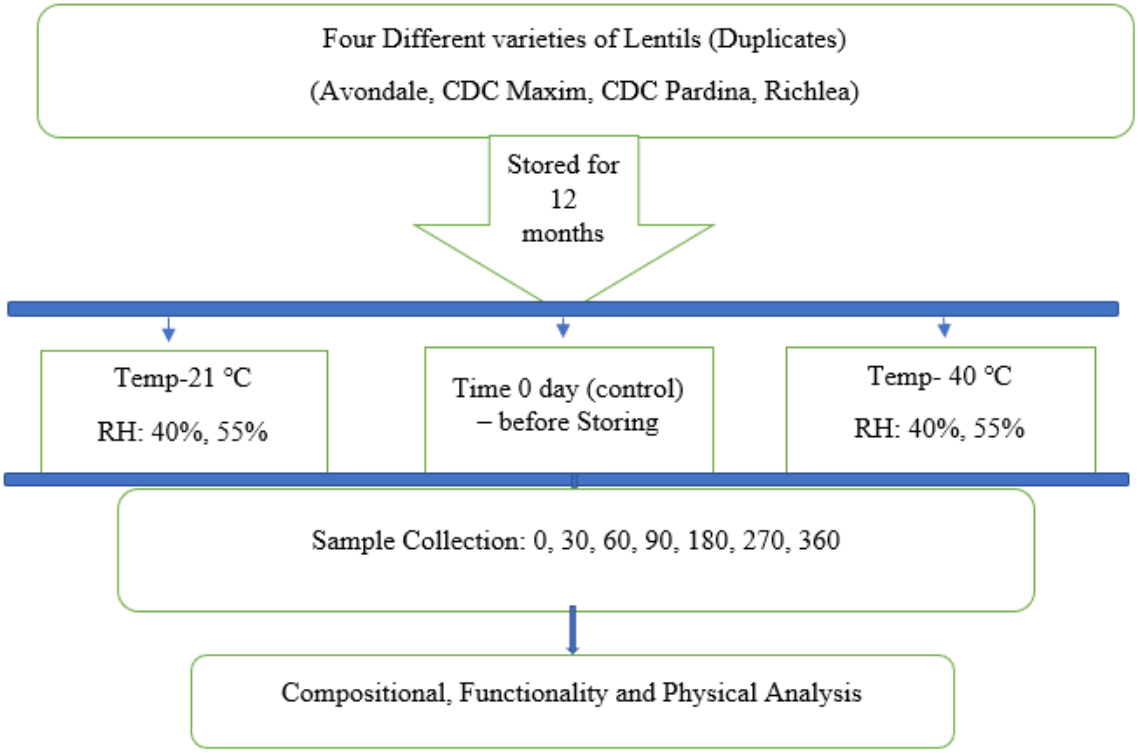

Figure 1: Overall experimental plan followed in this research.

3.2. Storage and Sample Preparation

3.2.1. Storage of Lentils

The main approach was to store the lentils at room temperature (21-22 °C) and 40 °C and different relative humidity (40% and 55%) in sealed containers. Accordingly, this range of relative humidities did not support the growth of mold as observed in preliminary studies and thus was selected for the current study. Samples were removed periodically for composition analysis and testing functionality. Lentils were collected and cleaned by mechanical and hand removal of foreign material and then split into two pools (i.e., subsample). The first pool of samples consisted of four varieties of lentils that were stored for twelve months at 21 °C and relative humidity levels of 40% and 55%. The second pool of lentils (same cultivars) was subjected to temperatures of 40 °C and relative humidity levels of 40% and 55% (Figure 2). The storage of the lentils was done in two replications following the specified sampling plan (Table 3).

Table 3: Sampling plan for lentils stored at varying temperatures and relative humidity

Varieties	Treatments	Number of Sampling	Amount of Lentils Taken per Sampling (g)	Sampling Time (Days)
4	21 °C, 40% RH	6	150	0, 30, 60, 90, 180, 270,360
4	21 °C, 55% RH	6	150	0, 30, 60, 90, 180, 270,360
4	40 °C, 40% RH	6	150	0, 30, 60, 90, 180, 270,360
4	40 °C, 55% RH	6	150	0, 30, 60, 90, 180, 270,360

3.2.2. Milling

Lentil samples collected were milled into flour using a UDY cyclone mill (UDY Corporation, Fort Collins, CO). The milling conditions include 12,600 rpm and a 0.5 mm screen. The milled flours of stored lentil cultivars were subjected to proximate composition and functionality analysis as described below.

3.3. Methods

3.3.1. Proximate Composition

3.3.1.1. Moisture Content

The official AACC International method 44-15.02 was used to determine the moisture content of flour (AACCI 2010). Lentil flour (2 g) was added to pre-weighed drying cups for each duplicate (W1). The flour and cup were weighed (W2) before placement into a 130 °C oven for 3 hours and again after cooling in a desiccator for approximately 20 minutes (W3). Moisture content was determined using the following formula:

$$\text{Moisture Content (\%)} = \frac{(\text{W2} - \text{W3})}{(\text{W2} - \text{W1}} \times 100$$

3.2.3.2. Protein Content

Samples were subjected to combustion to determine nitrogen content. The nitrogen content of lentil flour was used to calculate the protein content of the samples using the AACC Approved Methods of Analysis 46-30.01. (AACC 2010). A conversion factor of 6.25 was used to calculate protein.

3.2.3.3. Lipid Content

A modified AACC Approved Methods of Analysis official method 30-10.01 was used to determine lipid content was measured (AACC 2010). Before lipid extraction, 1.5 g of the sample was weighed into the filter bags (W2), which were then sealed and dried at 104

°C for 3 hours. Dried samples were weighed once more using the filter bag after being allowed to cool in the desiccator (W3). Hexane was employed to extract the lipid from the lentil flour after the samples were placed inside the Soxhlet device. Samples were subjected to solvent for extraction for 4 hours and 25 minutes. Samples were removed from the Soxhlet device, kept at room temperature for 5 min, and placed in a 103 °C in the oven for 30 min to remove any residual hexane. Samples were removed from the oven and allowed to cool and weighed (W4). The formula used for calculations was as follows:

$$\text{Lipid Content (\%)} = \frac{(\text{W3} - \text{W4})}{(\text{W2} - \text{W1}} \times 100$$

3.2.3.4. Ash Content

The inorganic residue that remains after the ignition or complete oxidation of organic matter in flour or food is known as ash. It denotes the total mineral content of any food. The AACC Approved Methods of Analysis method of 08-01.01 was used to determine ash content (AACCI 2010). The flour sample was heated to a high temperature. The empty crucible was weighed (W1), 1 g of flour was weighed, and the combined weight of the crucible and flour was recorded (W2). To avoid burning the sample, the oven was first set to 350°C for 1 hour, then 450°C for 1 hour, before being set to 590°C overnight. The crucible containing the ash was allowed to cool in a desiccator before being weighed again (W3). The ash content was determined using the following formula:

$$\text{Ash Content (\%)} = \frac{(\text{W3} - \text{W1})}{(\text{W2} - \text{W1}} \times 100$$

3.2.3.5. Total Starch Content

Total starch was calculated using the AACC Approved Methods of Analysis method 76-13.01 (AACC 2010). Megazyme International (Bray International) K-TSTA-50A/ K-TSTA-100A kits were used for the analysis. Sample (0.1g) was used in this method, and each sample was run in duplicate.

3.2.4. Functionality

3.2.4.1. Pasting Properties

Pasting profiles of lentil flour samples were determined using a Rapid Visco Analyzer (RVA) (Perten Instruments, Springfield, IL) using the modified AACC Approved Methods of Analysis 61-02.01. (AACC 2010). Briefly, the weights for flour (3.5 g) and water (25 g) were adjusted for flour moisture content. Furthermore, during a run, the temperature began at 50 ℃ and gradually increased to 95 ℃ over 4 minutes and 42 seconds, followed by a holding period until 7 minutes and 12 seconds into the run. The temperature was then reduced to 50 ℃ at 11 minutes and remained there until the end of the 23-minute run. The instrument recorded peak time, hot and cold paste viscosities, and breakdown viscosity. The starch prepared using the RVA was then stored at room temperature for 2 hours to cause gel formation. A texture analyzer was used to evaluate the textural properties of the gels formed in the canisters (Ta. Tx, Texture Technologies Corp, South Hamilton, MA). Each canister was placed upright on the metal plate, and the gel was compressed with a cylindrical plunger (diameter = 10 mm) at a speed of 4 mm/s to a distance of 15 mm and a trigger force of 2 g. The compression produced a force-time curve, from which the hardness (height of the first peak) was calculated.

3.2.4.2. Foaming Capacity and Stability

The foaming properties of lentil flour were determined using foaming capacity and stability (Stone et al., 2015). A slightly modified version of the procedure was used, a 1 % (w/w) protein solution (based on the weight protein content of the dry flour) was prepared with 10 mM sodium phosphate buffer (pH 7.00), and the resulting solution was stored overnight at 4 °C. After that, 15 mL of the protein solution was transferred to a narrow 400 mL glass beaker and foamed for 5 minutes with an Omni Macro homogenizer at 8000 rpm (Vfi), the volume of foam immediately after homogenization. The foam was transferred to a 100 mL graduated cylinder immediately after homogenization. The volume of foam was measured at time zero (Vfo) and again after 30 minutes (Vft). Foaming capacity (FC) and foaming stability (FS) were determined using the following equations respectively:

$$\text{FC}\ (\%) = \frac{\text{Vfi}}{\text{Vfo}} \times 100$$

$$\text{FS}\ (\%) = \frac{\text{Vft}}{\text{Vfi}} \times 100$$

3.2.4.3. Water Absorption Index (WAI) and Water Solubility Index (WSI)

A modified method (Simons et al. 2012) was used to calculate the WAI and WSI of lentil flours. The combined mass of lentil flour (2.5 g) was recorded after it was transferred to pre-weighed 50 mL centrifuge tubes. Water (30 mL) was added and vigorously shaken to break up lumps before being stirred with stir bars for 30 minutes. For 10 minutes, the mixture was centrifuged at 3000 rpm. The supernatant was decanted into pre-weighed beakers and placed in a 110 °C oven for 20 hours before being stored at 120 °C for 7 hours. The beaker containing solids was cooled and weighted. The difference compared to the pre-weighted beaker represents the solids that remained in the supernatant, which is

used to calculate WSI. WAI was calculated by weighing the tubes and the wet sediment contained within them. The WAI (g/g) and WSI (%) were calculated using the following formulas:

$$\text{WAI} = \frac{\text{Weight of the wet sediment (g)}}{\text{Initial weight of the dry flour (g)}} \times 100$$

$$\text{WSI} = \frac{\text{Weight of the solids in the supernatant (g)}}{\text{Initial weight of the dry flour (g)}} \times 100$$

3.2.4.4. Water Holding Capacity (WHC)

The procedure outlined in AACC Method 56-37 was used to calculate the WHC of lentil flour (AACCI 2010). A test tube was filled with the sample (1 g) (W1). The syringe barrel was filled with the test tube and filter cloth (put between the test tube and barrel) (W2). After removing the test tube, the flour was slowly and dropwise added to the DI water and swirled with a glass rod until wet. After one minute, the glass rod was withdrawn and cleaned with the filter cloth. The filter cloth was put at the test tube's end and retained within the barrel turned upside down. This syringe assembly was put in a 50 ml centrifuge tube and centrifuged for ten minutes at 300 g at room temperature. The final weight was taken after removing the syringe assembly from the centrifuge tube (W3) and WHC was calculated as:

$$\text{WHC} = \frac{(\text{W3} - \text{W2}) + (\text{W1} \times \text{mc})}{(1 - \text{mc})\text{W1}} \times 100$$

Where mc= initial moisture content of the sample.

3.2.4.5. Oil Absorption Capacity (OAC)

The oil absorption capacity was determined using the method described previously (Wang et al., 2020). The sample (0.5 g) was placed in a test tube. The filter paper, test tube with the sample, and syringe barrel were all weighed together. Canola oil (1.5 mL) was added to the test tube, and the mixture was vortexed for 5 seconds every 10 minutes for 20 minutes. The test tube containing the oil and sample was inverted into the syringe with the filter paper at the bottom of the test tube, and the assembly was immediately placed into a 50 mL centrifuge tube and centrifuged at 600 x g for 25 minutes. During centrifugation, free oil that was not bound to the flour passed through the filter paper and was collected in the centrifuge tube. After centrifugation, the entire assembly, including the syringe barrel, filter paper, test tube, sample, and oil absorbed, was weighed. During centrifugation, a sample blank with filter paper was also included to avoid the problem caused by some free oil entrapped in the filter paper and not collected at the bottom of the conical centrifuge tube. The oil absorption capacity was calculated as:

$$\mathrm{OAC\ (\ g\ oil\ /\ g\ sample)} = \frac{(\mathrm{W3} - \mathrm{W2} - \mathrm{W4})}{(1 - \mathrm{mc}/100)\mathrm{W1}} \times 100$$

Where W1 = weight of the sample before oil addition (g),

W2 = weight of the syringe barrel, filter paper, test tube, and sample (g),

W3 = weight of the syringe barrel, filter paper, test tube, sample, and oil absorbed after centrifugation (g),

W4 = weight of oil absorbed by the blank filter paper after centrifugation (g), mc = initial moisture content of the sample (%).

3.2.4.6. Emulsification

Emulsion activity (EA) and emulsion stability (ES) were determined as the emulsification properties (Yasumatsu et al., 1972). Using a slight modification to the method, 1.25 g of lentil flour was suspended in 48.75 g of 10 mM sodium phosphate buffer (pH 7.00) and refrigerated overnight at 4 ℃. In a beaker, 24.5 mL of flour solution was combined with 24.5 ml of canola oil. The solution was then homogenized for 3 minutes using an Omni Macro homogenizer at 8000 rpm. For EA, 10 mL of the homogenized solution was transferred to 15 mL centrifuge tubes, the height of the entire emulsion was measured, and the entire emulsion was centrifuged at 1315 x g for 5 minutes. After centrifugation, the heights of the emulsified layer were measured. For ES, the residual emulsion in the beaker was cooled to room temperature for 15 minutes in a cold-water bath after being heated to 80 ℃ in a water bath for 30 minutes. The produced emulsion was then diluted ten times into a 15 mL centrifuge tube, the height of the emulsion was measured, and the emulsion was centrifuged at 1315 x g for five minutes. The emulsified layer heights were noted. EA and ES were calculated using the following equations:

$$\text{EA}\ (\%) = \frac{\text{Height of emulsifed layer}}{\text{Height of entire emulsion in tube}} \times 100$$

$$\text{ES}(\%) = \frac{\text{Height of emulsifed layer}}{\text{Height of entire emulsion in tube}} \times 100$$

3.2.5. Physical Properties of Whole Seed

3.2.5.1. Color and Color Difference

Konica Minolta CR-410 Chroma meter (Konica Minolta, New Jersey, NJ) was utilized to determine the color and color difference of stored lentils. The instrument was first

calibrated using a standard white plate. After calibration, color measurements were randomly taken in duplicates on the lentils. L*, a*, and b* values were recorded to measure color where L* stands for lightness/darkness, a* stands for red/green and b* represents yellow/blue. A positive L* value is lighter, and a negative value is darker, a positive a* value is redder, a negative value is greener and a positive b* value is yellower, and a negative value is bluer. The color difference was then calculated through the difference in L*, a*, and b* values for 0 days and 30 days, 0 days and 60 days, and so on until 360 days using the following formula:

$$\text{Color difference } (\Delta E*) = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

3.2.5.2. Cook Firmness

To perform the cook firmness analysis, the AACC Approved Methods of Analysis method 56-36.01 (AACC 2010) method was applied. Using a texture analyzer (Ta. Tx, Texture Technologies Corp, South Hamilton, MA) this method assesses the firmness of cooked pulses. The maximum force needed to shear cooked pulses is what is referred to as the firmness of the cooked pulses, and it is stated as the maximum shear force per gram of cooked sample. This approach involved cooking 40 g of stored lentils for 25 minutes, then loading 7.5 ± 0.5 g of the cooked lentils into a Mini Kramer Shear Cell connected to a texture analyzer. System parameters were set up at a speed of 1.50 mm/s to a distance of 28 mm and a trigger force of 50 g with a load cell of 30 kg capacity to determine the firmness of cooked lentils. The maximum shear force measured was recorded. Firmness was reported as N/g.

3.2.6. Statistical Analysis

R studio software, version 1.4.1717, was used to evaluate the main effects (variety, days of storage, RH, and Temperature) and interactions using multivariate analysis of variance (MANOVA). Fisher's Least Significant Difference (LSD) at a 5% probability level was ascertained to do statistical comparisons within groups using analysis of variance (ANOVA), and the results are shown in bar graphs and tables in lowercase and capital letters.

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Lentils

3.1.1. Moisture Content of Lentils

Days of storage, temperature, relative humidity, and variety were significant (p-value <0.001) for the moisture content of lentil flour. All varieties had a similar initial moisture content at zero days of storage. Moreover, Maxim had a high moisture content of 9.67%, followed by Pardina 9.60 %, Avondale 9.52 %, and Richlea 9.20% (Table 4). Storage of lentils under diverse relative humidity storage at high temperature for 360 days resulted in higher moisture contents between different varieties of lentils. The moisture content of lentil flour was significantly higher at a high relative humidity of all varieties compared to low relative humidity (Table 4). Additionally, Pardina had the highest moisture content 8.20 to 10.20 % followed by Maxim 8.55 to 9.92 %, Avondale 8.50 to 9.90 %, and Richlea 8.30 to 9.33 %. Storage at high temperature and high relative humidity caused high moisture content with increasing days of storage i.e., 9.20 stored for 180 days and increased to 9.90 after 360 days (Table 5). Consequently, the interaction effect of days of storage and diverse relative humidity was significant (p-value < 0.001). Similarly,

Chidananda et al. (2014) reported an increase in the moisture content of different types of pulses including lentils occurred during storage for 30 days at different storage conditions. Moreover, the moisture content of green lentils went up from 11.9 % to 12.4 % after 30 days of storage.

The initial moisture content of the pulse, storage temperatures, relative humidity, and storage period all have a direct effect on respiration, which causes the release of water from the pulses (Chidananda et al., 2014). The rate of respiration increases as the temperature and moisture content rise during the storage period. Also, lentil seeds are highly hygroscopic, easily exchanging moisture with the environment (Hasan & Mohammad, 2018). The difference in the moisture content of different types of lentil flour held at various relative humidity and temperature settings can be attributed to these changes.

Table 4: Moisture content (%, ± standard deviation) of different varieties of lentil flour stored over 360 days at high temperature (40 °C) under diverse RH conditions.

Variety	**Control**	**LRH**	**HRH**
Avondale	9.52 ± 0.08 ^{c}	8.50 ± 0.05 ^{d}	9.90 ± 0.09 ^{b}
Maxim	9.67 ± 0.08 ^{c}	8.55 ± 0.05 ^{d}	9.92 ± 0.08 ^{b}
Pardina	9.60 ± 0.08 ^{c}	8.60 ± 0.04^{d}	10.20 ± 0.10 ^{a}
Richlea	9.20 ± 0.07 ^{c}	8.30 ± 0.04 ^{d}	9.33 ± 0.07 ^{c}

*Different lowercase letters represent significant differences among the varieties when stored at diverse storage conditions of RH based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%), HRH- High Relative Humidity (55%).

Table 5: Moisture content (%, ± standard deviation) of all lentil varieties at different sampling days stored over 360 days and different RH conditions at high temperature (40 °C).

Days of Storage	LRH	HRH
0 (control)	8.90 ± 0.25 ^{c}	8.90 ± 0.27 ^{c}
180	7.80± 0.35 ^{d}	9.20 ±0.50 ^{b}
270	7.82± 0.36 ^{d}	9.30 ± 0.55 ^{b}
360	7.85± 0.39 ^{d}	9.90 ± 0.60 ^{a}

*Different lowercase letters represent significant difference due to the diverse storage conditions of RH and different sampling days based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%).

3.1.2. Protein Content of Lentil Flour

Days of storage, temperature, and variety were significant (p-value <0.001) for the protein content of lentil flour. In contrast, relative humidity was not a significant factor on the protein content of lentil flour regardless of the variety, days of storage, and temperature (Table 6). The protein content (%) of lentil varieties were observed to be significantly different (p-value<0.001). The highest protein content was observed in the Maxim variety (24.69%) followed by Avondale (24.44%), Pardina (24.17%), and Richlea (22.95%) when stored for 360 days (Table 6). A reduction in the protein content impacted by the days of storage was observed as the protein content was significantly lower (p-value<0.001) after 360 days of storage than the time 0 (control) samples (Table 6). However, a slight impact was observed in the protein content of lentils stored at 40 °C compared to 0 (control) samples. Sravanthi et al.(2013) reported a decrease in the protein content of red lentils stored for 112 days in different storage conditions. Similarly, Bragança et al. (2020) reported a reduction in the protein content was identified,

especially in grains stored for 360 days at a temperature of 35 ℃. As a result, our result agreed with these results where a lower protein content occurred during over time of storage.

El-Refai et al. (1988) observed a significant decrease in the protein content of Faba beans stored for 9 months, which was attributed to the activity of proteolytic enzymes. The reduction was attributed to the extra energy needed to maintain its metabolism due to the high temperature's acute stress and increased metabolic rate. It obtained this energy from carbohydrates, particularly starch, which, when consumed, released protein that is closely associated with it (Bragança et al., 2020).

Table 6: Protein content (%, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Protein Content (%)**
Avondale	24.44 ± 0.40 [b]
Maxim	24.69 ± 0.40 [a]
Pardina	24.17 ± 0.37 [c]
Richlea	22.95 ± 0.45 [d]
p-value	<0.001
Days of Storage	**Protein Content (%)**
0 (control)	24.11 ± 0.40 [c]
180	24.57 ± 0.77 [a]
270	24.23 ± 0.77 [b]
360	23.99 ± 0.77 [d]
p-value	<0.001
Relative Humidity (RH)	**Protein Content (%)**
0 (control)	24.11 ± 0.40 [a]
40 % LRH	24.08 ± 0.81 [a]
55% HRH	24.05 ± 0.80 [a]
p-value	<0.001
Temperature	**Protein Content (%)**
0 (control)	24.11 ± 0.40 [a]
21 ℃ (RT)	24.11 ± 0.81 [a]
40 ℃ (HT)	24.01 ± 0.79 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

3.1.3. Starch Content of Lentil Flour

The four varieties were different in starch content. The highest starch content was observed in Richlea (42.72%) followed by Pardina (42.50%), Maxim (42.35%), and Avondale (40.55%) (Table 7). Days of storage and variety impacts on starch content were significant (p-value <0.001); however, similar starch contents were observed for samples stored at low and high temperature regardless of variety. Only a small difference in the starch content of lentils was observed between samples stored at 40% and 55% (Table 7). The starch content of lentil was significantly affected by the interaction of different cultivars, storage days, and different RH conditions (p-value< 0.005). Over 360 days, there was a noticeable higher starch content (%) of different varieties of lentil stored at HT (40℃) and various RH (Table 8). The starch contents of Avondale, Maxim, and Pardina varieties were lower compared to time 0 (control), i.e., 40.59 to 41.86%, 42.02 to 43.52%, 42.34 to 42.64%, respectively while the starch content of Richlea variety went down from 44.2 to 42.09% (Table 8). The starch content of the four different varieties was consistent until 90 days of storage with slight differences, but after 180 days, changes in the starch content were noticeable. Similarly, Berrios et al. (1999) reported a slight decrease in the starch content of black beans stored for two years. Aguilera et al. (2009), explained changes in starch content in lentils might be a result of different treatments such as soaking, cooking, and dehydration, thus high temperatures during storage caused an increase in the starch content. As a result, the available starch contents of treated lentils were significantly increased (Aguilera, Esteban, Benitez, et al., 2009).

Table 7: Starch content (%, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Starch Content (%)**
Avondale	40.55 ± 2.58 [d]
Maxim	42.35 ± 2.61 [c]
Pardina	42.50 ± 2.47 [b]
Richlea	42.72 ± 2.17 [a]
p-value	<0.001
Days of Storage	**Starch Content (%)**
0 (control)	42.30 ± 1.68 [a]
180	41.09 ± 2.00 [c]
270	41.40 ± 3.12 [b]
360	39.52 ± 1.91 [d]
p-value	<0.001
Relative Humidity (RH)	**Starch Content (%)**
0 (control)	42.30 ± 1.68 [a]
40 % LRH	42.27 ± 2.58 [a]
55% HRH	41.78 ± 2.44 [b]
p-value	<0.001
Temperature	**Starch Content (%)**
0 (control)	42.30 ± 1.68 [a]
21 ℃ (RT)	42.03 ± 2.32 [b]
40 ℃ (HT)	42.02 ± 2.78 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

Table 8: Starch content (%, ± standard deviation) of different varieties of lentil flour stored under diverse storage conditions at high temperature (40 °C).

Storage Conditions(Days-RH)	Avondale D	Maxim C	Pardina B	Richlea A
0 (control)	40.59 ± 2.00 ^{d}	42.02 ± 2.05 ^{b}	42.34 ± 2.01 ^{b}	44.42 ± 2.08 ^{a}
180-LRH	37.27 ± 1.90 ^{f}	41.04 ± 1.95 ^{b}	44.21 ± 2.17 ^{a}	42.43 ± 2.11 ^{ab}
270-LRH	42.28 ± 2.10 ^{ab}	37.77 ± 1.90 ^{c}	39.03 ±2.00 ^{c}	39.02 ±1.99 ^{c}
360-LRH	43.19 ± 2.18 ^{a}	43.26 ± 2.18 ^{a}	43.34 ± 2.18 ^{ab}	42.59 ± 2.19 ^{ab}
180-HRH	39.47 ± 1.80 ^{e}	41.04 ± 1.88 ^{b}	42.21 ± 2.01 ^{b}	42.78 ± 2.06 ^{ab}
270-HRH	40.86 ± 2.10 ^{cd}	42.11 ± 2.15 ^{b}	37.27 ± 2.01 ^{c}	37.88 ± 2.01 ^{c}
360-HRH	41.86 ± 2.12 ^{bc}	43.52 ± 2.18 ^{a}	42.64 ± 2.11 ^{ab}	42.09 ± 2.12 ^{C}

*Different lowercase letters in a column represent significant differences across storage days and RH within each variety. Different uppercase letters in a row represent significant differences between varieties. LRH-Low Relative Humidity(40%) and HRH-High Relative Humidity (55%).

3.1.4. Ash and Fat content of Lentil Flour

No significant changes occurred in the ash content of lentil stored for 360 days regardless of storing temperature, relative humidity, and variety. However, a slightly higher ash content was observed for samples stored for 270 days. The highest ash content was observed in Avondale variety (2.86%) followed by Pardina (2.70%), Richlea (2.51%), and Maxim (2.36%). Berrios et al. (1999) reported no change in the ash content of black beans stored for two years.

Days of storage and relative humidity had a significant impact on the fat content of lentil (p-value<0.001) whereas temperature and variety did not impact the fat content of lentil. The highest fat content observed in Maxim variety (0.94%) followed by Richlea (0.89%), Avondale (0.80%), and Pardina (0.79%). A noticeably higher fat content was

observed in lentils stored for 360 days at high relative humidity (55%), i.e., 0.82 to 1.21%, 0.88 to 1.18%, 0.78 to 0.84%, 0.84 to 1.27%, for Avondale, Maxim, Pardina, and Richlea, respectively. This result is different from results reported by Sravanthi et al. (2013) who observed that the fat content of red lentil flour remained the same for lower temperatures (10 ℃ and 20 ℃) throughout 16 weeks of storage. However, Nasar-Abbas et al. (2008a) indicated an increase in the fat content of faba bean stored at different temperatures for 12 months.

Instead of a true increase or decrease in value, the general changes in the proximate composition of the flours of various varieties of lentils stored under accelerated conditions may be the result of changes in the mass balance among constituents. For instance, a higher starch content resulted in lower concentrations of other constituents. Nevertheless, compositional changes were noted, and it was clear that temperature, relative humidity, and days affected the proximate composition of lentils. Therefore, it is expected to influence the functional properties of lentils.

3.1. Functional Properties

3.2.1. Oil Absorption Capacity (OAC)

The OAC of various varieties of lentils stored in different storage conditions was determined. There were not any significant differences in variety, temperature, and relative humidity on the OAC; however, the OAC of lentil flour was impacted significantly by days of storage (Table 9). The highest oil absorption capacity (g/g) of different varieties of lentil was observed in Pardina variety (0.43 g/g) followed by Richlea (0.42 g/g), Avondale (0.42 g/g), and Maxim (0.39 g/g). The OAC of lentil flour increased steadily over time when compared with the time 0 (control) samples (Figure 2).

Karki (2022) reported a similar trend where an increase in the oil absorption capacity of pea flour stored for 270 days. As a result, this increased OAC was attributed to protein hydrolysis and the exposure of internal hydrophobic sites. The protein content and distribution of hydrophilic and hydrophobic segments in flour are primarily influenced by the amino acid sequence, which can interact with water and oil. Non-polar amino acid side chains can form hydrophobic interactions with lipid hydrocarbon chains (Johnson, 1970; Kinsella, 1979). Thus, the observed higher OAC might suggest that changes in protein occurred during storage of the lentils.

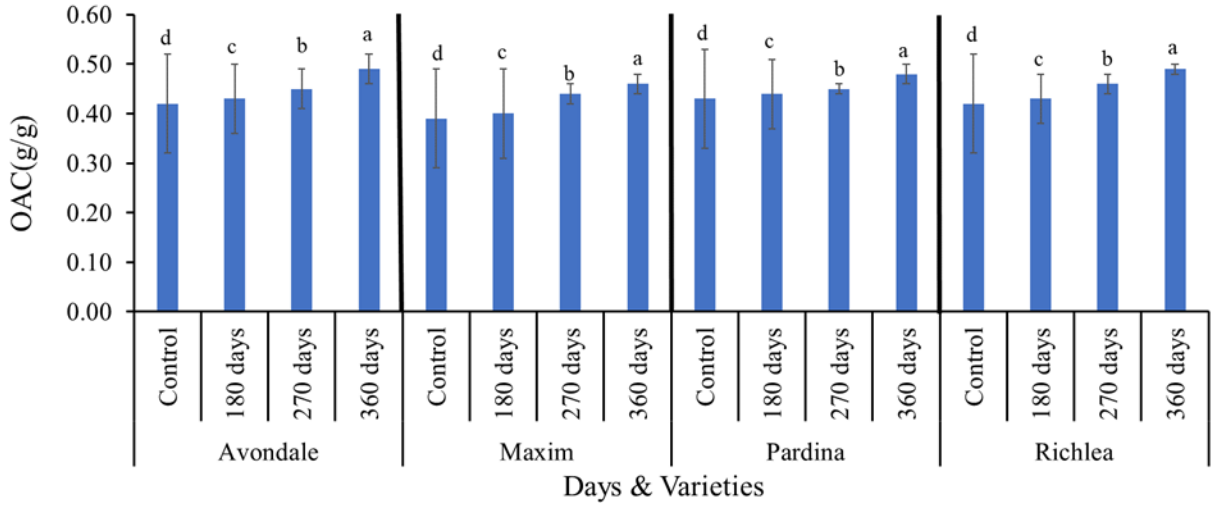

Figure 2: Oil absorption capacity (g/g) of lentil flours obtained from different varieties stored over 360 days. Different lowercase letters represent significant differences across storage days within each variety based on $\alpha = 0.05$. Error bars represent standard deviation.

Table 9: Oil Absorption Capacity (g/g, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Oil Absorption Capacity (g/g)**
Avondale	0.42 ± 0.08 [a]
Maxim	0.39 ± 0.06 [a]
Pardina	0.43 ± 0.08 [a]
Richlea	0.42 ± 0.08 [a]
p-value	<0.001
Days of Storage	**Oil Absorption Capacity (g/g)**
0 (control)	0.40 ± 0.21 [c]
180	0.42 ± 0.07 [c]
270	0.45 ± 0.03 [b]
360	0.48 ± 0.02 [a]
p-value	<0.001
Relative Humidity (RH)	**Oil Absorption Capacity (g/g)**
0 (control)	0.40 ± 0.21 [a]
40 % LRH	0.40 ± 0.04 [a]
55% HRH	0.41 ± 0.05 [a]
p-value	<0.001
Temperature	**Oil Absorption Capacity (g/g)**
0 (control)	0.40 ± 0.21 [a]
21 ℃ (RT)	0.41 ± 0.04 [a]
40 ℃ (HT)	0.41 ± 0.05 [a]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on at $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

3.2.2. Water Absorption Index and Water Solubility Index

The WAI of lentil flour samples stored at room temperature (RT, 21℃) and high temperature (HT, 40 ℃) were significantly different (p-value<0.001). To better understand the changes that occurred in the WAI and WSI, the data were divided into RT and HT. For the main effect of days of storage, varietal differences were observed for WAI of lentils stored at RT while difference due to relative humidity was not observed in the lentil (Table10). The effect of increasing days of storage and storage at RT regardless of the relative humidity resulted in significantly lower WAI in lentil flours (Table 10). The two-way interaction effect of days of storage and different varieties; and days of storage and different RH on the WAI of different varieties of lentils at HT was significant (p-value<0.001). Higher WAI resulted for lentils from increasing number of storage days at HT (Figure 3). Moreover, WAI was higher when compared with the time 0 (control) samples, i.e., 2.19 to 2.25 g/g, 2.25 to 2.26 g/g, 2.27 to 2.29 g/g, for Avondale, Maxim, and Richlea, respectively, but a decrease was observed in the Pardina variety, i.e., 2.35 to 2.31 g/g (Figure 3).

Table 10: Water Absorption Index (g/g, ± standard deviation) of lentils stored at room temperature (21℃) when factored for variety, day of storage, and relative humidity.

Varieties	**Water Absorption Index (g/g)**
Avondale	2.13 ± 0.06 [c]
Maxim	2.29 ± 0.06 [a]
Pardina	2.16 ± 0.07 [c]
Richlea	2.22 ± 0.13 [b]
p-value	<0.001
Days of Storage	**Water Absorption Index (g/g)**
0 (control)	2.27 ± 0.09 [a]
180	2.17 ± 0.09 [b]
270	2.21 ± 0.10 [b]
360	2.18 ± 0.12 [b]
p-value	<0.001
Relative Humidity (RH)	**Water Absorption Index (g/g)**
40 % LRH	2.19 ± 0.09 [a]
55% HRH	2.19 ± 0.11 [a]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, and different RH conditions, respectively based on at $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%).

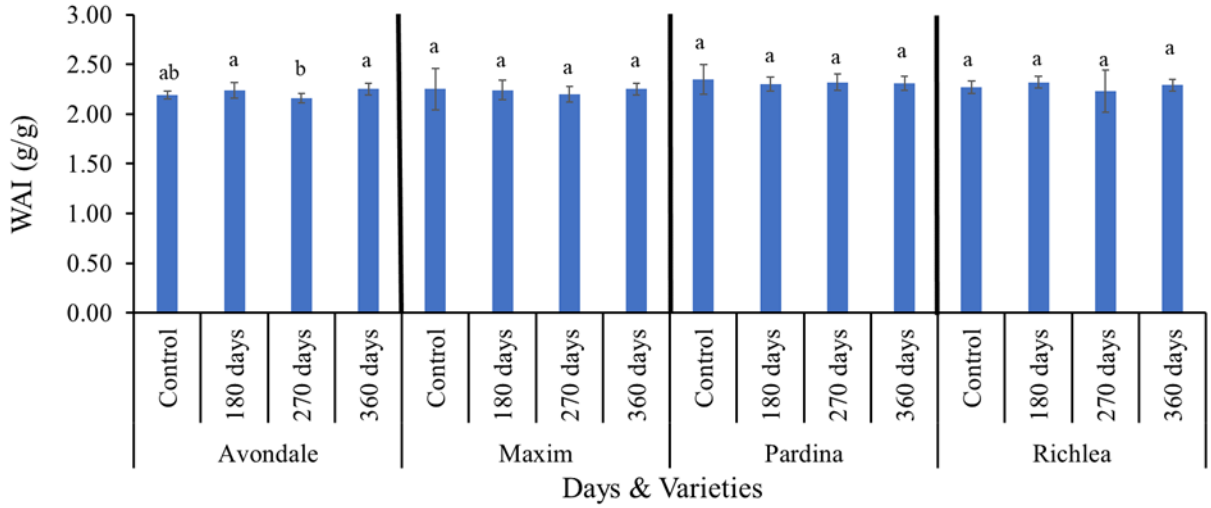

Figure 3: WAI (g/g) of lentil flours obtained from different varieties stored over 360 days at high temperature (40 °C). Different lowercase letters represent significant differences across storage days within each variety based on $\alpha = 0.05$. Error bars represent standard deviation.

The interactive effect of different varieties and different RH on the WAI of lentil flour stored at HT was significant (p-value<0.05). The water absorption index (WAI) of lentil flour stored at HT and high relative humidity (HRH, 55 %) was higher than WAI of lentil flour stored at HT and low relative humidity (LRH, 40 %)., i.e., 2.19 and 2.24 g/g, 2.22 and 2.24 g/g, 2.31 and 2.32 g/g, 2.26 and 2.31 g/g, for Avondale, Maxim, Pardina, and Richlea, respectively (Figure 4). In addition, the interactive effect of days of storage and different RH on the WAI of lentil flour stored at HT was significant (p-value<0.001). Higher WAI was observed in lentil flour stored at HRH within each sampling day however, this was not true for 270 days of sampling, i.e., storage at HRH resulted in higher WAI of lentil flour than at LRH (Figure 5).

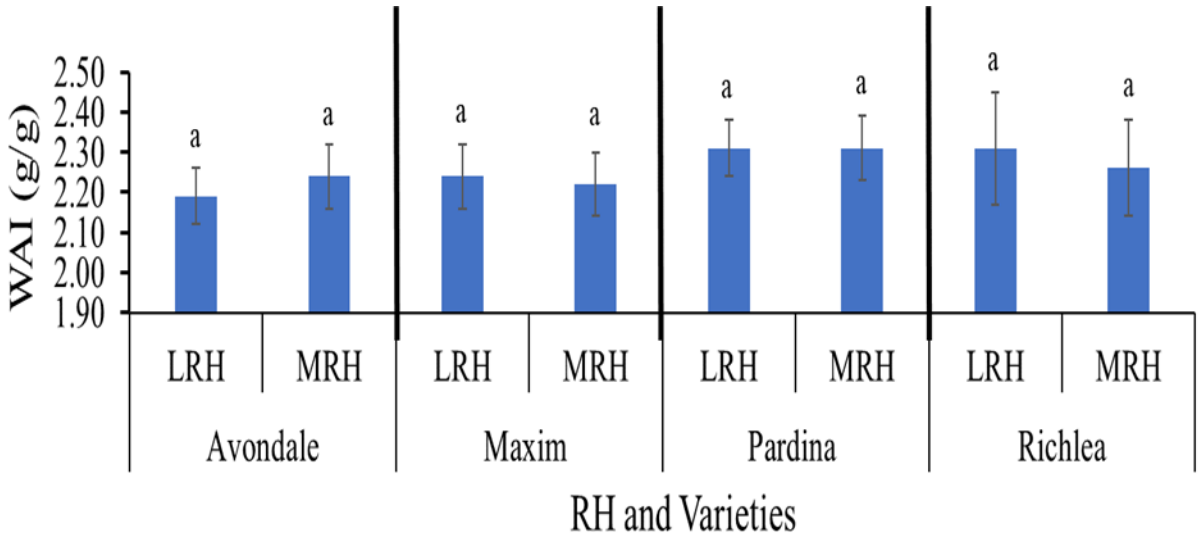

Figure 4:WAI (g/g) of lentil flours obtained from different varieties stored over 360 days at high temperature (40 °C). Different lowercase letters represent significant differences across relative humidity within each variety based on α = 0.05. Error bars represent standard deviation. LRH- Low Relative Humidity (40%), HRH- High Relative Humidity (55%).

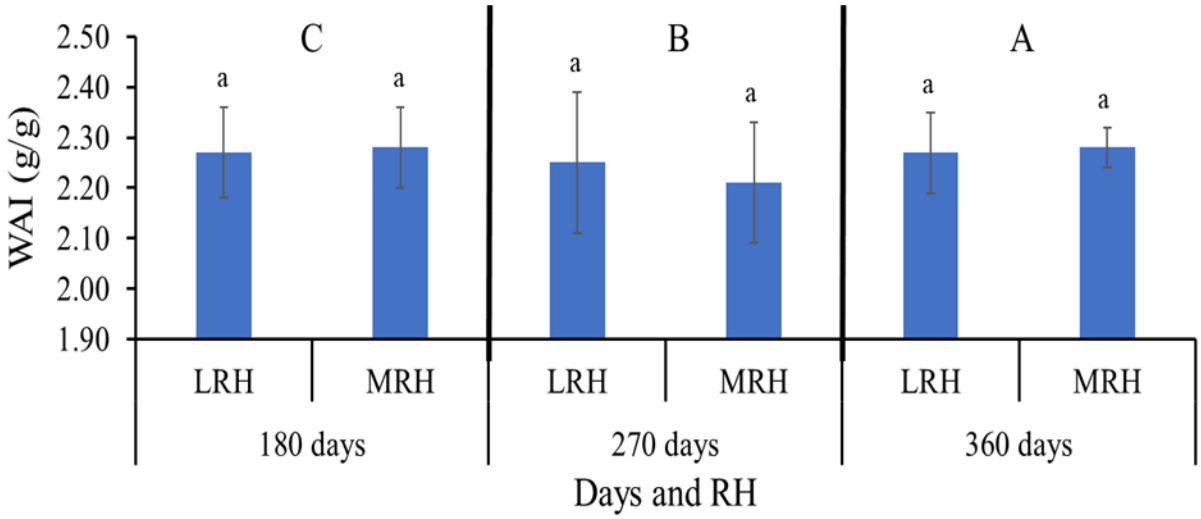

Figure 5: WAI (g/g) of lentil flours obtained from different varieties stored over 360 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference within days across diverse RH condition and across days of storage, respectively based on α = 0.05. Error bars represent standard deviation. LRH- Low Relative Humidity (40%), HRH- High Relative Humidity (55%).

The WSI of lentil samples stored at room temperature (RT, 21°C) and high temperature (HT, 40 °C) were significantly different (p-value<0.001). The main effect of days of storage, different varieties were observed in the WSI of lentils stored at RT while diverse relative humidity did not significantly impact the lentils (Table11). However, the WSI of lentil stored at 40 % relative humidity was slightly higher than samples stored at 55 % relative humidity. Increasing days of storage result in a decrease in the WSI index of lentil samples. Similar impacts occurred on the WSI of lentil samples stored at HT. Moreover, for the main effect of days of storage, differences in the WSI of different varieties existed when lentils were stored at HT whereas no difference in WSI was observed for lentils stored at different relative humidity (Table 11). The two-way interaction effect of days of storage and different varieties on the WSI of lentil flour stored at HT was significant (p-value<0.001). The general trend of decrease in WSI value was observed in different varieties with increasing days of storage., i.e., 23.71 and 21.30 g/g, 22.40 and 17.00 g/g, 20.85 and 19.02 g/g, 20.73 and 20.71 g/g, for Avondale, Maxim, Pardina, and Richlea, respectively (Figure 6).

Table 11: Water Solubility Index (g/g, ± standard deviation) of lentils stored at room temperature (21℃) when factored for variety, day of storage, and relative humidity.

Varieties	**Water Solubility Index (g/g)**
Avondale	24.37 ± 1.41 [a]
Maxim	20.04 ± 0.64 [c]
Pardina	22.44 ± 1.69 [b]
Richlea	20.71 ± 1.56 [c]
p-value	<0.001
Days of Storage	**Water Solubility Index (g/g)**
0 (control)	21.57 ± 1.90 [ab]
180	22.67 ± 2.06 [a]
270	21.22 ± 2.46 [b]
360	21.00 ± 1.93 [b]
p-value	<0.001
Relative Humidity (RH)	**Water Solubility Index (g/g)**
40 % LRH	22.39 ± 2.23 [a]
55% HRH	21.50 ± 2.13 [a]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, and different RH conditions, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%).

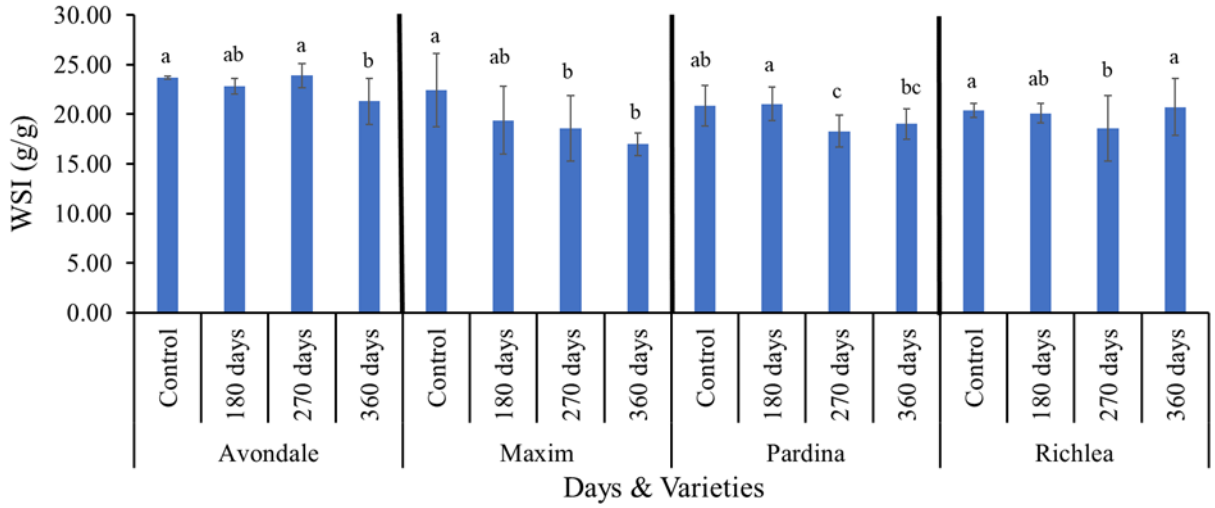

Figure 6:WSI (g/g) of lentil flours obtained from different varieties stored over 360 days at high temperature (40 °C). Different lowercase letters represent significant differences across storage days within each variety based on $\alpha = 0.05$. Error bars represent standard deviation.

To sum up, the WAI and WSI values of the lentil flour were highly influenced by the storage conditions. The WAI is a measurement of the flour's ability to absorb water and is reliant on the presence of hydrophilic groups that can bind to water molecules while the solubilization of the components of flour is related to the WSI index. Furthermore, due to lower ability of starch to absorb water due to crystallization (i.e., retrogradation) that occurred during storage, WAI and WSI values declined as storage time increased, behaving in a way that was consistent with the greater resistant starch content (González-Soto et al., 2007a). Therefore, protein degradation into peptides or free amino acids, as well as starch degradation, are thus the two most likely responsible factors for lower WSI in samples. A lower WSI value indicates that the starch is less soluble (González-Soto et al., 2007; Banks & Greenwood, 1971;Díaz et al., 2016).

3.2.3. Water Holding Capacity (WHC)

Days of storage, temperature, relative humidity, and different variety were significant (p-value <0.001) on the WHC of lentil flour. The four varieties had different WHC. The highest WHC (g/g) of different varieties of lentil was observed in the Richlea variety (1.20 g/g) followed by Maxim (1.14 g/g), Pardina (1.14 g/g), and Avondale (1.11 g/g). Increasing days of storage resulted in an increase in the WHC of lentil flour (Table 12). Also, an increase was observed in the WHC of lentil flour stored at high temperature (HT, 40 ℃) and high relative humidity (HRH, 55 %) (Figure 7). Water holding capacity is the ability to physically hold water and is a very important functional property required in flours for many food applications (Ma et al., 2011). Pathiratne et al. (2015) stated that high temperature caused higher WHC values in lentils and the combination effect of hydration and heat may have led to greater WHC of lentil flours, and thus these changes are attributed to protein denaturation. In addition, amino acid residues exposed as a result of protein denaturation have greater water-binding properties (Ma et al., 2011). Mwangwela et al. (2007) reported that the structural changes of the macro- and micro molecules in the seed particles caused by applied heat allow greater porosity and fluid entrapment while Kuntz (1971) demonstrated that higher WHC might be attributed to the increase in the amylose solubility and leaching and loss of the crystalline structure of starch.

Table 12: Water Holding Capacity (g/g, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Water Holding Capacity (g/g)**
Avondale	1.11 ± 0.12 [b]
Maxim	1.14 ± 0.11 [ab]
Pardina	1.14 ± 0.13 [ab]
Richlea	1.20 ± 0.12 [a]
p-value	<0.001
Days of Storage	**Water Holding Capacity (g/g)**
0 (control)	0.96 ± 0.10 [c]
180	1.19 ± 0.11 [a]
270	1.12 ± 0.11 [b]
360	1.18 ± 0.11 [ab]
p-value	<0.001
Relative Humidity (RH)	**Water Holding Capacity (g/g)**
0 (control)	0.96 ± 0.10 [c]
40 % LRH	1.14 ± 0.12 [b]
55% HRH	1.19 ± 0.10 [a]
p-value	<0.001
Temperature	**Water Holding Capacity (g/g)**
0 (control)	0.96 ± 0.10 [c]
21 ℃ (RT)	1.13 ± 0.09 [b]
40 ℃ (HT)	1.20 ± 0.12 [a]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

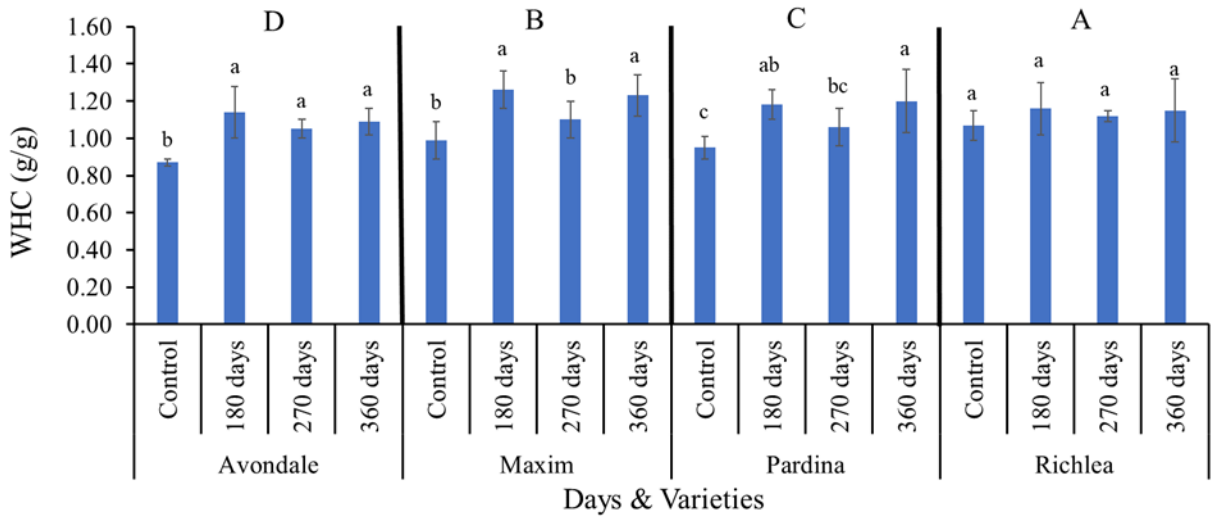

Figure 7: WHC (g/g) of lentil flours obtained from different varieties stored over 360 days at high temperature (40 °C) and high relative humidity (HRH 55 %). Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties based on $\alpha = 0.05$. Error bars represent standard deviation.

3.2.4. Foaming Capacity and Foaming Stability of Lentil Flour

The FC lentil varieties of stored in different storage conditions was determined. There were not any significant differences in FC due to temperature, and relative humidity effects; however, the FC of lentil flour was impacted significantly by days of storage and different variety. The interaction effect of days of storage and different varieties was significant (p-value<0.05) on the FC of lentil stored at accelerated conditions. Increasing days of storage of all varieties increased the FC content of lentil samples (Figure 8). When compared to the time 0 (control), FC of lentil flour increased significantly (p-value<0.001) over 360 days of storage from 108 to 148%, 133 to 141%, 115 to 150 %, and 120 to 146 %, for Avondale, Maxim, Pardina, and Richlea, respectively. The two-way interaction effect of days of storage and temperature was significant (p-value<0.005). Furthermore, the FC of lentil flour stored at HT, regardless of variety, was higher than RT after 360 days of storage (p-value<0.005) (Figure 9). These results agree

with the result reported by Karki (2022) where higher FC content was observed for pea samples stored at high temperature over 270 days.

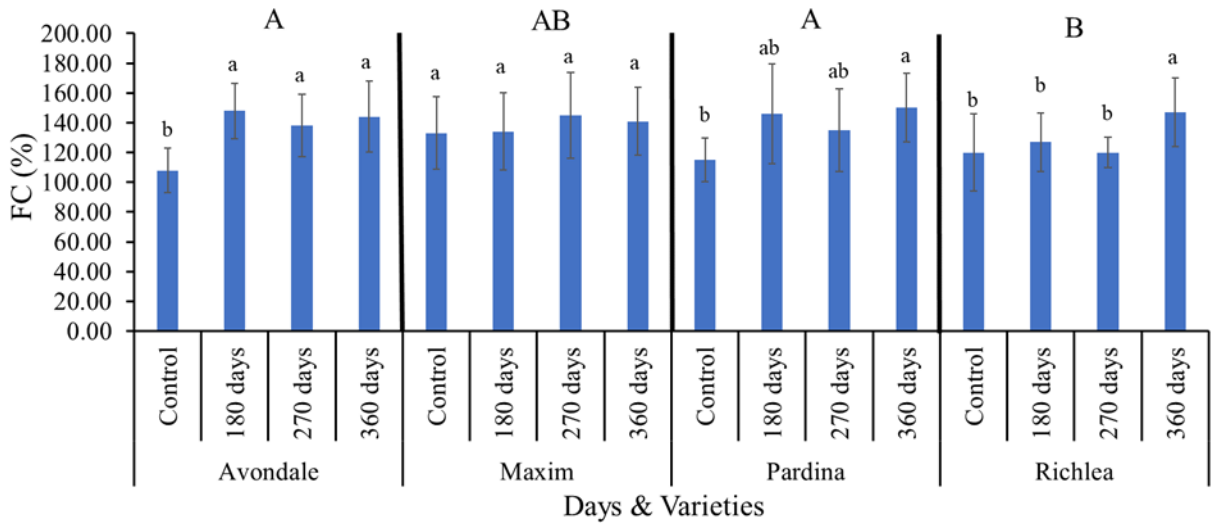

Figure 8: Foaming capacity (%) of lentil flours obtained from different varieties stored over 360 days. Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties based on α = 0.05. Error bars represent standard deviation.

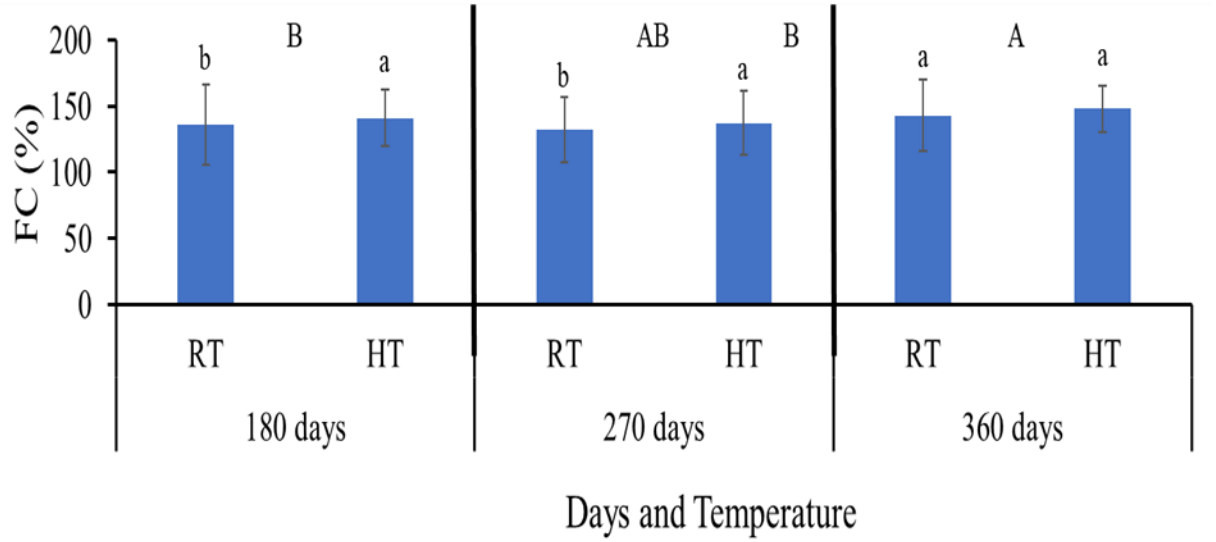

Figure 9: Foaming capacity (%) lentil flours obtained from different varieties stored over 360 days at RT (21 °C) and HT (40 °C). Different lowercase and uppercase letters represent significant difference between storage temperature within each sampling day and across days of storage, respectively based on α = 0.05. Error bars represent standard deviation.

The increase in FC was attributed to changes in protein structure. The main protein found in lentils is globulin. Globular proteins have a rigid, folded, and compact structure that is maintained by polar and non-polar interactions. The change in globulin structure during storage was most likely a conformation change or relaxation of globulins, which could explain the increase in FC (Ferreira et al., 2018; Sathe, 2008; Kinsella, 1979).

The foaming stability (FS) of lentil varieties stored in different storage conditions was determined. There were not any significant differences in FS due to temperature, and relative humidity effects; however, the FS of lentil was impacted significantly by days of storage and different variety. Increasing days of storage of all varieties resulted in a slight decrease in the FS content of lentil samples (Figure 10). When compared to the time 0 (control), FS of lentil flour decreased slightly (p-value<0.001) over 360 days of storage from 76 to 74%, 76 to 74%, 76 to 75 %, and 76 to 75 %, for Avondale, Maxim, Pardina, and Richlea, respectively. The two-way interaction effect of temperature and different varieties and relative humidity and different variety were significant (p-value<0.005). Furthermore, the FS of different varieties of lentil flour stored at HT was higher than RT after 360 days of storage (p-value<0.005) (Figure 11). In addition to the temperature, samples stored at high relative humidity (HRH) resulted in higher FS compared to low humidity (LRH) (Figure 12). Ferreira et al. (2018) documented that the increase in storage temperature developed a reduction in foam stability. As a result, the foaming capacity and foam stability always have an inverse relationship. High-foaming flours may produce large air bubbles surrounded by a thinner, less flexible protein film. Therefore, the air bubbles may collapse easily, lowering the foam's stability (Godswill et al., 2019; Jitngarmkusol et al., 2008).

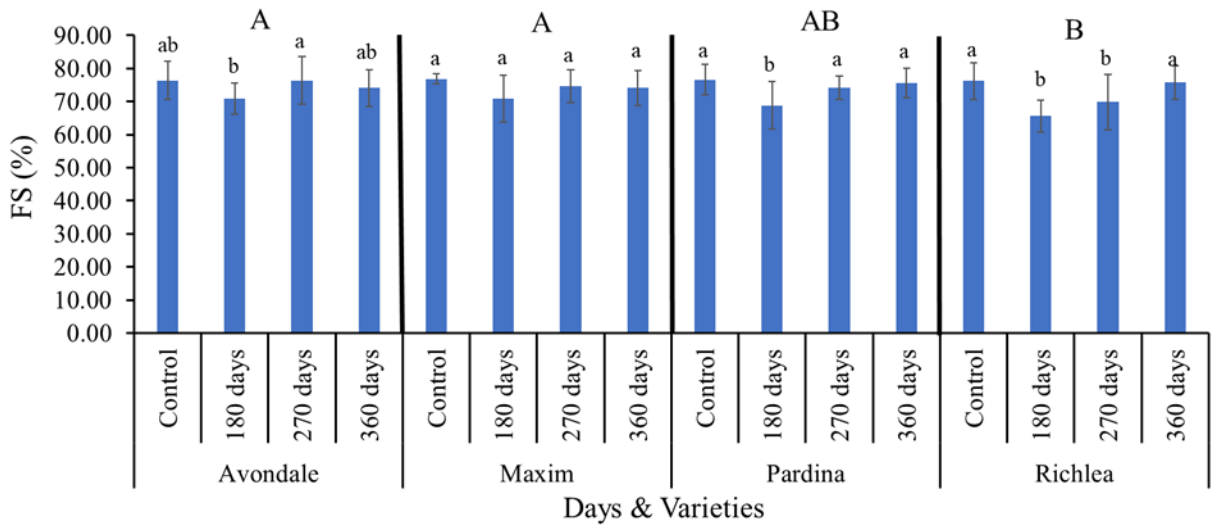

Figure 10:Foaming stability (%) lentil flours obtained from different varieties stored over 360 days. Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties based on α = 0.05. Error bars represent standard deviation.

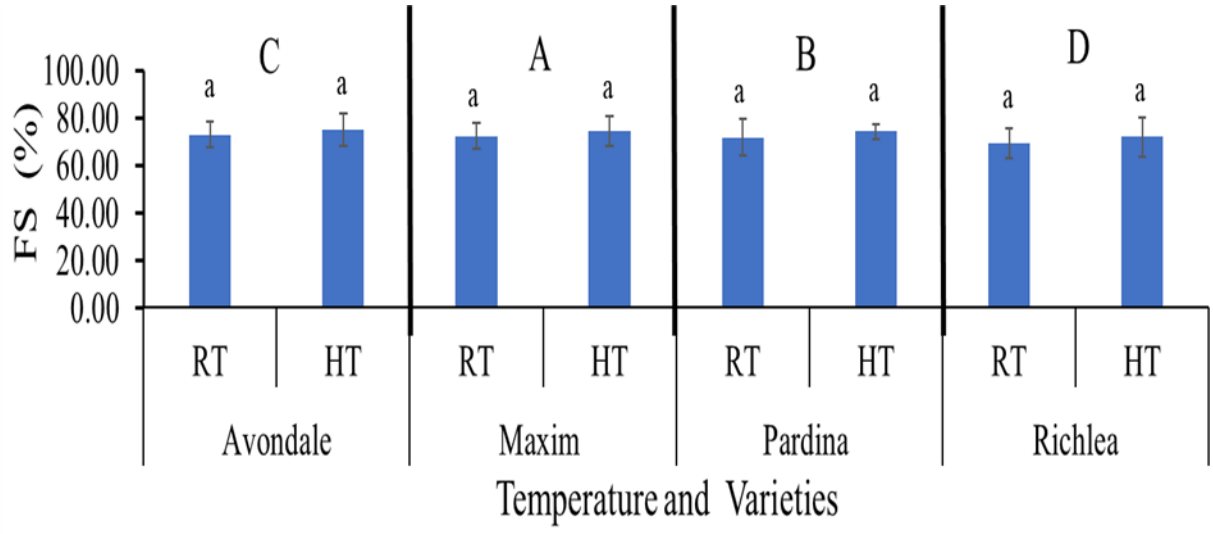

Figure 11: Foaming stability (%) of lentil flours obtained from different varieties stored over 360 days at RT (21 °C) and HT (40 °C). Different lowercase and uppercase letters represent significant differences between storage temperature within each sampling day and across different varieties, respectively based on α = 0.05. Error bars represent standard deviation. RT- Room Temperature and HT- High Temperature.

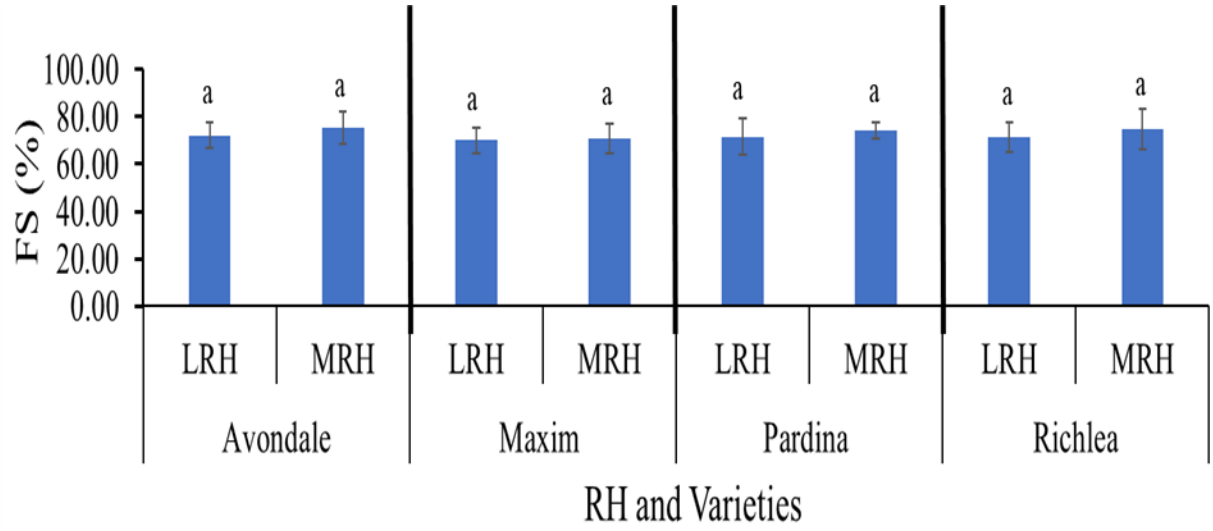

Figure 12: Foaming stability (%) of lentil flours obtained from different varieties stored over 360 days at LRH (40 %) and HRH (55 %). Different lowercase and uppercase letters represent significant differences between storage relative humidity and different varieties and across different varieties, respectively based on $\alpha = 0.05$. Error bars represent standard deviation.

3.2.5. Emulsion Activity and Emulsion Stability of Lentil Flour

The mean factors days of storage, temperature, relative humidity, and different variety significantly impacted the EA of lentils (p-value <0.001). The four varieties were different in the emulsion activity. The highest emulsion activity (g/g) of different varieties of lentil flour was observed in the Pardina variety (41.87 g/g) followed by Avondale (41.80 g/g), Richlea (41.65 g/g), and Maxim (41.61 g/g). Increasing days of storage caused an increase in the emulsion activity of lentil flour (Table 13). The temperature impacted the emulsion activity as well as the relative humidity. Furthermore, with increasing days of storage, an increase was observed in the emulsion activity of lentil flour stored at low temperature (RT, 21 ℃) (Figure 13). Also, an increase was observed in the emulsion activity of lentil stored at low relative humidity (LRH, 40 %) (Table 13). The two-way interaction effect of days of storage and relative humidity was

significant (p-value<0.005). Moreover, storage at low temperature and low relative humidity resulted in higher EA of lentil flour than at high relative humidity (HRH) at low temperature (Figure 14). Benítez, et al. (2009) reported an increase in the emulsion activity of lentils when the lentil was exposed to heat and the author explained that variations in EA during storage are possibly a result of interactions of various components of the flours that influence their emulsion properties. Boye et al. (2010) linked the changes in EA to protein denaturation that is important for the emulsifying properties of pulse proteins and may explain the increase in the results of this emulsion activity.

Table 13: Emulsion activity (g/g, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Emulsion Activity (g/g)**
Avondale	41.80 ± 0.75 [a]
Maxim	41.61 ± 0.67 [b]
Pardina	41.87 ± 0.72 [a]
Richlea	41.65 ± 0.77 [b]
p-value	<0.001
Days of Storage	**Emulsion Activity (g/g)**
0 (control)	40.39 ± 0.59 [c]
180	41.73 ± 0.61 [b]
270	41.69 ± 0.60 [b]
360	42.10 ± 0.58 [a]
p-value	<0.001
Relative Humidity (RH)	**Emulsion Activity (g/g)**
0 (control)	40.39 ± 0.59 [c]
40 % LRH	41.96 ± 0.62 [a]
55% HRH	41.73 ± 0.61 [b]
p-value	<0.001
Temperature	**Emulsion Activity (g/g)**
0 (control)	40.39 ± 0.59 [c]
21 ℃ (RT)	41.95 ± 0.62 [a]
40 ℃ (HT)	41.74 ± 0.61 [a]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

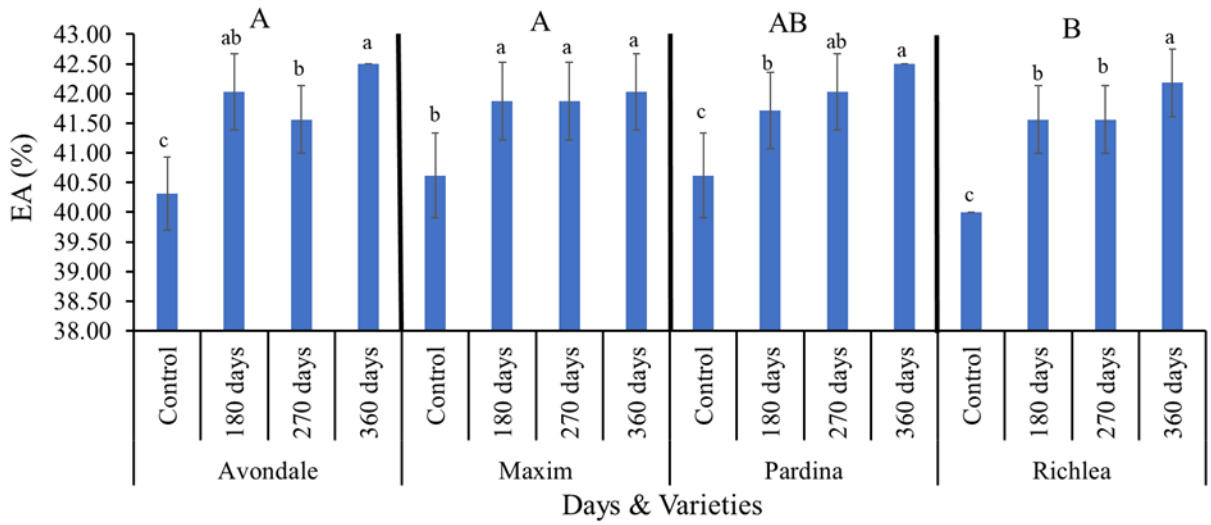

Figure 13: Emulsion activity (%) of lentil flours obtained from different varieties stored over 360 days at room temperature (21 °C). Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties based on α = 0.05. Error bars represent standard deviation.

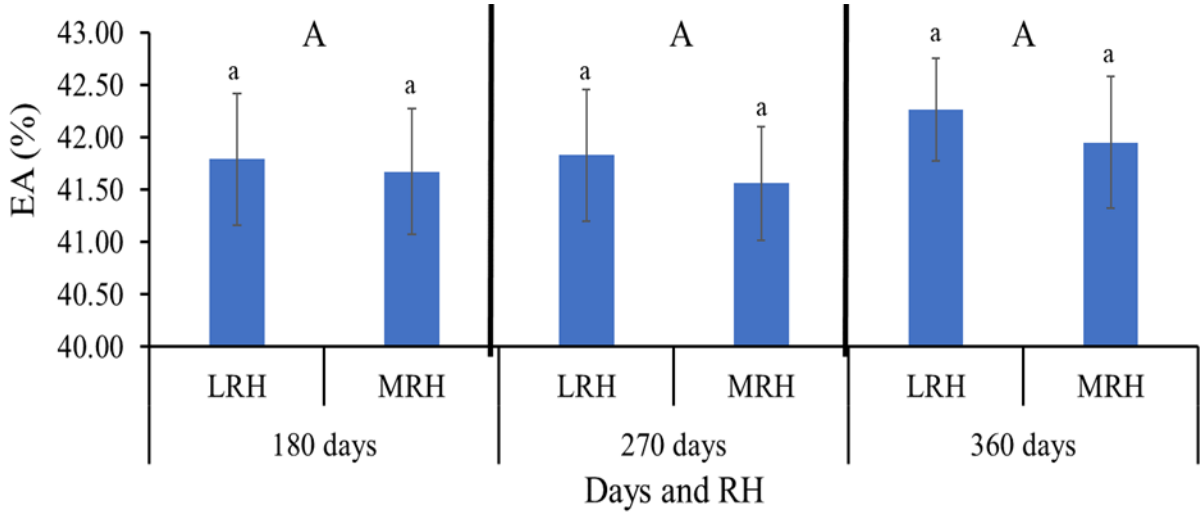

Figure 14:Emulsion activity (%) of lentil flours obtained from different varieties stored over 360 days at room temperature (21 °C) and diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase and uppercase letters represent significant differences across storage days and relative humidity and within each sampling day based on α = 0.05. Error bars represent standard deviation.

Days of storage, temperature, relative humidity, and different variety impacted the ES of lentil flour. The four varieties have relatively similar emulsion stability. The highest ES (g/g) of different varieties of lentil flour was observed in Richlea variety (47.76 g/g) followed by Pardina (47.74 g/g), Maxim (47.40 g/g), and Avondale (41.61 g/g). Increasing days of storage caused an increase in the emulsion stability of lentil flour (Table 14). The temperature impacted the emulsion stability. Furthermore, an increase was observed in the emulsion activity of lentil flour stored at low temperature (RT, 21 ℃) (Table 14). Also, an increase was observed in the emulsion activity of lentil flour stored at low relative humidity (LRH, 40 %) and high relative humidity (HRH, 55 %) when compared to control samples; however, a slight difference was observed between samples stored at LRH and HRH (Table 14). The two-way interaction temperature and relative humidity on different varieties were significant (p-value<0.005). Moreover, storage at low temperature and low relative humidity resulted in higher ES of lentil flour (Table 15). Boye et al. (2010) indicated that differences in protein content and variations in the molecular structure of the soy proteins and alteration caused temperature may explain the increase in emulsion stability. Also, Godswill et al. (2019) indicated that An increased number of non-polar amino acid residues on the surface of protein will reduce the energy barrier to adsorptions which depends on the protein structure, and thus, the changes structure likely improved ES.

Table 14: Emulsion stability (g/g, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Emulsion Stability (g/g)**
Avondale	47.40 ± 1.01 [b]
Maxim	47.69 ± 1.08 [a]
Pardina	47.74 ± 1.05 [a]
Richlea	47.76 ± 0.96 [a]
p-value	<0.001
Days of Storage	**Emulsion Stability (g/g)**
0 (control)	44.92 ± 0.96 [c]
180	48.06 ± 0.62 [a]
270	47.75 ± 0.63 [b]
360	47.81 ± 0.62 [b]
p-value	<0.001
Relative Humidity (RH)	**Emulsion Stability (g/g)**
0 (control)	44.92 ± 0.96 [c]
40 % LRH	41.96 ± 0.63 [a]
55% HRH	41.73 ± 0.65 [b]
p-value	<0.001
Temperature	**Emulsion Stability (g/g)**
0 (control)	44.92 ± 0.96 [c]
21 ℃ (RT)	47.95 ± 0.68 [a]
40 ℃ (HT)	47.79 ± 0.59 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

Table 15: Emulsion stability (ES) (g/g, ± standard deviation) of different varieties of lentil flour stored at diverse storage conditions.

Storage Conditions (Temperature-RH)	Avondale	Maxim	Pardina	Richlea
RT-LRH	47.91 ± 0.62 [a]	48.22 ± 0.61 [a]	48.21 ± 0.65 [a]	48.22 ± 0.64 [a]
RT-HRH	47.39 ±0.71 [b]	47.91 ± 0.64 [b]	47.91 ± 0.81 [b]	47.91 ± 0.61 [b]
HT-LRH	47.81 ± 0.50 [b]	47.91 ± 0.61 [b]	47.70 ± 0.48 [b]	47.70 ± 0.48 [b]
HT-HRH	47.39 ± 0.49 [b]	47.70 ± 0.48 [b]	48.12 ± 0.65 [a]	48.02 ± 0.64 [a]

*Different lowercase letters in a column represent significant differences within each variety based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

3.3. Starch Functionality

3.3.1. Final viscosity (FV) of Lentil Flour

The main factors days of storage, temperature, relative humidity, and different variety were significantly affected the final viscosity of lentil (p-value <0.001). Changes in the FV of lentil varieties were observed under different storage conditions. The main effect of days of storage, temperature, relative humidity, and different varieties was observed in the FV of lentil flour stored at diverse storage conditions (Table 16). The four varieties had different FV. The highest FV (cP) of different varieties of lentil flour was observed in the Richlea variety (2641 cP) followed by Pardina (2641 cP), Maxim (2181 cP), and Avondale (2175 cP). Storing lentil samples at low relative humidity (LRH 40 %) resulted in FV as well as storing at low temperatures (RT 21 °C). RVA plots in appendix figures 1- 4 show that the viscosity increased after lentils were stored 360 days of storage.

Table 16: Final viscosity (cP, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Final Viscosity (cP)**
Avondale	2175 ± 337 [d]
Maxim	2181 ± 316 [c]
Pardina	2615 ± 402 [b]
Richlea	2641 ± 333 [a]
p-value	<0.001
Days of Storage	**Final Viscosity (cP)**
0 (control)	2225 ± 325 [d]
180	2624 ± 350 [a]
270	2346 ± 397 [b]
360	2284 ± 424 [c]
p-value	<0.001
Relative Humidity (RH)	**Final Viscosity (cP)**
0 (control)	2225 ± 325 [c]
40 % LRH	2424 ± 460 [a]
55% HRH	2412 ± 371 [b]
p-value	<0.001
Temperature	**Final Viscosity (cP)**
0 (control)	2225 ± 325 [c]
21 ℃ (RT)	2554 ± 399 [a]
40 ℃ (HT)	2281 ± 390 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

The two-way interaction of the days of storage and different varieties, days of storage, and RH were significant (p-value<0.001) for the FV of lentil flour stored at high temperature (HT 40 ℃). The FV of lentil samples stored at HT for 360 days at diverse storage conditions showed significant differences among the varieties as well as within the varieties. Moreover, the FV decreased with increasing days of storage on lentil samples stored at high temperature (40 ℃) (Figure 15). Significant reduction in FV of stored lentil samples over 360 days was observed compared with the control lentil samples from 2093 to 1884 cP, 2265 to 1856 cP, 2266 to 2169 cP, and 2277 to 2168 cP, for Avondale, Maxim, Pardina, and Richlea, respectively. However, similarly, final viscosity decreased with increasing days of storage at diverse RH conditions, where samples stored at high relative humidity (HRH 55 %) had a lower value within each sampling day (Figure 16). The reduction observed in the FV agreed with the findings of lower viscosity in the FV of different cultivars of peas stored at high temperature and high relative humidity for 270 days (Karki, 2022). In addition, Rupollo et al. (2011) reported a similar reduction in FV in carioca beans stored at 25 ℃ for 360 days. The viscosity properties depend on the extent of starch granule swelling, the resistance of the swollen granules to dissolution by heat, the presence of soluble starch, and the interaction or cohesiveness between the swollen granules, and thus the heat might change the structure of the starch and could be responsible for the viscosity reduction (Naivikul & D'Appolonia, 1979).

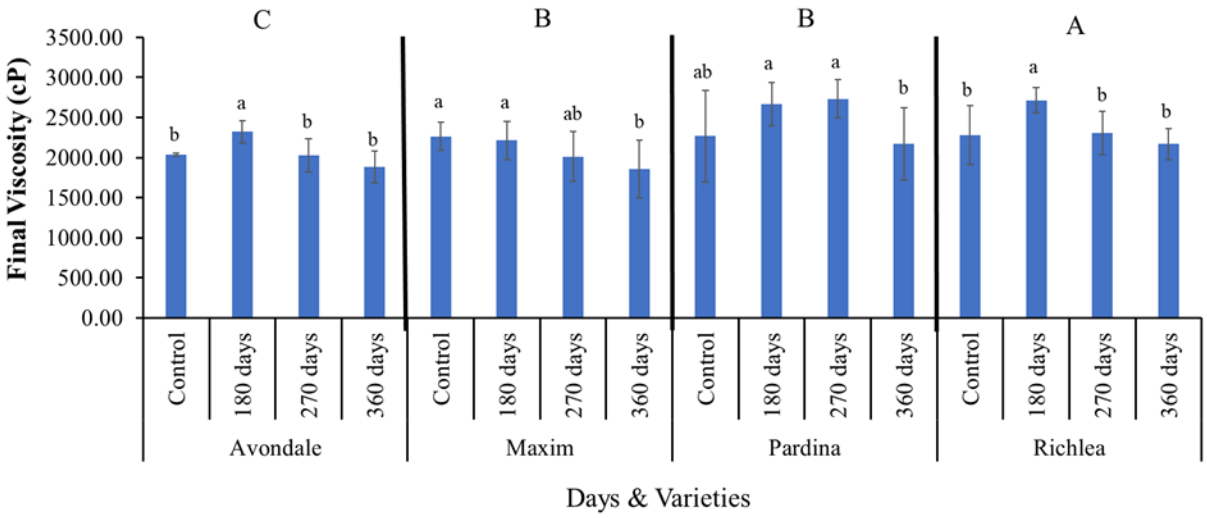

Figure 15: Final viscosity (cP) of lentil flours obtained from different varieties stored under diverse storage conditions over 360 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties, respectively based on α = 0.05. Error bars represent standard deviation.

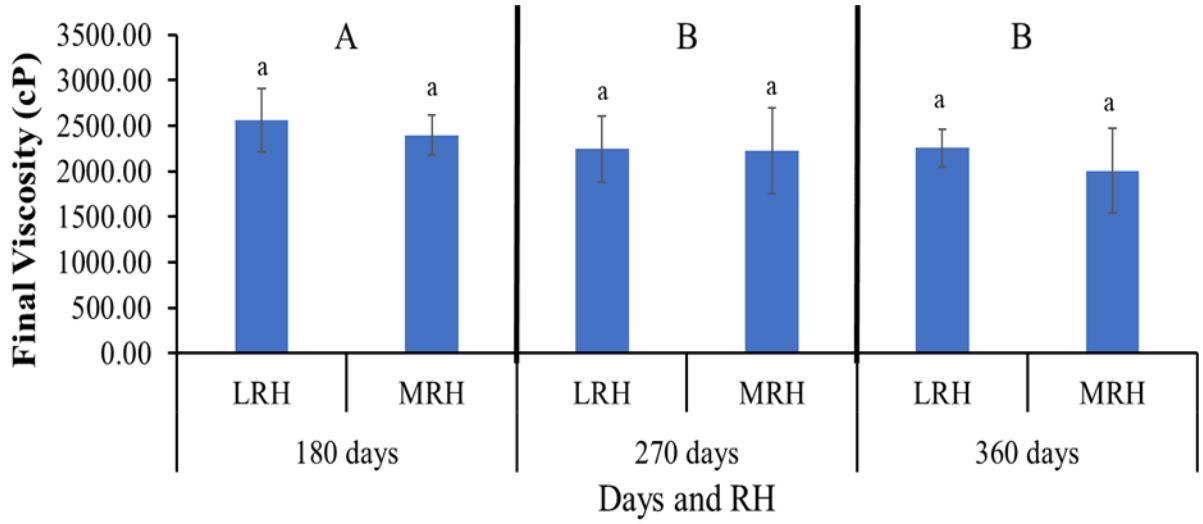

Figure 16: Final viscosity (cP) of lentil flours obtained from different varieties stored over 360 days at high temperature (40 °C) under diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase and uppercase letters represent significant differences across storage days and relative humidity and within each sampling day based on α = 0.05. Error bars represent standard deviation.

3.3.2. Setback Viscosity (SV) of Lentil Flours

Days of storage, temperature, relative humidity, and different variety impacted the setback viscosity of lentil flour. The main effect of days of storage, temperature, relative humidity, and different varieties was observed in the SV of lentil flour stored at diverse storage conditions (Table 17). The four varieties were different in the SV. The highest SV (cP) of the lentil varieties was observed in the Richlea (1179 cP) followed by Pardina (1079 cP), Maxim (950 cP), and Avondale (875 cP). Lentil samples stored at low relative humidity (LRH 40 %) resulted in high SV compared to high relative humidity (HRH 55 %) as well as stored at low temperatures (RT 21 ℃) (Table 17). The interaction effect of days of storage and different varieties on the SV of lentil stored at high temperature (HT 40 ℃) was significant (p-value<0.005). The SV of lentil decreased significantly after 360 days of storage at HT compared to control samples, i.e., Avondale (913 to 696 cP), Maxim (1069 to 664 cP), Pardina (890 to 777 cP), and Richlea (985 to 952 cP) (Figure 17).

Table 17: Setback viscosity (cP, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Setback Viscosity (cP)**
Avondale	875 ± 223 [d]
Maxim	950 ± 247 [c]
Pardina	1079 ± 249 [b]
Richlea	1179 ± 233 [a]
p-value	<0.001
Days of Storage	**Setback Viscosity (cP)**
0 (control)	964 ± 217 [c]
180	1128 ± 234 [a]
270	1008 ± 275 [b]
360	940 ± 261[d]
p-value	<0.001
Relative Humidity (RH)	**Setback Viscosity (cP)**
0 (control)	964 ± 217 [c]
40 % LRH	1054 ± 287 [a]
55% HRH	997 ± 244 [b]
p-value	<0.001
Temperature	**Setback Viscosity (cP)**
0 (control)	964 ± 217 [c]
21 ℃ (RT)	1156 ± 219 [a]
40 ℃ (HT)	886 ± 238 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

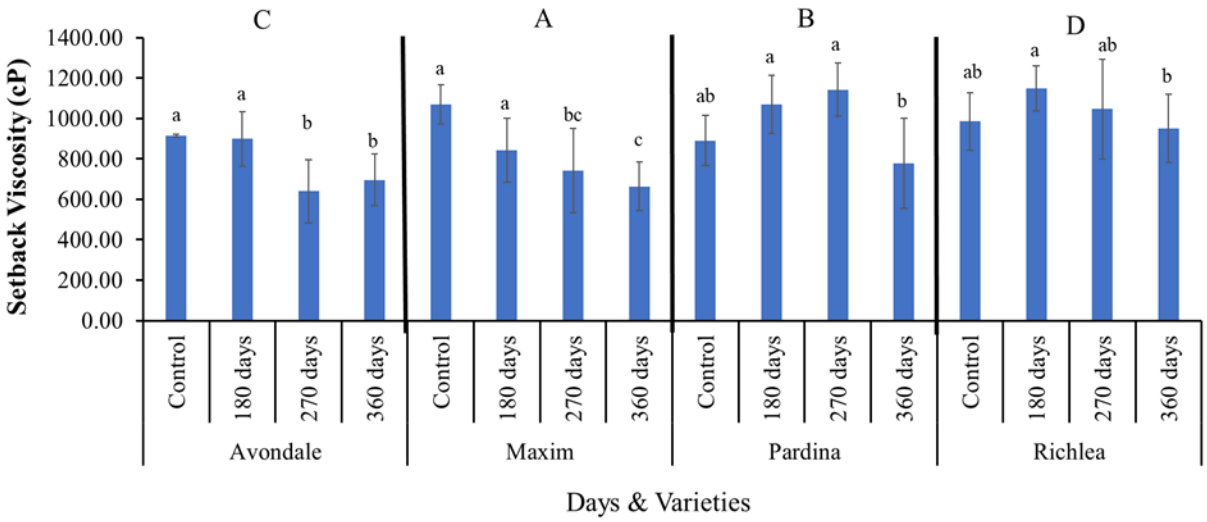

Figure 17: Setback viscosity (cP) of lentil flours obtained from different varieties stored under diverse storage conditions over 360 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties, respectively based on $\alpha = 0.05$. Error bars represent standard deviation.

The reduction observed in the SV agreed with the findings of lower SV of different cultivars of peas stored at high temperature and high relative humidity for 270 days (Karki 2022). Rupollo et al. (2011) reported a similar result occurred in carioca beans stored at different temperature (5 °C, 15 °C and 25°C) for 360 days, where the SV was mostly impacted and reduced in the carioca beans flour stored at 5 °C, 15 °C, and 25°C. The changes in the molecular structure crystalline region caused by the amylose content, as well as the reduction in relative crystallinity, were identified as important factors causing lower SV(Ferreira et al., 2017). Moreover, Ferreira et al. (2017) reported that weaker gels formed as a result of reduced FV and SV of starch were caused by changes in the amylopectin chains of the starch from the stored beans (such as breakage and release of amylopectin short chains). Moreover, days of storage, temperature, relative humidity, and variety impacted the gel strength of lentil flour. The highest gel strength (cP) was observed in the Richlea variety (314 cP) followed by Maxim (280 cP), Pardina

(279 cP), and Avondale (244 cP). Increasing days of storage resulted in a reduction in the gel strength of lentil flour (Table 18). Less gel strength was observed in the lentils stored at HT and HRH over 360 days (Table 18).

Table 18: Gel Strength (cP, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Gel Strength (cP)**
Avondale	244 ± 77 [d]
Maxim	280 ± 74 [b]
Pardina	279 ± 59 [c]
Richlea	314 ± 50 [a]
p-value	<0.001
Days of Storage	**Gel Strength (cP)**
0 (control)	280 ± 42[b]
180	290 ± 38 [a]
270	279 ± 65 [c]
360	244 ± 85 [d]
p-value	<0.001
Relative Humidity (RH)	**Gel Strength (cP)**
40 % LRH	287 ± 70 [a]
55% HRH	271 ± 73 [b]
p-value	<0.001
Temperature	**Gel Strength (cP)**
21 ℃ (RT)	313 ± 43 [a]
40 ℃ (HT)	246 ± 79 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

3.3.3. Peak Viscosity (PV) of Lentil Flour

Days of storage, temperature, relative humidity, and different variety were significant (p-value <0.001) on the PV of lentil. Changes in the PV of different varieties of lentil flour were observed due to different storage conditions. The main effect of days of storage, temperature, relative humidity, and different varieties was observed in the PV of lentil flour stored at diverse storage conditions (Table 19). The four varieties had different PV. The highest PV was observed in the Richlea variety (1741 cP) followed by Pardina (1679 cP), Avondale (1514 cP), and Maxim (1499 cP). Storing lentil samples at high relative humidity (HRH 55 %) resulted in high PV (Table 19) as well as storing at low temperature (RT 21 ℃). In contrast to FV and SV, PV increased during storage at different storage conditions. The two-way interaction of days of storage and relative humidity was significant (p-value<0.001) on the PV of lentil flour stored at high temperature. Storing lentil samples at HRH tended to result in flours having higher PV than the samples stored at LRH at high temperature (Figure 18). Also, the two-way interaction of relative humidity and different varieties was significant (p-value<0.001) for the PV of lentil flour from lentils stored at HT. The varieties tended to have higher PV when stored at HRH and high temperature (Figure 19).

Table 19: Peak Viscosity (cP, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Peak Viscosity (cP)**
Avondale	1514 ± 345 [c]
Maxim	1499 ± 362 [d]
Pardina	1679 ± 277 [b]
Richlea	1741 ± 207 [a]
p-value	<0.001
Days of Storage	**Peak Viscosity (cP)**
0 (control)	1312 ± 188 [d]
180	1593 ± 233 [c]
270	1633 ± 278 [b]
360	1672 ± 409 [a]
p-value	<0.001
Relative Humidity (RH)	**Peak Viscosity (cP)**
40 % LRH	1590 ± 277 [b]
55% HRH	1676 ± 347 [a]
p-value	<0.001
Temperature	**Peak Viscosity (cP)**
21 ℃ (RT)	1801 ± 245 [a]
40 ℃ (HT)	1465 ± 289 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

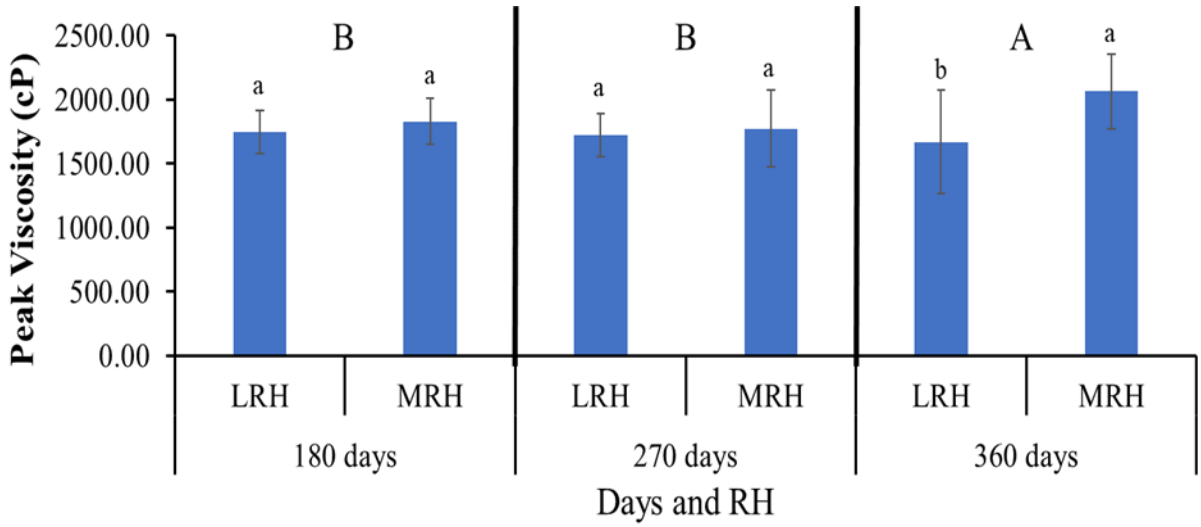

Figure 18: Peak viscosity (cP) of lentil flours obtained from different varieties stored over 360 days at high temperature (40 °C) under diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase and uppercase letters represent significant differences across storage days and relative humidity and within each sampling day based on α = 0.05. Error bars represent standard deviation.

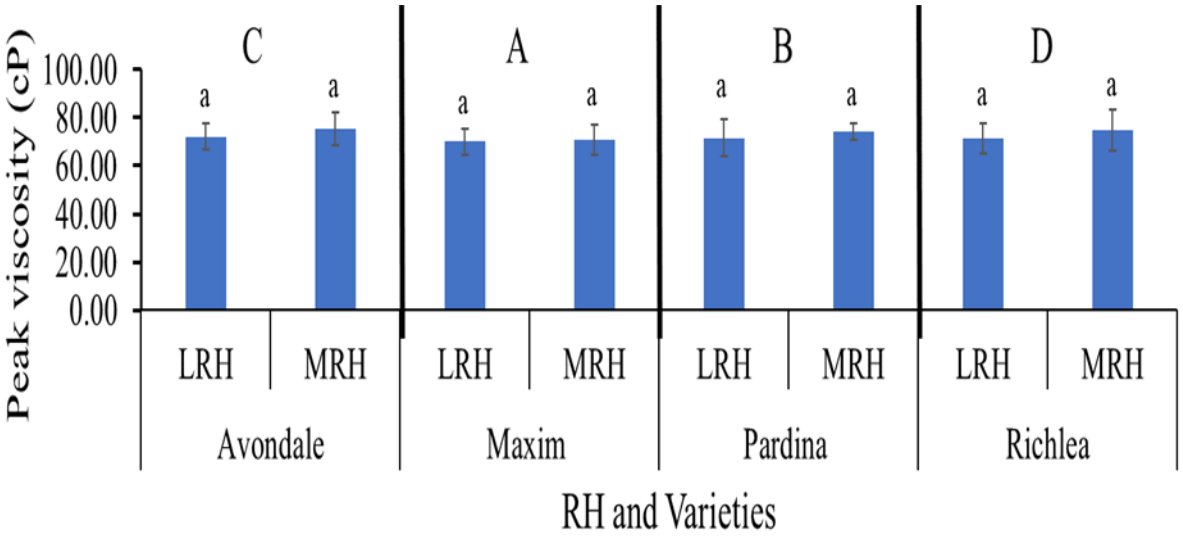

Figure 19: Peak viscosity (cP) of lentil flours obtained from different varieties stored at different RH and high temperature (40 °C) under diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase and uppercase letters represent significant differences across different RH within each variety and across varieties, respectively based on α = 0.05. Error bars represent standard deviation.

Ferreira et al. (2017) stated that a higher moisture content led to higher PV in the starch isolated from beans stored at 17% moisture, and 14 % moisture at 32 °C for 12 months. This assumption supports our results where high relative humidity led to high moisture content, and this could be attributed to the increase in peak viscosity. Furthermore, the swelling power of starch was determined to be high, and it was documented that the high swelling power could be one of the reasons for the increase in PV of the starch isolated from the beans (Ferreira et al., 2017).

3.4. Physical Properties of Lentils

3.4.1. Moisture Content (MC) of Lentils

Days of storage, temperature, relative humidity, and different variety impacted the moisture content of lentil seeds. Differences in the MC of varieties of lentil seeds were observed in response to different storage conditions. The main effect of days of storage, temperature, relative humidity, and different varieties was observed in the moisture content of lentil seeds stored at diverse storage conditions (Table 20). The four varieties had different moisture contents. The highest moisture content (%) was observed in the Avondale variety (10.49 %) followed by Maxim (10.04 %), Richlea (9.76 %), and Pardina (9.40 %). Increasing days of storage caused an increase in the moisture content of lentil seeds (Table 19). Storing lentil seeds at high relative humidity (HRH 55 %) resulted in higher moisture content as well as storing at low temperature (RT 21 °C) (Table 20). An increase in the moisture content occurred over the storage time. The interactive effect of days and varieties was significant (p-value<0.001) when stored at RT (Figure 20) while no significant impact was observed in HT. However, the interactive effect of RH and varieties was significant (p-value<0.001) when stored at RT and HT storage

conditions. The moisture content of all the varieties of lentil seeds stored at HRH was significantly higher than those stored at LRH, regardless of the variety when stored at HT and RT (Figures 21 and 22).

Table 20: Moisture content (%, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Moisture Content (%)**
Avondale	10.49 ± 1.03 [a]
Maxim	10.04 ± 0.92 [b]
Pardina	9.40 ± 1.40 [d]
Richlea	9.76 ± 1.19 [c]
p-value	<0.001
Days of Storage	**Moisture Content (%)**
0 (control)	9.45 ± 0.82 [c]
180	9.86 ± 1.16 [b]
270	10.05 ± 1.21 [a]
360	9.98 ± 1.31 [ab]
p-value	<0.001
Relative Humidity (RH)	**Moisture Content (%)**
40 % LRH	9.43 ± 1.01 [b]
55% HRH	10.50 ± 1.19 [a]
p-value	<0.001
Temperature	**Moisture Content (%)**
21 ℃ (RT)	10.63 ±0.99 [a]
40 ℃ (HT)	9.29 ± 1.06 [b]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

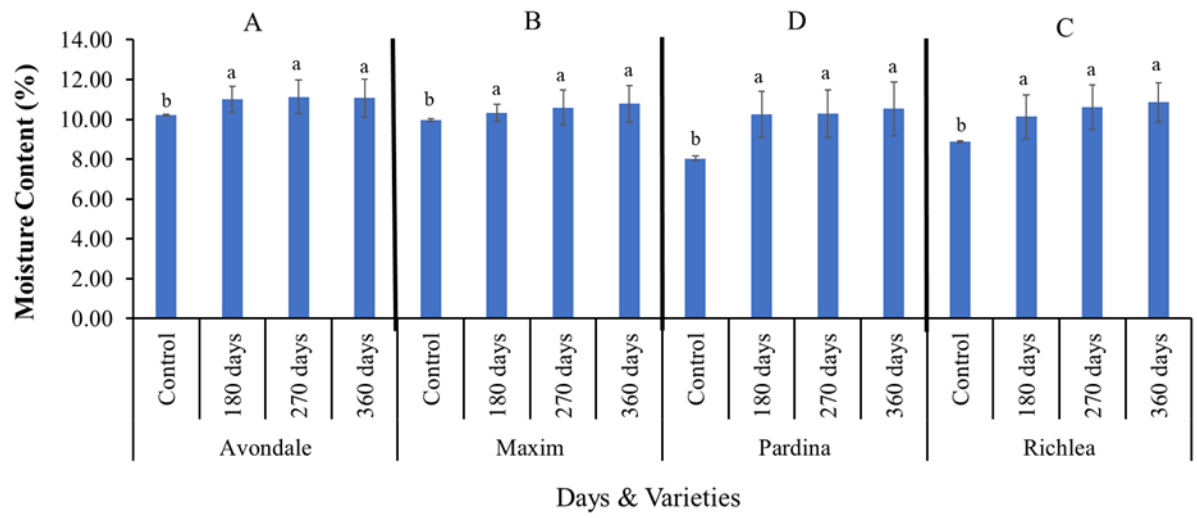

Figure 20: Moisture content of lentil seeds obtained from different varieties stored over 360 days at room temperature (21 °C). Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties, respectively based on α = 0.05. Error bars represent standard deviation.

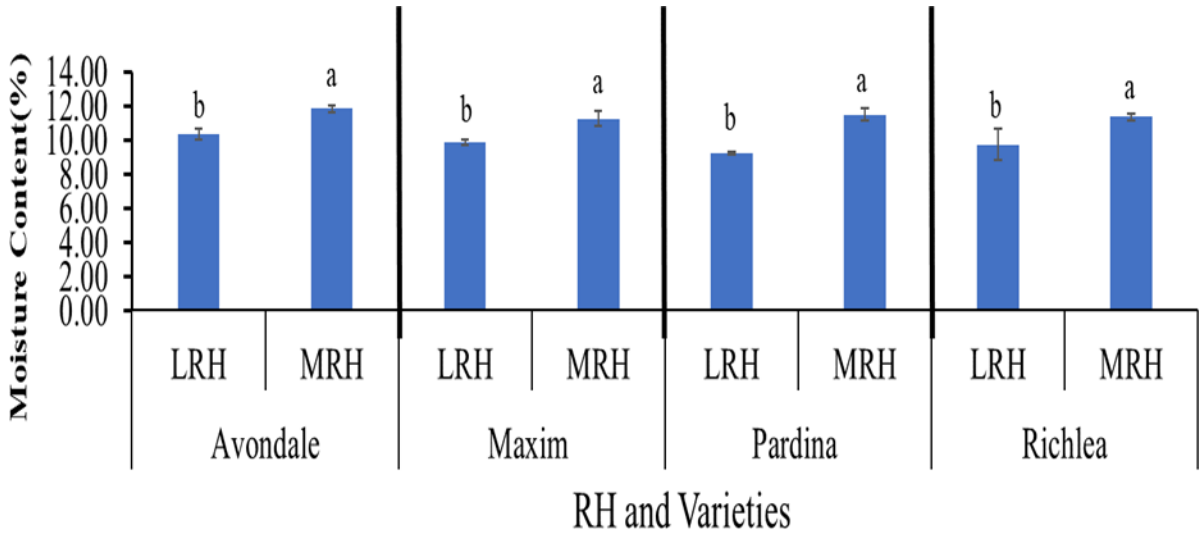

Figure 21:Moisture content of lentil seeds obtained from different varieties stored at different RH and at room temperature (21 °C) under diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase letters represent significant differences across different RH within each variety based on α = 0.05. Error bars represent standard deviation.

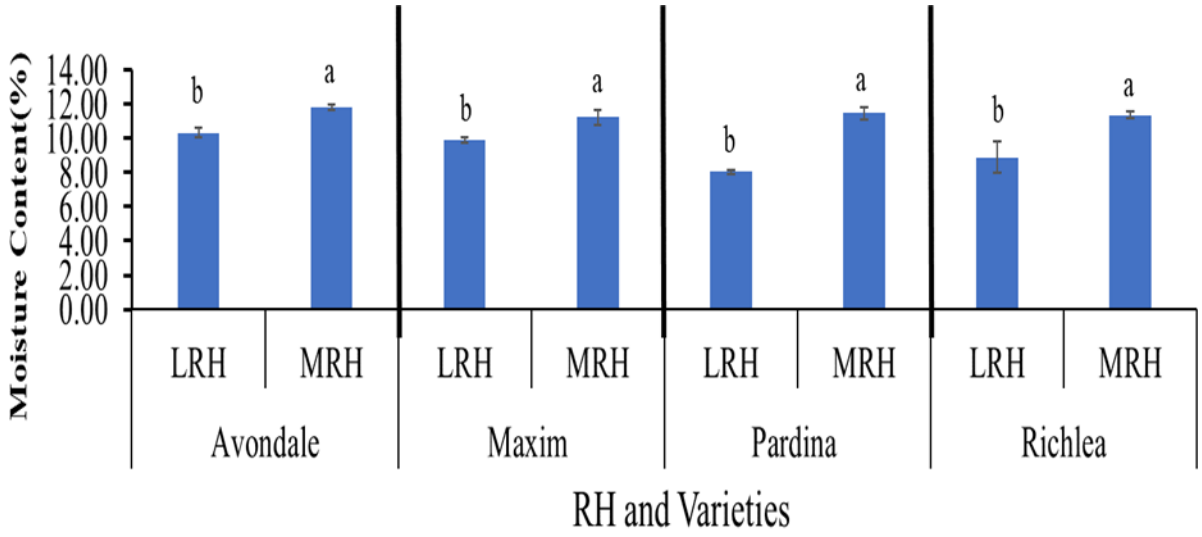

Figure 22: Moisture content of lentil seeds obtained from different varieties stored at high temperature (40 ℃) under diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase letters represent significant differences across different RH within each variety based on $\alpha = 0.05$. Error bars represent standard deviation.

Our results were similar to those previously reported for pea (Karki 2022) where an increase occurred in the moisture content of peas stored over 270 days at room temperature (21 ℃) and also, pea samples stored over 270 days under high relative humidity (75%) resulted in high moisture content than low relative humidity (40%). Chidananda et al. (2014) linked the respiration rate of the pulses is responsible for the increase in the moisture content of pulses and the authors indicated that moisture content increased significantly over time could possibly be due to the release of water during respiration.

3.4.2. Cook Firmness of Lentil

Impact of days of storage, temperature, relative humidity, and variety on the cook firmness of lentil seeds were significant (p-value <0.001). Changes in the cook firmness of different varieties of lentil seeds were observed due to different storage conditions. The main effect of days of storage, temperature, relative humidity, and varieties was observed in cook firmness of lentil seeds stored at diverse storing conditions (Table 21). The cooked firmness was significantly different among varieties. The highest cook firmness (N/g) was observed in the Maxim variety (29.96 N/g) followed by Richlea (23.21 N/g), Avondale (19.91 N/g), Pardina (17.99 N/g). Storing lentil seeds at high relative humidity (HRH 55 %) resulted in high cook firmness (Table 21) as well as storing at high temperature (HT 40 ℃). Furthermore, an increase in the cook firmness was observed in the lentil seeds as the days of storage increased (Table 21). A slight change occurred in the cook firmness of lentil seeds stored at room temperature (21℃) and low relative humidity (40 %) while a significant change occurred in the lentil seeds stored at high temperature (40 ℃) and low relative humidity (40 %). Moreover, an increase in the cook firmness occurred over the storage time up to 270 days but dropped at 360 days, the interactive effect of days and varieties was significant (p-value<0.001) when stored at HT (Figure 23). Also, an increase was observed in the cook firmness of lentil seeds stored at high temperature (40 ℃) and low relative humidity (40 %) (Figure 24). The two-way interaction of relative humidity and days of storage was significant (p-value<0.001) for the cook firmness of lentils stored at HT. Lentil seeds tended to have higher cook firmness with increasing days of storage and relative humidity (Figure 25).

Table 21: Cook firmness (N/g, ± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Cook Firmness (N/g)**
Avondale	19.91 ± 8.21 [c]
Maxim	29.96 ± 8.16 [a]
Pardina	17.99 ± 7.22 [d]
Richlea	23.21 ± 6.85 [b]
p-value	<0.001
Days of Storage	**Cook Firmness (N/g)**
0 (control)	16.82 ± 2.61 [d]
180	17.37 ± 5.64 [c]
270	25.48 ±7.62 [b]
360	26.90 ± 10.06 [a]
p-value	<0.001
Relative Humidity (RH)	**Cook Firmness (N/g)**
40 % LRH	22.56 ± 8.01 [b]
55% HRH	23.94 ± 9.86 [a]
p-value	<0.001
Temperature	**Cook Firmness (N/g)**
21 ℃ (RT)	19.95 ± 6.27 [b]
40 ℃ (HT)	26.55 ± 10.05 [a]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

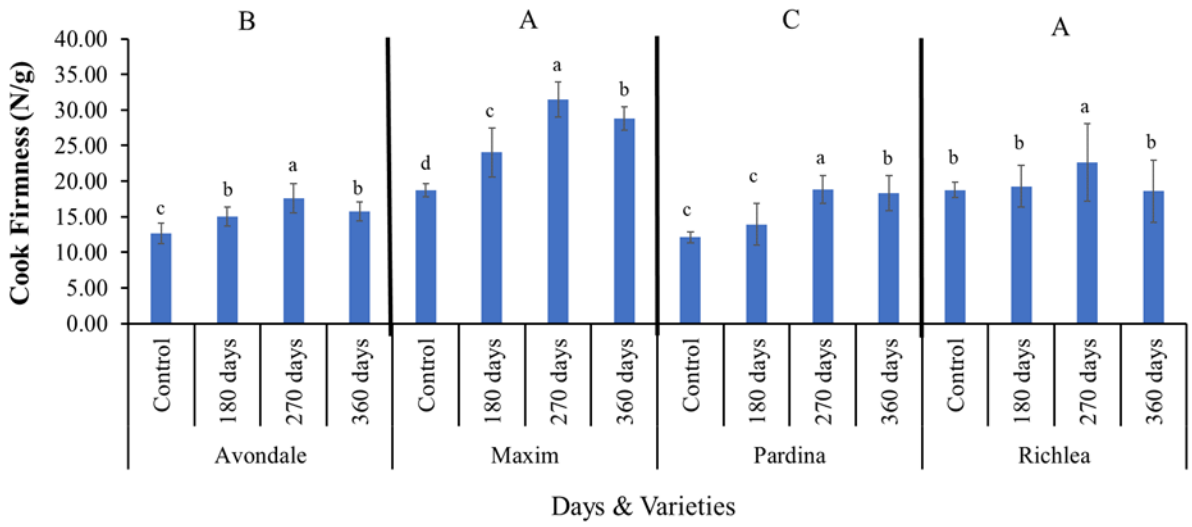

Figure 23: Cook Firmness of lentil seeds obtained from different varieties stored over 360 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties, respectively based on α = 0.05. Error bars represent standard deviation.

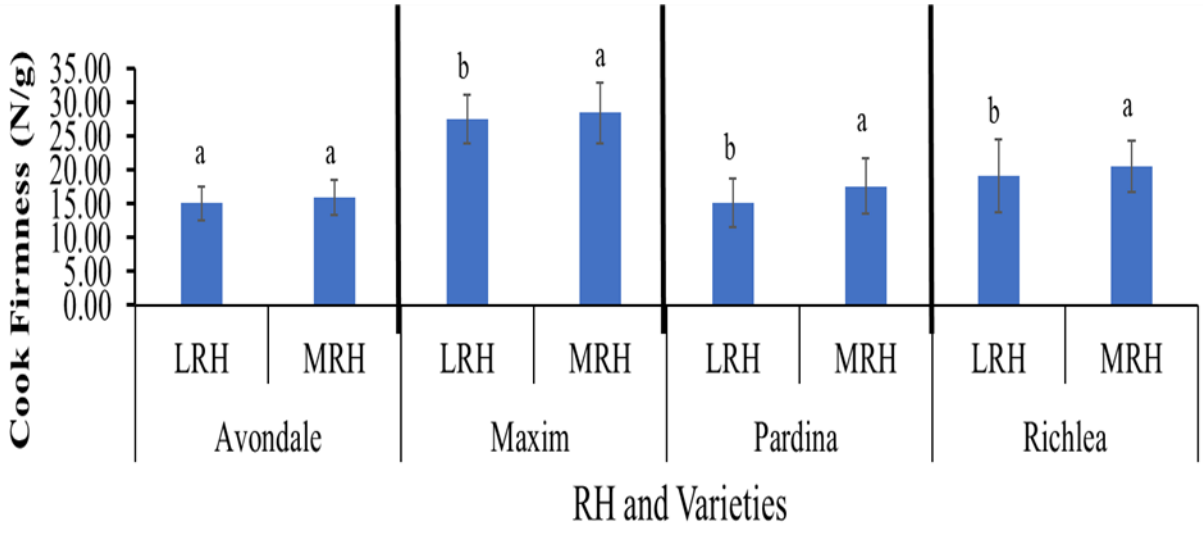

Figure 24: Cook Firmness of lentil seeds obtained from different varieties stored at high temperature (40 °C) under diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase letters represent significant differences across different RH within each variety based on α = 0.05. Error bars represent standard deviation.

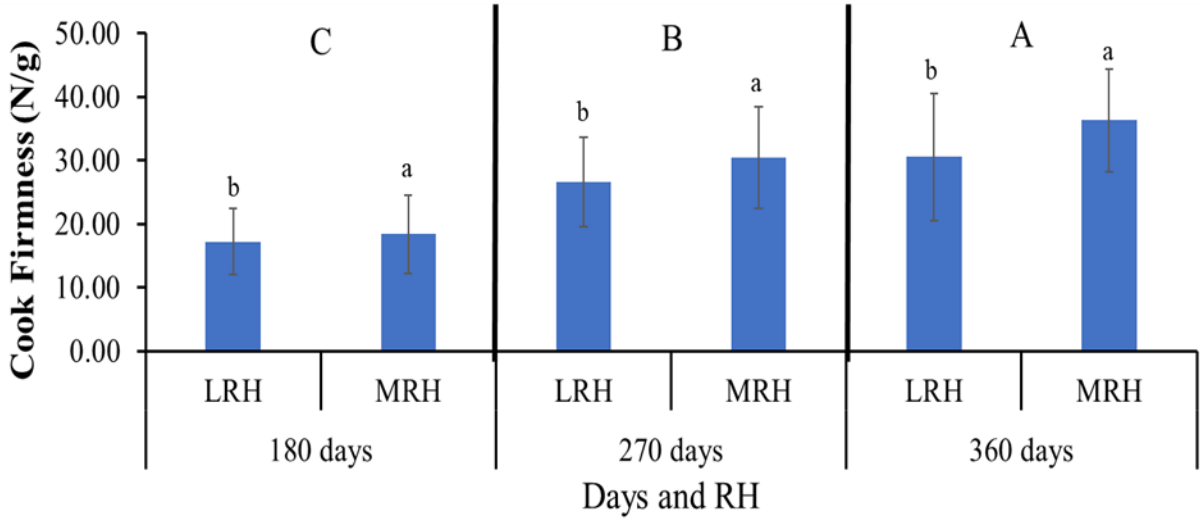

Figure 25: Cook Firmness of lentil seeds obtained from different varieties stored over 360 days at high temperature (40 °C) under diverse relative humidity low relative humidity LRH (40 %) and high relative humidity (55 %). Different lowercase and uppercase letters represent significant differences across storage days and relative humidity and within each sampling day based on $\alpha = 0.05$. Error bars represent standard deviation.

Our results agreed with the findings of (Reyes-Moreno et al., 2000a) where cooking time increased on chickpeas samples stored at 33-35 °C compared to low temperature. Similar to our results, (Karki 2022) indicated that no change was observed in the peas sample stored at low temperature over 270 days. Nasar-Abbas et al. (2008) stated that the cause of faba bean hardening has been identified as high-temperature storage. Storage at temperatures above 30 °C for one year resulted in a harder texture than storage at 25 °C. Seed hardening due to accelerated temperature storage most likely contributed to decreased hydration and swelling capacity (Nasar-Abbas et al., 2008). The hard-to-cook (HTC) defect in the pulses was attributed to the physical and chemical changes during storage. Moreover, the solubilization of pectic substances by the enzyme phytase results in cooked pulses with a hard texture, and also the HTC defect in common beans was

linked to a decrease in protein and starch digestibility, and a decrease in phytic acid content, resulting in an increased cooking time (Hohlberg & Stanley, 1987; Martín-Cabrejas et al., 1997).

3.4.3. Color and Color Difference of Lentil

Days of storage, temperature, relative humidity, and variety impacted the color content of lentil seeds. An increasing trend of color difference was observed for each variety of lentil samples stored at high temperature HT (40 ℃) and room temperature RT (21 ℃). However, HT had a greater impact on color than RT storage. An increase in the color difference of lentil samples stored under different storage conditions (Table 22). The highest color difference occurred at 360 days of storage. Similarly, increasing temperature of storage and relative humidity increased the color difference. Stored samples at HT and HRH had a greater color difference than RT and LRH (Table 22). The color difference was significantly different among the four varieties (Table 22). Furthermore, the visual color difference in the varieties of lentil seeds stored at HT and HRH was significant after 360 days (Figure 26). The color of all varieties became darker after 360 days of storage at high temperature and diverse relative humidity. There was not any significant difference either visually or statistically of color difference on the lentil seeds stored at RT. However, days of storage, relative humidity, and varieties were significant (p-value <0.001) on the color difference of lentil seeds stored at high temperature. The interaction effect of days of storage and varieties was significant (p-value<0.001) when stored at HT. All varieties showed an increase in color difference with increasing days of storage (Figure 27). Furthermore, Richlea had the highest color difference (7.40) followed by Avondale (7.22), Maxim (4.01), and Pardina (2.91). The

interaction effect of RH and varieties when stored at HT was significant (p-value<0.05) for color difference. The color difference for all varieties increased as RH increased (Figure 28). Also, the interaction effect of the days of storage and diverse RH condition on the color difference. The color difference value was higher for lentil seeds stored at high temperature and high relative humidity for 360 days (Figure 29). Additionally, a trend toward greater color difference value was observed as storage days increased (Figure 29).

Table 22: Color difference (± standard deviation) of lentils when factored for variety, day of storage, relative humidity, and storage temperature.

Varieties	**Color Difference**
Avondale	4.22 ± 3.70 [a]
Maxim	2.38 ± 1.96 [b]
Pardina	2.08 ± 1.64 [c]
Richlea	4.34 ± 3.66 [a]
p-value	<0.001
Days of Storage	**Color Difference**
180	2.63 ± 2.08 [c]
270	3.29 ± 3.02 [b]
360	3.83 ± 3.76 [a]
p-value	<0.001
Relative Humidity (RH)	**Color Difference**
40 % LRH	3.03± 2.78 [b]
55% HRH	3.48 ± 3.31 [a]
p-value	<0.001
Temperature	**Color Difference**
21 ℃ (LT)	1.12 ± 0.94 [b]
40 ℃ (HT)	5.38 ± 2.96 [a]
p-value	<0.001

*Different lowercase letters in a column represent significant differences within each variety, storage days, different RH conditions, and storage temperature, respectively based on $\alpha = 0.05$. LRH-Low Relative Humidity (40%) and HRH-High Relative Humidity (55%). RT- Room Temperature and HT- High Temperature.

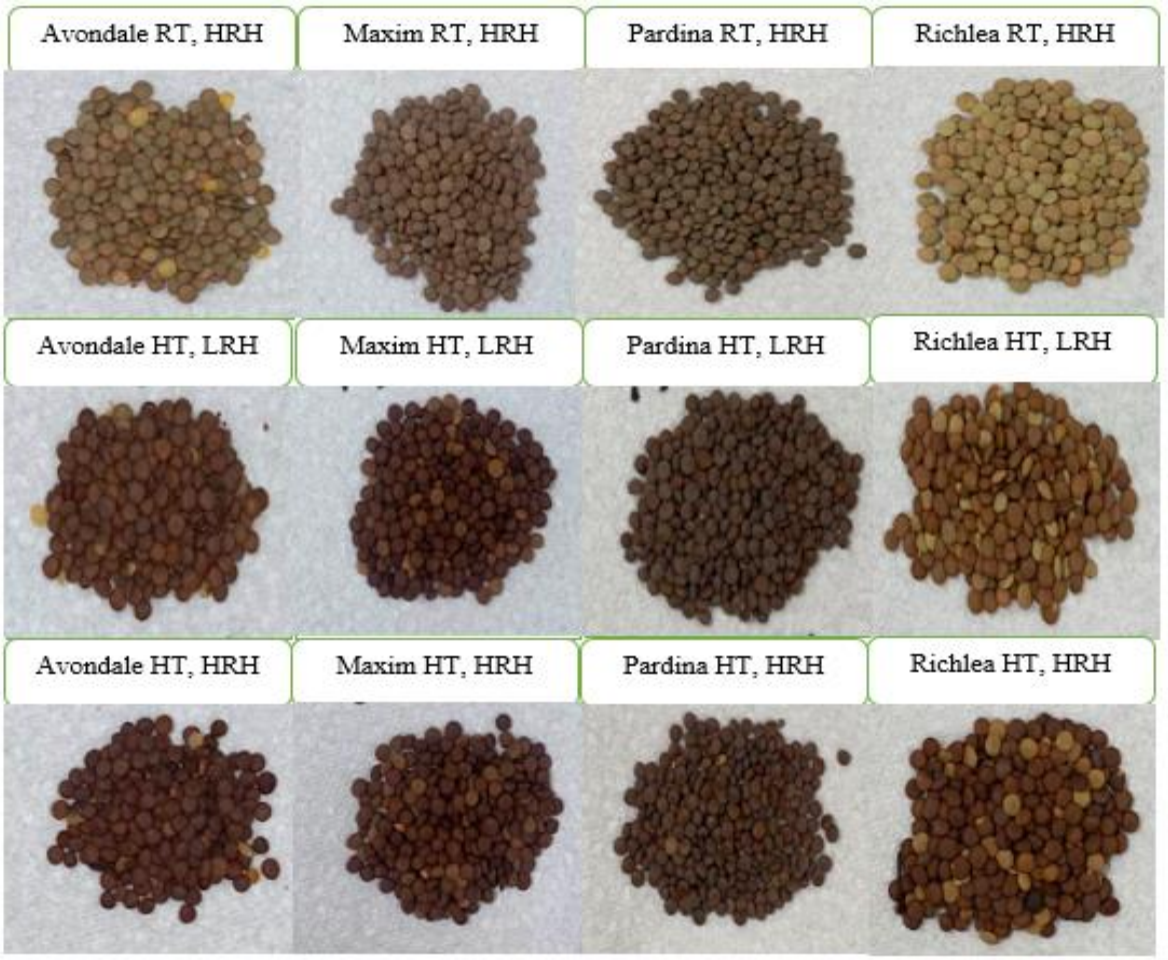

Figure 26: Differences in color of lentil seeds stored under diverse storage conditions for 360 days. Storage Conditions: RT- Room Temperature (21 °C) HT- High Temperature (40 °C); LRH-Low Relative Humidity (40%), HRH- High Relative Humidity (55%).

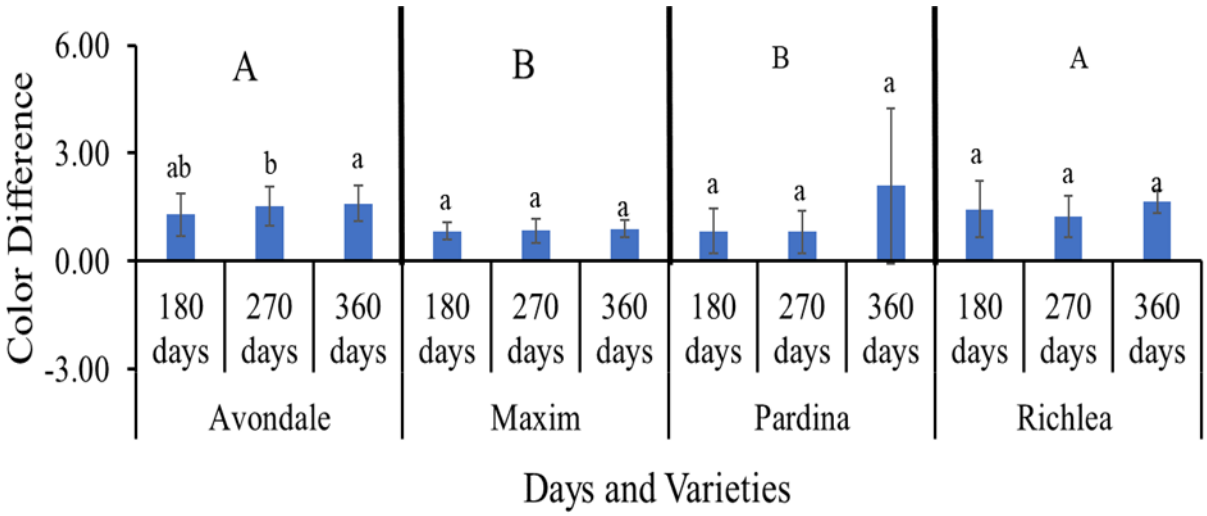

Figure 27: Color difference of different varieties of lentil seeds stored over 360 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant differences across storage days within each variety and across varieties, respectively based on $\alpha = 0.05$. Error bars represent standard deviation.

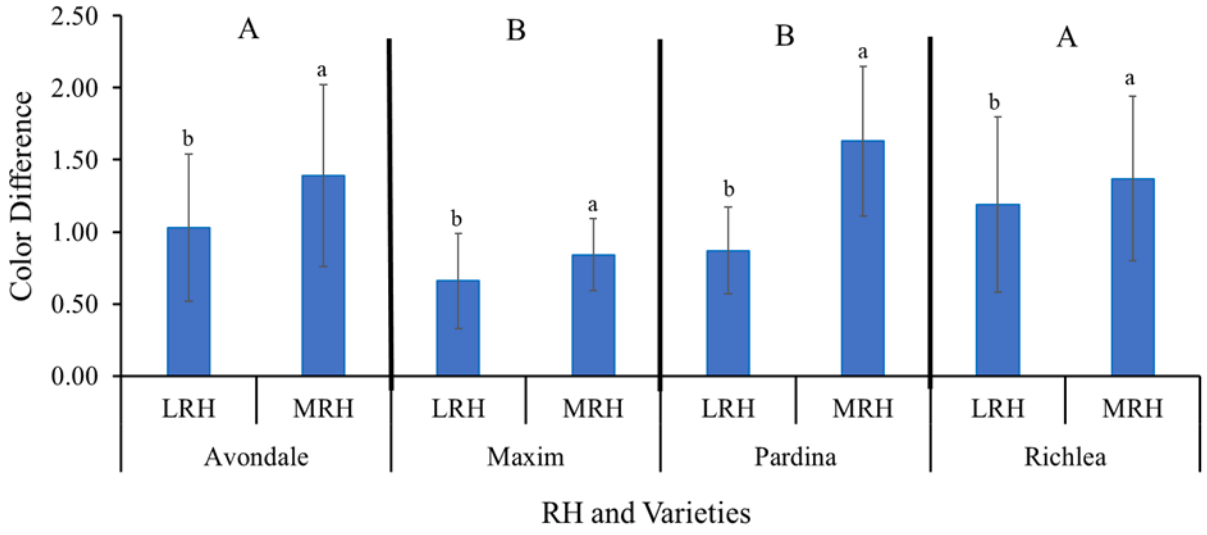

Figure 28: Color difference of lentil seeds stored at different RH and high temperature (40 °C). Different lowercase and uppercase letters represent significant differences across different RH within each variety and across varieties, respectively based on $\alpha = 0.05$. Error bars represent standard deviation. LRH-Low Relative Humidity (40%), HRH-High Relative Humidity (55%).

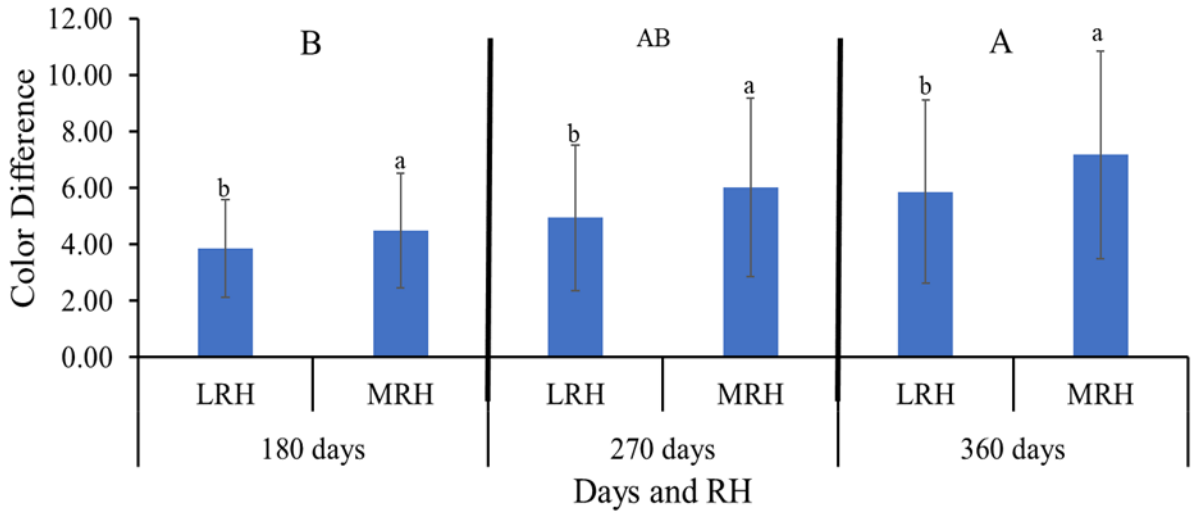

Figure 29: Color difference of lentil seeds stored at diverse RH over 270 days of storage at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across different RH within each day of storage and across days of storage, respectively based on $\alpha = 0.05$. Error bars represent standard deviation. LRH- Low Relative Humidity (40%), HRH- High Relative Humidity (55%).

The color difference was caused by differences in the L*, a*, and b* values of lentil seeds. L* values (i.e., an indicator of lightness) while a* (i.e., an indicator of red/green coordinate values) and b* (i.e., an indicator of yellow/blue coordinate values). Similarly to our results, Sopiwnyk et al. (2020) reported significant changes in the color of some different types of pulses: whole yellow pea, split yellow pea, whole navy bean, and decorticated red lentil, that were stored for 0–24 months under ambient warehouse conditions. Accordingly, L* values were slightly lower for whole yellow pea at 18 and 24 months of storage, and b* values were lower at 12 and 24 months than for most other storage periods and no consistent trend was observed for a* values. Additionally, changes in all three-color values were observed for a whole navy bean. Starting at 3 months of storage, the whole navy bean had decreased L* values compared to time zero values and continued to decrease during storage. (Karki 2022) reported similar results where an

increasing trend of color difference was observed for dry peas stored at HT (40 ℃) and HRH (75 %) over 270 days, and also an increase in the color difference was observed with increasing days of storage. The color changes observed in stored pulse flours are most likely due to enzymatic browning and a possibility of the Maillard reaction, which is thought to occur during pulse storage (Martín-Cabrejas et al., 1997). Reyes-Moreno et al. (2000) stated that browning in stored pulses can be attributed to the non-enzymatic darkening due to the polymerization reaction of phenolic compounds. Non-enzymatic browning is a reaction that causes food to deteriorate, not only by causing a characteristic brown color to appear but also by hardening the samples (Karathanos et al., 2007). In general, color changes of legumes are due to temperature, seed moisture, and light during storage.

4. SUMMARY AND CONCLUSION

The storage of lentil varieties under varying temperature (21 & 40 ℃) and relative humidity (40 & 55 %) conditions for an extended time influenced the nutrient composition, functionality, and physical characteristics. The nutrient composition of flour from different varieties of lentils was evaluated after 360 days of storage under various RH and temperature conditions. The moisture and starch content of lentil flour was observed to be higher with increasing days of storage, increasing relative humidity, and increasing temperature while a reduction in the protein content was observed. A slight increase was observed in the ash content of lentil samples stored for 270 days whereas a noticeable increase was observed in the fat content of lentil flour stored for 360 days at high relative humidity (55%) and high temperature (40 ℃). This outcome suggested that

the long-term storage of lentils high temperature (40 ℃) (HT) and diverse relative humidity (40 and 55 %) can alter the nutrient composition of lentil flour.

The functional properties of lentil flour were affected by long-term storage of different varieties, various temperatures (21 & 40 ℃), and relative humidity (40 & 55 %). The oil absorption capacity of lentil varieties increased steadily over time. Increasing days of storage and storage at RT (21 ℃) resulted in a significantly lower WAI in lentil flours while stored samples at high temperature (40 ℃) resulted in a higher water absorption index. Also, the WAI of lentil flour stored at HT (40℃) and high relative humidity (HRH, 55 %) was higher than the WAI of lentil flour stored at HT and low relative humidity (LRH, 40 %). On the other hand, the WSI of lentil flour stored at 40 % relative humidity was slightly higher than samples stored at 55 % relative humidity. Also, increasing days of storage resulted in a decrease in the WSI index of lentil samples whereas increasing days of storage caused an increase in the water-holding capacity of lentil flour. The FC of lentil flour increased with increasing days and temperature while a slight decrease occurred in the foaming stability of lentil samples stored at 21 ℃ with increasing days. Emulsion activity (EA) of lentil flour stored at low temperature (21 ℃) and low relative humidity (40 %) resulted in higher EA of lentil flour than at high relative humidity (55 %) at low temperature. Similarly, stored lentil samples at low temperature (21 ℃) and low relative humidity (40%) resulted in higher emulsion stability. This outcome suggested that the long-term storage of lentil not only impact the functionality of lentils but also can improve some functionality.

The long-term storage of different varieties of lentils in accelerated temperature and relative humidity conditions influenced starch functionalities. The harshest storage

conditions were high temperature (40 ℃) and high relative humidity (55%) which had the greatest impact on starch properties. Overall, final viscosity and setback viscosity decreased as storage conditions changed, while peak viscosity and hot paste viscosity increased. Less gel strength of lentil flour stored at high temperature over 360 days was observed in the lentil flour as well as lentil flour stored at high relative humidity (55%) over 360 days. The observations suggested for safe and long-term storage lentils should be stored at RT (21 ℃) and LRH (40%) conditions.

The physical properties were influenced by the long-term storage of different varieties of lentils in the accelerated conditions of temperature and relative humidity. The high temperature (40 ℃) and high relative humidity (55%) of storage were the harshest based on having the most significant impact on the physical properties. Changes in the cook firmness of different varieties of lentil seeds were observed due to different storage conditions. Storing lentil seeds at high relative humidity (HRH 55 %) resulted in high cook firmness as well as storing at high temperature (HT 40 ℃). Increasing the time of cooking results in economic losses. Lentil storage at high temperatures (40 ℃) and RH (55%) results in the bleaching and browning of lentil seeds, which can affect their visual quality and significantly lower the market value of lentils.

In conclusion, the main effects of days of storage, temperature, relative humidity, and variety impacted the overall quality of lentils. The harshest storage conditions for lentils were observed to be the increased time of storage, the high temperature (40℃), and high relative humidity (55%) while a low temperature (21℃) and low relative humidity (40%) had a lower impact in the overall quality of lentils. These findings may be of interest to the plant-based food market, where lentils can be alternative to meat

products and flour. Also, it might be helpful in many food applications and to growers and handlers to have a better understanding of lentil varietal behavior. The outcome of this research provides proper storage guidelines for lentils to maintain their quality and enhance their value.

5. FUTURE WORK RECOMMENDATION

The fact that adverse storage conditions caused compositional, functional, and physical changes in lentils suggests that storage may affect the phytochemical composition of lentils. Moreover, the effect of storage conditions on the phytochemical composition and bioactive compounds of different varieties of lentils needs to be investigated further. Changes in volatile compounds in lentil cultivars as affected by storage conditions need to be studied. How the starch and protein attribute to the change of pasting properties need to be sufficiently evaluated and, the impact of storage on the physicochemical and functional characteristics of isolated lentil starch and protein needs to be investigated. The phenomena of browning on the lentil seeds during storage need to be investigated sufficiently.

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Appendix

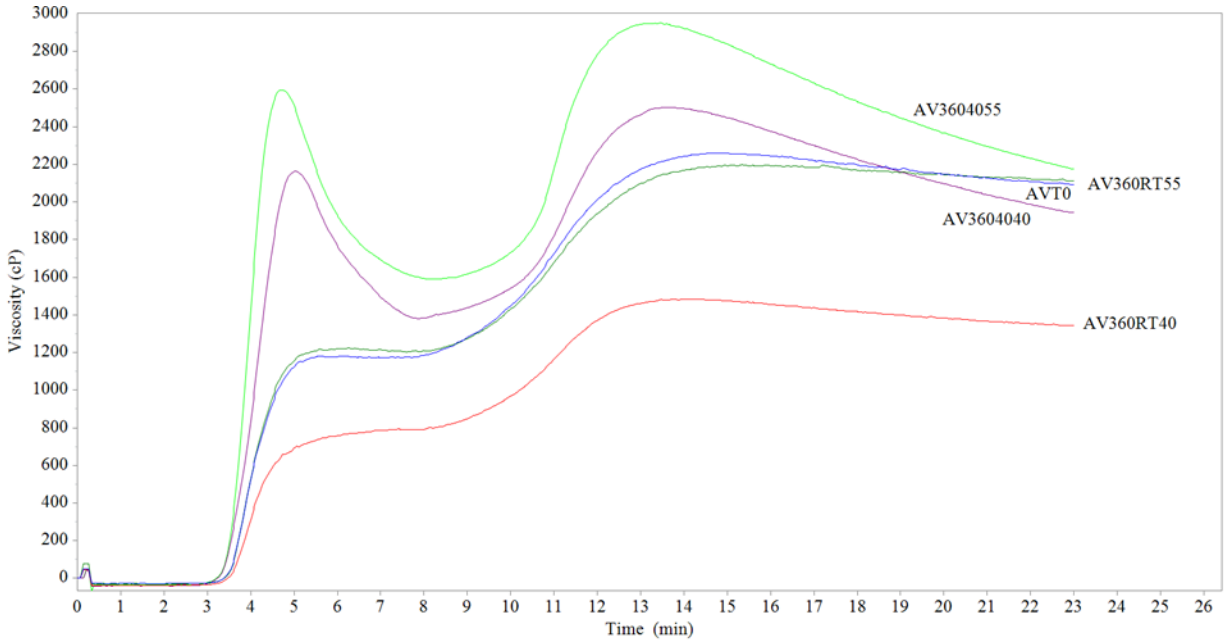

Figure 1: Rapid visco analyzer (RVA) plot of flour from Avondale variety, time 0 and 360 days at 21 °C and 40 °C under 40% and 55% RH.

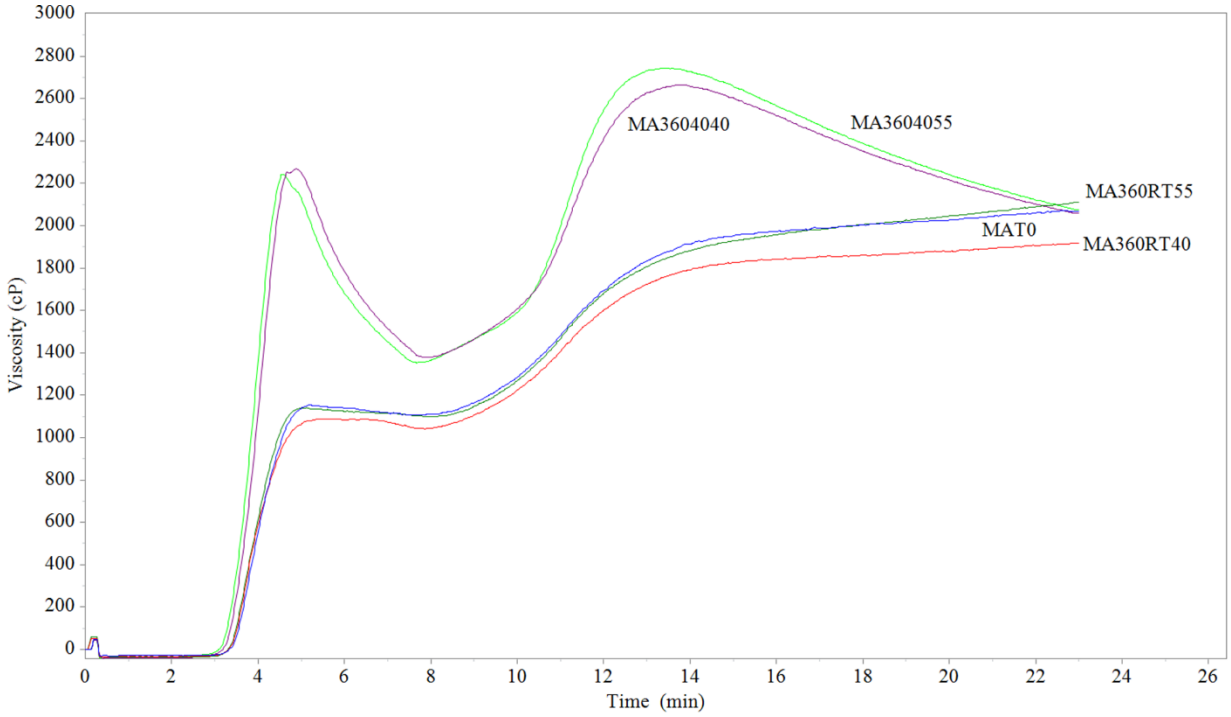

Figure 2: Rapid visco analyzer (RVA) plot of flour from Maxim variety, time 0 and 360 days at 21 °C and 40 °C under 40% and 55% RH.

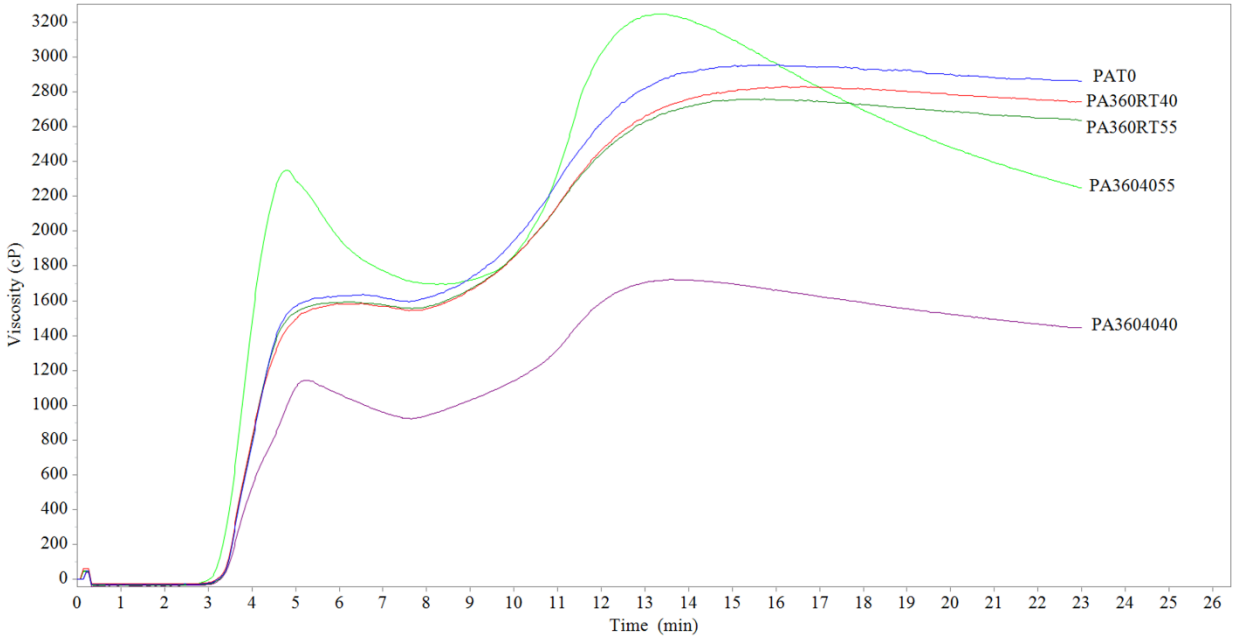

Figure 3: Rapid visco analyzer (RVA) plot of flour from Pardina variety, time 0 and 360 days at 21 °C and 40 °C under 40% and 55% RH.

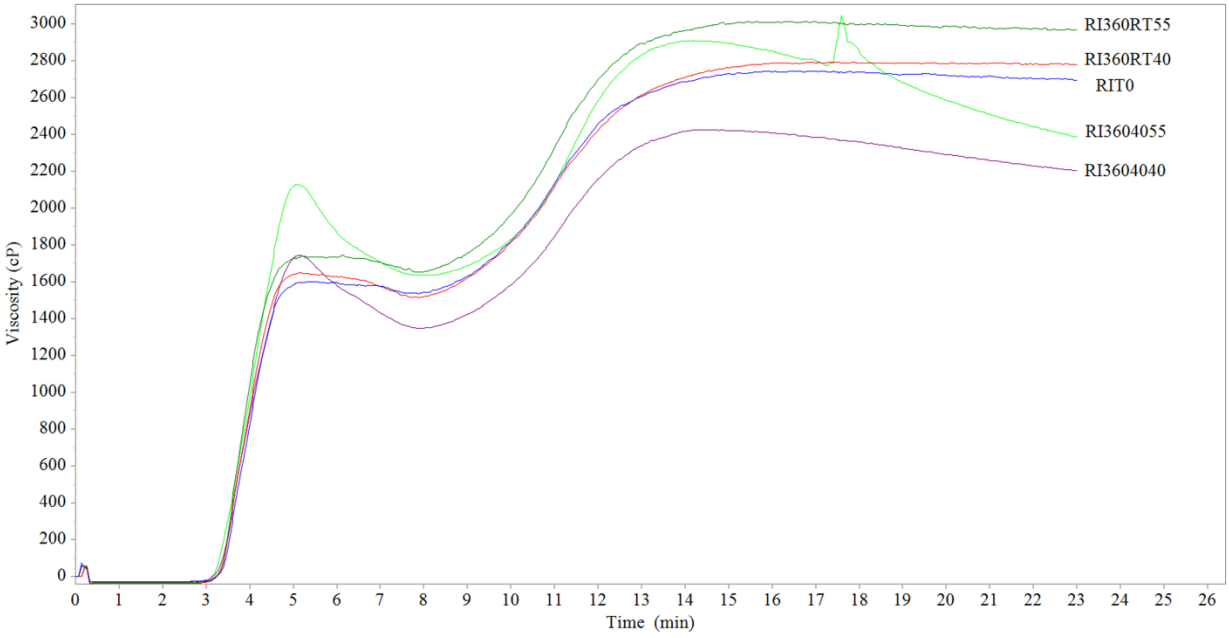

Figure 4: Rapid visco analyzer (RVA) plot of flour from Richlea variety, time 0 and 360 days at 21 °C and 40 °C under 40% and 55% RH.