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IMPACT OF STORAGE CONDITIONS AND VARIETIES ON THE COMPOSITION,
PHYSICAL PROPERTIES, AND FUNCTIONALITY OF DRY PEAS

BY

SUSHMITA KARKI

A thesis submitted in partial fulfillment of the requirements for the

Master of Science

Major in Biological Sciences

Specialization in Food Science

South Dakota State University

2022

THESIS ACCEPTANCE PAGE

Sushmita Karki

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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ACKNOWLEDGMENTS

I owe my deepest gratitude to my advisor Dr. Clifford Hall, for his excellent guidance, patience, understanding, and encouragement during the conduct of this research as well as my graduate studies at South Dakota State University. I am honored to have Dr. Hall as my advisor, thank you for being a wonderful advisor, every single meeting with you have guided me so well for the things coming up. I would like to thank Dr. Vikram Mistry, Dr. Srinivas Janaswamy and Dr. Brent Turnipseed for their valuable suggestions in this research and for serving on my committee.

I am highly indebted to my parents (Gautam Karki and Shanti Karki), my siblings (Sujita Karki and Subham Karki) and entire Karki family, for their unconditional love, blessings, and encouragement, thank you for trusting me and my decision to begin this journey and guiding me in your own possible ways, none of this could have happen without your immense support.

I would like to sincerely thank Dwarika Bhattarai for his time and help for the data analysis, huge data in this research was impossible to analyze without your support. Thank you so much. I would also like to express my thanks to Dr. Serap Vatansever for her support and guidance for the experiments required in this research.

Lastly, special thanks to my lab mates Dana Edleman, Abdulmalik AlbuTuwaybah, Hussain Al Naser, Shirin Kazemzadeh Pournaki, Jessica Christoffer and Jeffery Lehman for providing much appreciated friendly, supportive, and enjoyable lab environment, I really enjoyed sharing lab with you all.

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ABSTRACT

IMPACT OF STORAGE CONDITIONS AND VARIETIES ON THE COMPOSITION, PHYSICAL PROPERTIES, AND FUNCTIONALITY OF DRY PEAS

SUSHMITA KARKI

2022

Dry peas are sustainable, healthy, and nutritious pulse crop and are excellent source of protein and complex carbohydrates as well as micronutrients. The effects of storage conditions on composition of beans and cereals have been widely reported. The information gained-contributed significantly to better storage practices for these commodities. However, literature addressing the chemical composition, functionality, and physical characteristics of dry pea seeds upon storage is scarce. This study was designed to address the impact of storage conditions and varieties on the nutrient profile, functionality, and physical properties of dry pea seeds. Six different varieties of dry peas were stored for 270 days under two temperatures (21 and 40 °C) and three relative humidities (RHs; 40, 65 and 75%) in a sealed glass container. Peas were removed at 30, 60, 90, 180 and 270 days and time 0 served as the control sample to evaluate nutrient composition and functionality of pea flour and physical properties of whole seed. PCA revealed that varieties differ in physical properties and nutrient composition, but behavior of changes were similar while stored under diverse conditions. Storage of peas in the adverse conditions altered the nutrient composition and functionality of flours, starch functionality being most affected (i.e., lower final viscosity and setback viscosity; higher

peak viscosity compared to control sample). With increasing days of storage, peas stored under 65% and 75% RHs at 40 °C had significant color change, i.e., green peas faded to creamy yellow and yellow peas to dark yellow suggesting browning and bleaching of the dry peas. Extended period of storage at 75% RH and 40 °C were observed to be the harshest storage conditions. The findings of this study support physical properties of dry peas can differ remarkably upon the varying storage environments and different cultivar types. Storage conditions can substantially impact nutrient profile, and functionality of dry pea flours while different pea varieties do not have significant impact on these, that can further affect food formulations important for the food industry. Based on this study, storage of pea at 55% RH or lower and 21 °C is recommended for long-term storage.

1. INTRODUCTION

1.1. General Introduction

Dry peas (*Pisum sativum* L.), also known as dry, smooth or field peas, are the naturally dried seeds that exist as yellow or green cotyledon varieties and are grown around the world as a cool-season pulse crop (Dahl et al. 2012). Pulses are the edible seeds of leguminous crops and are most important sources of human food having generous nutritional, agronomic, and economic perks (Chen et al. 2012; Miller et al. 2015; Muramoto et al. 2011). Dry peas are inexpensive and rich source of nutrients, including protein (20-25%), complex carbohydrates (starch, 30–50% and dietary fiber, 3–27%), and antioxidants that are important to improve metabolic health (Vatansever et al. 2021).

Pulses, like dry peas, after harvest are stored on farm or in warehouse storage facilities and then processed. Different physiochemical changes occurring during storage can accelerate qualitative, quantitative, and economic losses. Pulses are typically harvested at 20-22% moisture content, dried, and stored at 12-14% moisture content for 10 months with no losses (Chidananda et al. 2014). The biotic and abiotic elements in the storage structure, primarily temperature and moisture content, as well as relative humidity, have a role in the safe storage of pulses. Therefore, monitoring and management of these parameters will contribute to safe storage with lower qualitative and quantitative losses during extended period (Alagusundaram et al. 1990; Chidananda et al. 2014; Jayas 2012).

A survey of the literature shows that researchers have focused on how to interpret the genetic, agronomic, and environmental impacts on functional properties and nutrient composition of pulses. However, little effort has been made to address the impact of

storage on functional and compositional changes of pulses. There is only limited information available regarding changes that take place under various pulse storage conditions. Changes in bean and cereal composition under various storage environments has been extensively investigated whereas, pulses like pea have not been given much attention. The storage impact on dry beans has been reported, but the studies to-date lack a systematic reporting of composition and functionality, and some only report a few nutrients and many lack information on functionality. It is for these reasons that this study has been conducted.

1.2. Research Objectives

The goal of this research was to address the gap in knowledge related to the storage impact on composition and functional and physical properties of different varieties of dry peas by completing the following objectives:

- 1) To store different varieties of dry pea samples in different treatment conditions, i.e., accelerated temperature and relative humidities, for nine months.
- 2) To determine the effect of storage and variety on the proximate composition of the stored dry peas.
- 3) To characterize the storage and variety impacts on the functionality of the dry peas.
- 4) To establish the impact of diverse storage conditions and variety on the physical properties of the dry peas.

We hypothesis that diverse storage conditions will impact the nutrient composition and functional properties of dry peas. High storage temperatures and relative humidity will impact the nutrient, functional and physical characteristics of dry pea to a greater extent

than storage at low temperature and relative humidity. Variety will not impact nutrient, functional properties, and physical properties of dry pea under similar storage conditions.

2. LITERATURE REVIEW

2.1. Introduction to Pulses and Dry Peas

Pulses are defined by Food and Agriculture Organization (FAO) of the United Nations as a type of leguminous crop that are harvested solely for the dry seeds that are consumed directly. Pulses grow in pods and come in various shapes, sizes, and colors. FAO have acknowledged 11 different types of pulses: dry beans, dry broad beans, dry peas, chickpeas, cow peas, pigeon peas, lentils, Bambara beans, vetches, lupins and puls. Dry pea (*Pisum Sativum* L.) is one of the most common pulse crops in the world based on total production in comparison to other pulse crops. It accounts for 8 to 14.6% of the total world production of pulses (Joshi and Rao 2017). Dry peas have either green or yellow cotyledon (Dahl et al. 2012); however, the composition between the two varieties market classes is similar (Hall et al. 2017).

Historical data shows that dry peas were primarily grown in the United States in the Palouse region of Washington and Idaho (Simsek et al. 2009). However, in 1990, North Dakota and Montana began producing. In 1991, approximately 647 hectares of dry peas were planted in North Dakota and in 2006, 247,000 hectares, which was 66% of the US production. More than 70% of the total dry pea production in the United States is exported to India, China and Spain for food and feed processing (Simsek et al. 2009). World production of dry peas in 2018 was more than 13.5 million tons, the major producers being Canada, Russia, and China (FAOSTAT 2020). The total dry pea production of the United States in 2020 was 941,571 metric tons (Hall 2020).

2.2. Economic Importance of Dry Peas

2.2.1. Food Industries

Pea protein is one of the popular, easily available, economic and sustainable additive or supplement in global food industry (Shand et al. 2007; Tulbek et al. 2017). Several studies have been conducted to investigate the formulation of innovative food and beverages like bread (Espinosa-Ramírez et al. 2018; Pico et al. 2019; Sahagún et al. 2020), pasta (Linares-García et al. 2019), meat products (Baugreer et al. 2018), baked goods (Gularte et al. 2012; Matos et al. 2014), snacks (Morales-Polanco et al. 2017; Philipp et al. 2018) and beverages (Akin and Ozcan 2017; Ben-Harb et al. 2020; Yin et al. 2015) and demonstrated the potential of pea protein to enhance nutritional and functional properties of the product depending on the amount incorporated. Furthermore, gluten-free product can be prepared with pea ingredients for example, gluten-free muffins prepared using pea protein isolates possess comparable characteristics to those made from the wheat gluten (Shevkani and Singh 2014).

Global interest in pea fiber, coming from recovered pea hulls, that result from split pea production and protein fractionation, is increasing due to increased interest in the fiber fortification, and improving shelf-life of food products. It can be considered as the best choice to formulate low carbohydrate and low-calorie food products such as for bread, snack foods, biscuits, crackers, pasta, tortillas, and dietary supplements (Damian and Olteanu 2014). When pea fiber was incorporated in yogurt making, yogurt with high viscosity and the reduction in syneresis compared to control was observed (Damian and Olteanu 2014). Pea pods, the by-products of pea processing can be of importance because of the dietary fiber, protein, and minerals content in the pods. The pods were utilized to

formulate fiber enriched instant pea soup powder that helps to enhance the functional properties of soup and is a good way to utilize a waste product (Hanan et al. 2020).

Starch is used to develop edible films that are biocompatible, non-toxic, economic, and environment friendly, in addition they also have similar properties as synthetic polymers, i.e., they are odorless, transparent tasteless, semi-permeable to CO₂ and resistant to O₂ diffusion. Comparatively, due to the high amount of amylose content in pea starch, it has been reported to produce films with improved physical and mechanical properties than other traditional starches (Saberri et al. 2016). Application of pea starch in the development of food products like bread (Lu et al. 2018) and noodles (Li and Vasanthan 2003; Wang et al. 2012) improved quality characteristics.

2.2.2. Feed Industries

Dry peas are energy and protein rich pulses that can be used as an animal or livestock feed and are comparable with other feeds such as barley, corn, canola meal and sunflower meal (Anderson et al. 2002). It has been reported (Anderson et al. 2002) that dry peas are excellent supplement and palatable feedstuffs for beef cattle, dairy cattle, poultry, swine and sheep. Several studies have been conducted to confirm the inclusion of dry pea to feed pigs without affecting their performance (Stein et al. 2004; Stein et al. 2006).

Lactating dairy cows fed ground dry peas to replace soybean meal and corn grain were not negatively impacted by pea consumption. The ground peas did not affect milk yield or composition (Vander Pol et al. 2008). Similarly, dry peas can replace other feeds in the diet of beef cattle without affecting animal gain and carcass quality (Fendrick et al. 2005). In addition, dry peas have potential application as aquaculture feed. It has been successfully used to substitute wheat in seabass feed where no impact on growth

performance, carcass quality and organoleptic properties were observed (Adamidou et al. 2009). Additionally, research conducted on blue shrimp diet with a dry pea as an ingredient also supported that dry pea is a suitable and acceptable ingredient for shrimp feed (Cruz-Suarez et al. 2001). Furthermore, dry peas are a protein rich nutritious and palatable feed for rainbow trout (Thiessen et al. 2003). Overall, animal studies support the inclusion of peas in animal diets. Humans can also benefit from pea consumption.

2.3. Health Benefits of Dry Peas

2.3.1. Glycemic Index

Pulses are considered as a part of a healthy diet, as they have a very low-glycemic index and are very high in dietary fiber. Pulses contribute to reducing blood lipids that are harmful and a major risk factor for cardiovascular disease when they are consumed consistently (Mizelman et al. 2020). The concept of glycemic index (GI) can be explained as the potential of carbohydrate rich foods to increase blood sugar or glucose levels once consumed (Wolever et al. 1991). It can be affected by the rate of digestion or absorption of the carbohydrate containing foods. Low glycemic index diet is of importance to improve metabolic control of hyperlipidemia in diabetic patients (Goñi and Valentín-Gamazo 2003). Thus, consumption of low GI food is suggested to control diabetes by American Diabetes Association (ADA) and Canadian Diabetes Association (CDA). Moreover, lower GI also helps to reduce insulinemic and glycemic responses to food and protect against colon cancer. Low, medium and high GI foods are assigned values of 55 or less, 56-69, and 70 or greater, respectively (Singh et al. 2021). Different types of pulse based ingredients have been used to develop pulse-fortified low GI foods such as pastas, breads, crackers, extruded snacks, cookies, cereal bars, and

muffins. Lower GI results in foods with pulse ingredients was demonstrated where mean reductions of 10.8 ± 2.7 GI units and 4.8 ± 2.6 GI units were observed *in vitro* GI and *in vivo* GI testing, respectively proving the role of pulse flours on reducing glycemic index (Fujiwara et al. 2017). Similarly, GI of whole yellow pea flour incorporated as a functional ingredient to produce pasta, banana bread and biscotti was determined and compared with whole wheat flour. Results confirms the utilization of dry peas can be beneficial to produce low GI products (Marinangeli et al. 2009). Comparison of glycemic response of yellow pea flour, pea starch and maize starch was completed directly on these ingredients without incorporation into food items. Yellow pea flour and pea starch were found to have better (i.e., lower value) glycemic response than maize starch (Seewi et al. 1999). The composition of dietary fibers may have caused the differences in GI, where higher amounts of dietary fiber caused lower GI and vice-versa (Trinidad et al. 2010).

2.3.2. Cardiovascular Disease

Diets rich in fiber have been proven to help lowering blood pressure, improve serum lipid levels and reduce inflammation (Slavin 2008). Epidemiological evidence shows by consuming legumes four times or more per week compared with once a week reduced coronary heart disease and cardiovascular disease risk (Bazzano et al. 2001). The presence of antioxidant components in the pulses may have helped to reduce cardiometabolic risk. In addition, folic acid present in the pulses reduces homocysteine levels that helps to reduce the risk of stroke (Rebello et al. 2014).

2.3.3. Obesity

Rebello et al. (2014) reported that pulse consumption may influence satiety that can help consumers to overcome environmental cues to eat or help them to comply their calorie

restriction. Meal with lentils and yellow peas reduced appetite and energy intake when compared to a meal that consists of macaroni and cheese. Similarly, increase in duration of satiety was noticed when the consumption of pea fiber enriched bread was compared to regular bread (Lunde et al. 2011). However, no effect of pea protein was observed on satiety when compared with egg albumin, casein, gelatin, soya protein and wheat gluten (Lang et al. 1998).

2.4. Nutritional Composition of Dry Peas

Dry pea is a significant source of protein, complex carbohydrates, vitamins, and minerals (Table 1). It is relatively high in crude protein (14-31%), total carbohydrates (55-72%), which includes mainly starch (30-49%) and total dietary fibers (3-20%), vitamins (e.g., folate) and minerals but are low in fat (Hall et al. 2017).

Table 1: Proximate composition of different market classes of dry pea grown in the USA in 2020.

Proximate Composition (%)	Green Pea Mean (SD)	Yellow Pea Mean (SD)	Winter Pea Mean (SD)	Marrowfat Pea Mean (SD)
Moisture	9.2 (1.3)	9.9 (1.1)	7.8 (0.9)	6.8 (0.5)
Ash	2.6 (0.3)	2.4 (0.6)	2.5 (0.1)	2.5 (0.1)
Fat	1.6 (0.6)	1.7 (0.6)	1.7 (0.4)	2.1 (0.2)
Protein	23.5 (1.3)	21.4 (1.3)	21.3 (1.3)	23.5 (1.3)
Total Starch	45.1 (3.0)	43.9 (3.0)	46.1 (2.4)	43.9 (3.0)

Source: Hall 2020

2.4.1. Carbohydrate

Dry peas are of great nutritional importance because of their high content of complex carbohydrates (i.e., starch and dietary fibers). The most significant component of dry pea

is starch, which accounts for 36.9-49.0% of the total nutrient composition (Dahl et al. 2012). Environmental and cultivar effects were found to be substantial in dry pea starch as variability in starch composition was observed among samples (Wang and Daun 2004).

Chemically, starch is composed of two types of glucan polymers (i.e., amylose and amylopectin). Amylose is a simple linear glucan molecule of α -(1-4)-linked D-glucose units whereas, amylopectin is more complex highly branched molecule with α -(1-4)-linked glucopyranosyl units in a chain that are connected by α -1,6 linkages (Vanier et al. 2017). Compositionally, pea starch is composed of 35-65% amylose with the remaining content composed of amylopectin (Zhou et al. 2019). Diverse pea cultivar, growing location and year were documented to have an effect on the starch content of the pea. Significant interactive effect of cultivar-by-year and cultivar-by-location was found in the starch content. The highest starch content was observed in Cooper and Cutlass varieties and the lowest was in CDC striker. Also, peas grown in 2006 and 2007 were significantly different in their starch content, peas from 2006 having the higher value of starch. Similarly, pea samples from the Melfort location had the highest starch content, whereas pea samples from Indian Head and Swift Current had the lowest (Wang et al. 2010).

Moreover, the composition of dry peas also contains dietary fibers in the form of seed coat, i.e., pea hull fiber and the cotyledon, that ranges from 14-26% of dry weight basis (Brummer et al. 2015; Dahl et al. 2012). The Food and Drug Administration (FDA) defines dietary fiber as the “non-digestible soluble and insoluble carbohydrates and lignin that are intrinsic and intact in plants” (FDA 2018). Total dietary fiber is composed of

insoluble dietary fiber and soluble dietary fiber. Insoluble and soluble fiber contents of dry pea range between 8.7-12.9% and 0.6-3.7%, respectively (Stoughton-Ens et al. 2010).

2.4.2. Protein

Dry peas have gained prominent interest as a high quality plant-based proteins due to its high protein content (i.e., 23-31%) that can vary among cultivars (Boukid et al. 2021).

Pea protein can be classified into four major groups. The major proteins are globulin that is a salt-soluble and the main storage protein found in dry peas that accounts for 55-65% of total protein content. Globulin dissociates into subunits at different pH values and ionic strength. Albumins, i.e., the metabolic and enzymatic proteins, are the other major pea protein that ranges from 18-25% in a dry pea seed. Prolamin and glutelin are present as a minor protein and observed in a lower quantities, i.e., 4-5% and 3-4% respectively (Lu et al. 2020; Vatansever et al. 2020). Pea protein contains a high concentration of lysine, which is a deficient amino acid in cereal grains. Arginine, phenylalanine, leucine, and isoleucine are present in high amounts in globulin. Albumin is high in tryptophan, lysine, and threonine. However, dry pea proteins are low in sulfur containing amino acids; methionine and cysteine (Bahnassey et al. 1986; Vatansever et al. 2020). Pea proteins are gaining popularity globally because of its nutritional and health benefits, economic, sustainable, and availability. Furthermore, pea proteins have less allergic controversies and high digestibility in comparison to other plant proteins (Lu et al. 2020).

2.4.3. Lipids

Lipid account for a very small amount in the nutrient composition of dry peas. Lipid content of dry peas was noted to be 0.6-3.9% (Hall 2020). Limited research on pea lipid content have been published because most of the attention are focused on dry pea as a

rich source of protein, starch, fiber and micronutrients but low in lipid. Dry pea lipid is mainly composed of triacylglycerides and phospholipids. The lipid content was reported to have a distribution of high amount of phospholipids (52.2 to 61.3%) and triacylglycerides (31.2 to 40.3%) whereas, diacylglycerols (2-4%), free fatty acids (1.3-2.7%), steryl esters (0.8-2.4%) and hydrocarbons (0.5-0.9%) were noted to be present in small amounts (Yoshida et al. 2007). In terms of fatty acid content, linoleic and oleic acids are the main unsaturated fatty acids while palmitic acid is the predominant saturated fatty acid present in the dry peas (Kukavica et al. 2007). Moreover, the fatty acid profile of dry peas contains 10.65% palmitic, 3.29% stearic, 28.25% oleic, 47.59% linoleic, 9.29% linolenic and 0.22% arachidic acids (Ryan et al. 2007).

2.4.4. Other Minor Constituents

Other important minor components in dry peas are vitamins, minerals, and bioactive compounds. Dahl et al. (2012) reported that potassium is available in high amount (i.e., 1.04% of dry weight basis) in dry pea seeds. Other minerals include phosphorous (0.39%), magnesium (0.10%), and calcium (0.08%) are the other minerals present in dry peas. Furthermore, U.S. grown field peas are a rich source of iron (46–54 mg kg⁻¹), zinc (39–63 mg kg⁻¹), and magnesium (1350–1427 mg kg⁻¹) (Amarakoon et al. 2012).

Dry pea is the dietary source of vitamins. Vitamin B- Folic acid or folate, is one of the important dietary components that is required to form red and white blood cells and epithelial cells of the digestive tract. The concentration of folate ranges from 23.7 to 55.6 µg/100 g in the yellow pea and 24.9 to 64.8 µg/100 g in the green pea (Han and Tyler 2003). Moreover, bioactive compounds such as phenolic compounds,

oligosaccharides, saponins, phytate, enzyme inhibitors, and lectins are also present in dry peas (Patterson et al. 2017).

2.5. Functionality of Dry Pea Flour

Dry pea flours are considered as one of the suitable ingredients to develop innovative products such as pasta, noodles, snacks, plant-based meat alternatives, and baked goods due to its functional properties (Ren et al. 2021). In the food system, functional properties are defined as the important physicochemical properties that affects the functionalities of proteins of foods while preparing, processing and storage (Kinsella 1982). It also describes how the ingredient behaves during preparation, cooking and how the final products are affected in terms of its appearance, structure, feel and taste. Functional properties include, water absorption index, water solubility index, water holding capacity, oil absorption capacity, emulsion activity, emulsion stability, foaming capacity and stability (Awuchi et al. 2019). Components such as carbohydrates, proteins, fats and oils, moisture, fibers, ash of the flour and the physical structure of these components are factors influencing the functional properties of flours (Awuchi et al. 2019).

2.5.1. Water Holding Capacity (WHC)

Water holding capacity (WHC), also termed as water hydration capacity, water absorption capacity and water binding capacity, refers to the amount of water that is taken up by flour or food per gram of protein or the water retention ability of proteins against gravity separation to achieve the desirable consistency. When water is added to the flour, hydration process begins when hydrophilic interactions occur among molecules of starch and protein and hydrogen bonds with the molecules of water (Awuchi et al. 2019; Lam et al. 2018). The interaction of ion-dipole, dipole-dipole and dipole-induced

dipole interactions causes water binding (Vatansever et al. 2020). Furthermore, WHC is also influenced by the amino acid composition. According to Lam et al. (2018), the capacity of water molecules to bind differs among the groups, i.e., backbone peptide groups, amide groups, hydroxyl groups and nonpolar residues of amino acids. WHC of an ingredient is very important functional property required as it determines the quality of finished product in terms of mouthfeel, texture, and flavor retention. Very low or high WHC can negatively impact food formulations and affect the textural quality of the product. Furthermore, WHC is important in the development of baked foods as it can affect several parameters like proofing, loaf volume, bread yield, bread crumb, shelf-life, and machinability while bread making (Awuchi et al. 2019). Over absorption and under absorption of water by flour can lead to several quality problems while developing foods. Over absorption of flour by water can cause wet and sticky dough that results in poor machinability and sometimes over fermentation whereas final product can have excessive volume, mold issues and poor symmetry. Similarly, under absorption of water can create stiff and dry dough, proofs slower, dry ingredients may not be dispersed well because of less water and the end finished product can have firm and dense texture, may stale quickly, and have a low volume (AIBInternational 2018).

2.5.2. Oil Absorption Capacity (OAC)

Oil absorption capacity (OAC) or oil holding capacity (OHC) is one of the important functional properties of flour. Lam et al. (2018) defines OAC as the amount of oil that a flour can absorb per gram of protein. Factors such as protein conformation, amino acid composition, and surface polarity or hydrophobicity tend to contribute to OAC of protein in food system (Awuchi et al. 2019). Higher protein composition of flour result in higher

oil absorption value of the flour. Binding of the aliphatic chains of lipid to the nonpolar side chains of amino acids caused lipid and protein content in the flour to interact (Lam et al. 2018).

OAC of flour tends to influence the mouthfeel, flavor, texture, and yield of the final product. The OAC is the essential functional property of flour that are important for producing doughnuts, pancakes, baked goods, desserts, confectioneries, beverages, salad dressings, meats extenders, and meat analogues and enhances the sensory attributes of the finished product (Vatansever et al. 2020; Wang et al. 2020). Moreover, higher OAC of flour will result in improved palatability, extension of shelf life, and flavor retention when used in the preparation of meat or bakery products that requires fat absorption (Chandra et al. 2015). Oil absorption capacity (OAC) are calculated in a percentage value as well as in g/g value depending on the methods followed for the determination of OAC (Wang et al. 2020, Ferreira et al. 2018).

2.5.3. Foaming Properties

In the food system, foaming properties of protein are of importance to produce variety of foods. The term foam refers to the two-phase system made up of air cells that are separated by a thin continuous liquid layer called the lamellar phase. Food foams are often complicated systems that contain a combination of gases, liquids, solids, and surfactants (Zayas 1997b). Lam et al. (2018) stated that foams are dispersion of gas bubbles in a liquid or solid phase. Foaming capacity (FC) is the amount of interfacial area that can be created by whipping the flour. Foam stability (FS) is defined as the time needed to lose 50% of either liquid or volume of foam. It is the stabilization of foam against stress by the protein (Awuchi et al. 2019; Lam et al. 2018).

Protein content of flour is primarily responsible for foaming properties. Awuchi et al. (2019), reported that usually, FC and FS are dependent on the interfacial film formed by the proteins that maintains the air bubble suspension that tends to slow down the rate of coalescence. FC and FS are supposed to have inverse relationship that means when the flour has high FC, there can be large air bubbles that result in thin and less flexible protein film. The large bubbles may collapse very easily, which results in lower stability of the foam (Jitngarmkusol et al. 2008). The flours to be incorporated as an ingredient for the development of baked products such as angel food cakes, and muffins must possess good foaming properties (El-Adawy and Taha 2001).

2.5.4. Emulsifying Properties

In food emulsion system, emulsion can be explained as the heterogenous combination or dispersion of two or more immiscible liquids with the help of mechanical agitation. The types of emulsions include oil-in-water, for instance milk and mayonnaise, and the other is water-in-oil type, such as butter and margarine (Awuchi et al. 2019; Lam et al. 2018). The oil-in-water exhibits a creamy texture whereas, water-in-oil emulsion tend to have a greasy texture. Emulsifying properties are the key functional properties for the innovation of plant-based products and are described as emulsion capacity (EC), emulsion activity (EA) and emulsion stability (ES). EC is the measure of oil (mL) that is emulsified by 1 g protein under certain conditions that is dependent on the shape, charge, and hydrophobicity distribution of the protein molecules, neutrality of dipoles, and hydration of polar groups. ES can be defined as the potential of the emulsion to resist changes caused by the mechanism of creaming, coalescing, and flocculation. The properties of proteins and emulsification condition, protein source and its concentration, pH, ionic

strength, and viscosity of the system are some parameters influencing EC and ES. EA is the emulsifying properties that is present as the maximal interfacial area (cm²) of a stabilized emulsion per 1 g of protein (Zayas 1997a).

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

The water absorption index (WAI) of flour is the measure of volume occupied by the granule or starch polymer after swelling when the flour is treated in excess water. Water solubility index (WSI) is defined as the amount of small molecules such as polysaccharides solubilized from the granule upon the addition of excess of water (Yousf et al. 2017). While producing an extruded snack product, WAI and WSI are considered as the critical functional quality characteristics. High WAI values represent the availability of large starch molecules in the flour or the product. Similarly, high WSI means the presence of dextrinized starch molecules in the product (Oikonomou and Krokida 2011). In addition, WAI of flour is related to molecules such as starch and protein and their hydrophilicity and gelation capacity and WSI is related to the solubility of molecules (Du et al. 2014).

2.5.6. Pasting Properties

Starch is one of the most important ingredients in food products. Starch undergoes changes as a result of being hydrated under thermal conditions is referred to as gelatinization. This process leads to swelling of starch granules and leaching out of some molecules like amylose and amylopectin from swollen granules causing an increase in viscosity; which is called pasting of starch (Debet and Gidley 2006). Pasting is an important functionality as it impacts the sensory characteristics of a food. The degree of pasting that results from the application of heat in the presence of water that influences

the final texture, digestibility, and the end-use in products (Ocheme et al. 2018). Different instruments are used to evaluate the pasting properties of starch such as amylograph (e.g., Brabender Amylograph), dynamic rheometer equipped with a starch pasting cell, Ottawa starch viscometer, Rapid-Visco Analyzer (RVA), and consistometer (Balet et al. 2019).

RVA is the heating and cooling viscometer used to measure the change in viscosity of an aqueous suspension starch or flour over a given period under constant shear applied through a paddle. A pasting profile of the starch is constructed using changes in the viscosity of the starch suspension as a function of temperature and time (Liu et al. 2019). Balet et al. (2019) reported that the RVA test is comprised of five different stages, i.e., addition of water to sample, heating, holding at the maximum temperature, cooling, and the final stage as a holding stage. The standard RVA pasting profile consists of initial temperature that is set at 50 °C; holding time of 1 min at 50 °C; heating over 3 min 42 s to 95 °C; holding for 2 min 30 s at 95 °C; cooling over 3 min 48 s to 50 °C; and the last stage holding for 2 min at 50 °C.

Peak viscosity, trough viscosity (also known as hot paste viscosity), breakdown viscosity, setback viscosity, final viscosity (also known as cold paste viscosity), peak time and pasting temperature are parameters obtained during the RVA test (Ohizua et al. 2017) and can be plotted as viscosity or resistance encountered during the test (Figure 1). According to Balet et al. 2019, the maximum viscosity recorded during heating is called peak viscosity. It is also an indirect measure of water holding capacity of starch. Ohizua et al. (2017) indicated trough viscosity as the ability of the paste formed by heating to resist breakdown while breakdown viscosity is the flour's ability to resist heating and shear stress while cooking. Furthermore, final viscosity is defined as the capacity of

starch to form viscous paste after cooking and cooling and setback viscosity measures the retrogradation tendency of starch. The time at which the peak viscosity occurs is the peak time and pasting temperature is the temperature when the viscosity of starch tends to increase.

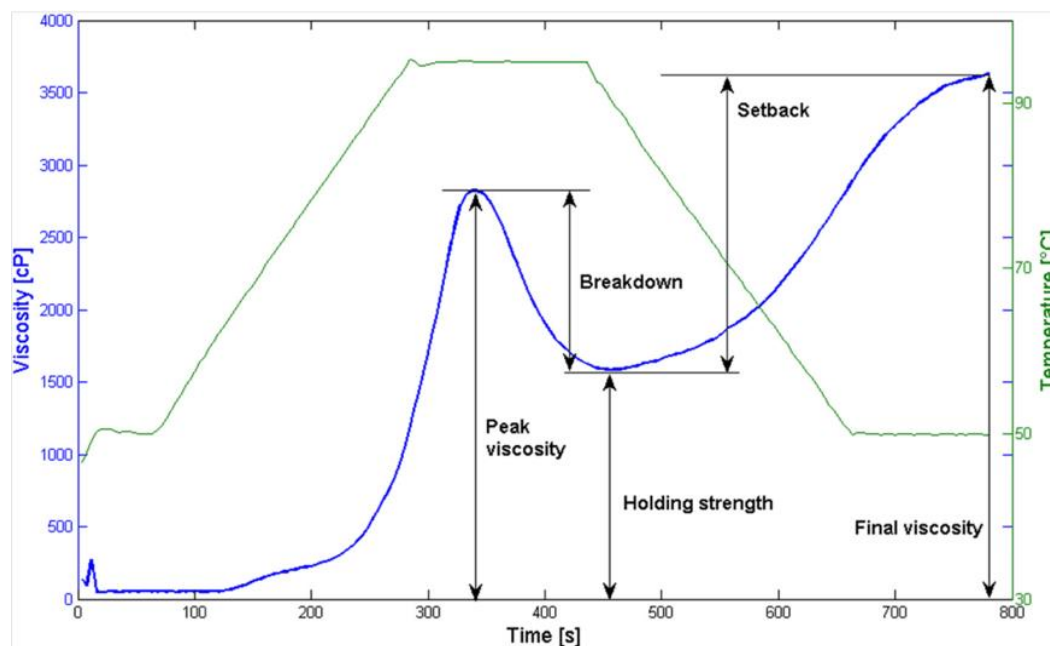


Figure 1. Typical rapid visco-analyzer (RVA) profile of heat-treated flour (150 °C, 15 min) in water. Source: (Keppler et al. 2018)

2.6. Physical Properties and Varieties of Dry Peas

Dry peas exist in green and yellow cotyledon varieties and have four different market classes, i.e., yellow pea, green pea, winter pea and marrowfat pea (Hall 2020). Globally, various kind of dry pea cultivars are available for cultivation. However, US producers mainly grow Ariel, Arcadia, Cruiser, Banner, Columbian, CDC Striker, and K-2 green pea cultivars and CDC Meadow, CDC Golden, DS Admiral, CDC Agassiz, Delta, and Bridger yellow pea cultivars, which is 65-70% of total varieties of pea production in the United States (Tulbek et al. 2017).

Physical properties of dry peas vary extensively between the varieties. Test weight helps to indicate sample density, size, and shape. Hall (2020) reported that test weight of dry peas grown in the USA in 2020 ranged from 46.3 to 67 lbs./Bu (Table 2). The 1000 kernel seed weight of dry peas ranged from 145 to 318 g. The 1000 seed weight varied among cultivars. Yellow pea was observed to have the highest 1000 seed weight followed by green pea, marrowfat pea and winter pea having the lowest. In addition, swelling capacity, water hydration capacity and cook firmness of dry pea cultivars were determined (Hall 2020). Water hydration capacity of all dry peas ranged from 68 to 119%, the swelling capacity ranged from 89% to 159% and the cooked firmness ranged from 11.5 to 48.3 N/g with a mean value of 24.9 N/g. These parameters varied depending upon the different market class and variety. Seed weight and volume and the hydration and swelling capacity are the factors affecting cooking time. Seed with dense cotyledon have less swelling capacity which leads to the longer cooking time (Özer et al. 2012). Factors such as cultivar, growing location and year of cultivation effect cooking time and firmness of dry peas significantly (Wang et al. 2010).

Table 2: Physical Properties of different market classes of dry pea grown in the USA in 2020.

Physical Properties	Green Pea Mean (SD)	Yellow Pea Mean (SD)	Winter Pea Mean (SD)	Marrowfat Pea Mean (SD)
Test Weight (lbs./Bu)	64 (2)	63 (2)	65 (0.4)	62 (1)
1000 seed wt. (g)	220 (31)	244 (28)	175 (12)	300 (26)
Water Hydration Capacity (%)	99 (7)	93 (7)	96 (5)	111 (6)
Unhydrated Seeds (%)	2 (2)	2 (3)	1 (1)	0 (0)
Swelling Capacity (%)	120 (12)	116 (12)	119 (8)	136 (3)
Cooked Firmness (N/g)	21.7 (4)	27.2 (6.6)	21.6 (1.6)	23.6 (5.1)

Source: Hall 2020

2.7. Storage Impact on Chemical Composition of Pulses

Pulses are generally harvested when the moisture content is about 20 to 22% then dried and can normally be stored for 10 months at 12 to 14% moisture content with no losses. The biotic and abiotic elements in the storage structure, primarily temperature and grain moisture content, as well as relative humidity, plays an important role in the safe storage of pulses (Jayas 2012).

Faba bean seeds were studied after storage in three different storage conditions for nine months (El-Refai et al. 1988). Slight decrease in protein content of faba beans was observed after nine months of storage, which was attributed to the activity of proteolytic enzymes. However, no significant differences in the crude protein content were observed in other research studies on storage of common beans or black beans (Berrios et al. 1999; Molina et al. 1975; Nyakuni et al. 2008). Pea stored for seven months at 90% relative humidity (RH) caused alterations in protein composition, i.e., decrease in albumin,

increase in vicilin and no change in legumin (Górecki et al. 1985). Sievwright and Shipe (1986), studying black bean storage at 30 °C and 40 °C and 80% RH, also documented poor protein digestibility of the stored beans. Moreover, a significant reduction in digestibility of protein was confirmed in common beans (Nyakuni et al. 2008) and chickpeas (Reyes-Moreno et al. 2001).

Some changes in the starch content and starch functionality were also reported in stored pulses. El-Refai et al. (1988) reported the gradual reduction in the starch content of faba beans after the storage for three, six and nine months. A significant decrease in the starch digestibility was reported by Nyakuni et al. (2008) for beans after the six months of storage. Adzuki beans were studied after storage in accelerated condition of temperature and RH (Yousif et al. 2003). These authors reported an increase in starch gelatinization onset temperature and gelatinization peak temperature for the adzuki beans stored for 6 months at 30 °C. Similarly, storage of adzuki beans at 40% RH led to high starch gelatinization onset temperature and gelatinization peak temperature compared to 65% RH. While studying the impact of storage on common black beans, (Garcia-Vela and Stanley 1989), high starch gelatinization temperature in the beans stored at high temperature, i.e., at 30 °C compared to those stored at 15 °C were observed. Changes in the crystallinity of the starch granules was the possible reason given for such changes in the starch gelatinization onset temperature and gelatinization peak temperature of the stored beans (Yousif et al. 2003). Reyes-Moreno et al. (2001), also confirmed increase in starch gelatinization temperature of stored chickpea compared to the chickpea without storage.

Phytochemical composition of pulses also was affected by the unfavorable storage conditions. For instance, the research conducted on chickpea stored at accelerated condition, i.e., 33– 35 °C, RH-75% for 160 days (Reyes-Moreno et al. 2000), showed significant reduction in phytic acid content of stored chickpea. In addition to this, tannin content was also determined and found to decrease in the seed coat while it increased in the cotyledon of the stored chickpeas. Similar reductions in phytic acid of black beans were reported by Sievwright and Shipe (1986). In addition, Nyakuni et al. (2008) observed reduction in the phytic acid of stored common bean. Similarly, a reduction in phytic acid content and an increase in tannins concentration was reported after long term storage of dry beans (Martín-Cabrejas et al. 1997). The greatest reduction in phytic acid was reported in the cowpeas and dry beans that were stored at 29 °C and 65% RH; proving high temperature and RH caused phytic acid to decrease (Hentges et al. 1991). However, El-Refai et al. (1988) found no significant changes in the phytic acid content of stored faba beans.

Limited compositional changes due to storage have been reported for peas. Instead, compositional changes in other pulses might be an indicator for potential compositional changes in peas. Loss in lipid content was reported in navy and pinto beans during a 65-day storage (Chen 1991) whereas, Berrios et al. (1999) mentioned no reduction of lipid in black bean after two years of storage at 4.5 °C. Gradual decrease in ash content of faba beans stored for 9 and 12 months with increasing temperature was reported while no noticeable changes were observed in phosphorus, iron, calcium and magnesium contents of faba beans (El-Refai et al. 1988; Nasar-Abbas et al. 2008). In addition, increased sugar content in the faba beans stored over nine months was observed,

while increased sugar contents were reported in navy beans stored for 65 days (Chen 1991; El-Refai et al. 1988).

Several storage studies on pulses indicate changes in the physical properties of pulses. Storage of legumes at adverse conditions, i.e., high temperature ($>25\text{ }^{\circ}\text{C}$) and high RH ($>65\%$) will reduce the quality and develop hardening phenomenon which is characterized by longer cooking times of legumes. This is known as hard-to-cook (HTC) defect or hard-shell effect (HS) (Martín-Cabrejas et al. 1997; Njoroge et al. 2015). HS effect is considered to arise because of the restriction of moisture migration during processing of legumes (soaking and cooking) due to biochemical changes like oxidation of tannins, formation of protein–tannin complexes or biophysical changes like size reduction leading to the impermeability of the seed coat or outer shell to water. Seeds do not tend to soften while soaking or do not become tender after cooking for certain time are known to have the HTC defect. Legumes having these defect require high energy processing to facilitate softening. Even after softening, the beans are less acceptable among consumers because of their poor texture and low nutritional quality (Njoroge et al. 2015; Shiga et al. 2004). In addition, (Shimelis and Rakshit 2005) proposed that the hardness of beans and increased cooking time was the result of the reduction in water permeability that influenced the hydration capacity of seed. Faba beans (Nasar-Abbas et al. 2008) stored at high temperature ($>37\text{ }^{\circ}\text{C}$) were found to have low hydration and swelling coefficients compared to beans stored at low temperature ($<25\text{ }^{\circ}\text{C}$).

In addition to texture issues, changes in the color of legumes due to the long-term storage at high temperature and RH have been observed. For example, darkening of kidney beans stored at high temperature and RH was observed, while there was no

difference in color of beans stored at low temperature and RH for 1 year (Hughes and Sandsted 1975). As per Reyes-Moreno et al. (2000), storage at high temperature (33-35 °C) and RH (75%) for 160 days caused darkening of testa color of chickpea. Similarly, testa color of faba beans was affected by the storage temperature and days of storage (Nasar-Abbas et al. 2009). After 12 months of storage, faba bean seeds changed color from beige to medium brown that were stored at low temperature, i.e., <25 °C and at high temperature, i.e., >37 °C, seeds color changed to dark reddish brown or almost black. Collectively, the evidence supports changes in pulse chemistry and functionality, but a lack of complete compositional analysis suggest that additional research is needed. Furthermore, data on compositional changes of peas stored under different environmental conditions has not been reported and will be the focus of the research presented in the remaining parts of this thesis.

3. MATERIALS AND METHODOLOGY

3.1. Materials

3.1.1. Dry Pea Samples

Six different varieties of dry pea samples, i.e., yellow pea (Agassiz and Salamanca), green pea (Arcadia and Ginny) and winter pea (Keystone and Vail) were collected in duplicate from seed handlers in 2020. These duplicates were maintained throughout the storage and analytical aspects of the study (Figure 2).

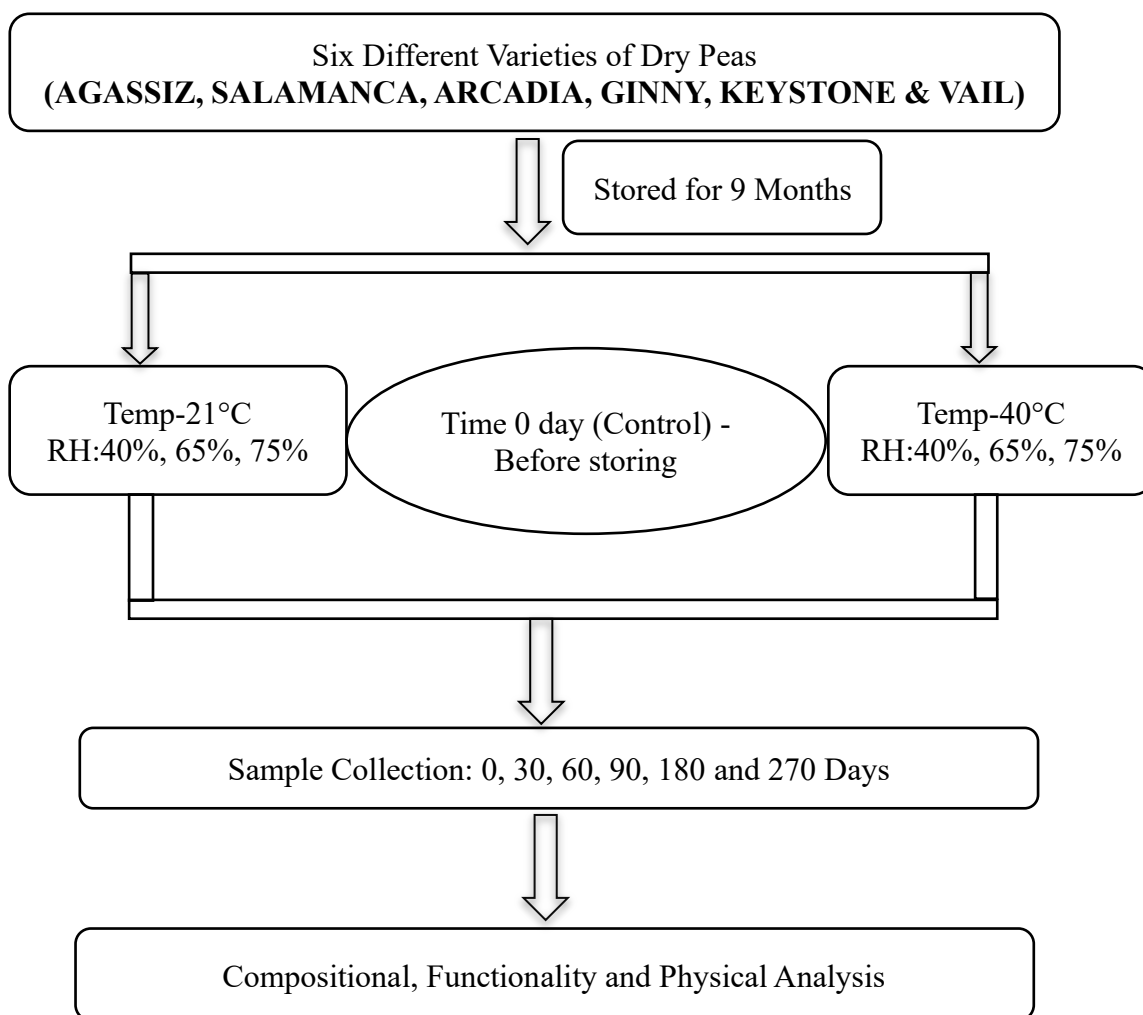


Figure 2: Overall experimental plan followed in this research.

3.2. Methods

3.2.1. Storage of Dry Peas

Dry peas collected were cleaned by mechanical and hand removal of foreign material and split peas and sub-divided into two pools (i.e., subsample). The first pool of samples comprised of six varieties of peas and stored for nine months at 21 °C and relative humidities of 40%, 65% and 75%. The second pool of peas (same cultivars) were subjected to temperature of 40 °C and several relative humidities of 40%, 65% and 75% (Figure 2). For the ease of explanation, relative humidities of 40%, 65% and 75% were assigned as LRH, MRH and HRH and for temperature 40 °C as HT and 21 °C as RT in the result and discussion section. The storage of the peas was done in two replications following the specified sampling plan (Table 3).

Table 3: Sampling plan for dry peas stored at varying temperature and relative humidity (RH).

Varieties	Treatments	Number of Sampling	Amount of pea taken per Sampling (g)	Sampling time (Days)
6	21 °C, 40% RH	5	150	30, 60, 90, 180, 270
6	21 °C, 65% RH	5	150	30, 60, 90, 180, 270
6	21 °C, 75% RH	5	150	30, 60, 90, 180, 270
6	40 °C, 40% RH	5	150	30, 60, 90, 180, 270
6	40 °C, 65% RH	5	150	30, 60, 90, 180, 270
6	40 °C, 75% RH	5	150	30, 60, 90, 180, 270

3.2.2. Milling

At incremental time periods, i.e., 30, 60, 90, 180, 270 days of storage, subsamples of dry peas were collected and milled using a UDY cyclone mill into flour for further analysis. The milling conditions include 12,600 rpm (standard operating condition set by manufacturer, UDY Corporation, Fort Collins, CO) using a 0.5 mm screen. Proximate composition and functionality analysis were completed on the milled flours of stored dry pea cultivars.

3.2.3. Proximate Composition

3.2.3.1. Moisture Content

Moisture content of flour was determined using the official AACC International method 44-15.02 (AACCI 2010). For each duplicate, 2 g of pea flour was added to pre-weighed drying cups (W_1). The flour and cup were weighed (W_2) prior to placement into a 130 °C oven for 3 hours and again after cooling in a desiccator (W_3). Moisture content was determined using the following formula:

$$\text{Moisture Content (\%)} = \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100$$

3.2.3.2. Protein Content

The nitrogen content of the pea flour was used to determine the protein content in the samples using combustion method following the AACC International method 46-30.01 (AACCI 2010). A conversion factor of 6.25 was used.

3.2.3.3. Lipid Content

Lipid content was obtained using the official AACC International method 30-10.01 (AACCI 2010). Weight of the filter bags (W_1) was taken, 1.5 g of sample was weighed in the filter bags (W_2) sealed and dried at 104 °C for 3 hours before lipid extraction. Dried

samples were allowed to cool in the desiccator and weighed again with the filter bag (W_3). The samples were inserted into the Soxhlet apparatus, hexane was used to extract lipid from the pea flour. Lipid extraction was carried out for 4 hours and 25 min. Samples were removed from the Soxhlet, kept in room temperature for 5 min and placed in a 103 °C in oven for 30 min to remove any residual hexane. Samples were removed from the oven and allowed to cool and weighed (W_4).

The formula used for calculations was as follows:

$$\text{Lipid Content (\%)} = \frac{(W_3 - W_4)}{(W_2 - W_1)} \times 100$$

3.2.3.4. Ash Content

Ash content was obtained using the official AACC International method of 08-01.01 (AACCI 2010). Ash can be defined as the inorganic residue that remains after the ignition or complete oxidation of organic matter in flour or food. It represents the total minerals content in any food (Marshall 2010). Sample flour was heated at high temperature. Weight of the empty crucible was taken (W_1), 1 g of flour was weighed, and the weight of the crucible and flour was noted (W_2). To prevent burning of the sample, the oven was first brought to 350 °C for 1h, then 450 °C for 1h, before being left at 590 °C overnight. The crucible with the ash was allowed to cool in a desiccator and weighed again once cool (W_3). The ash content was determined using the following formula:

$$\text{Ash Content (\%)} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

3.2.3.5. Total Starch Content

Total starch was determined using the official AACC International method 76-13.01 (AACCI 2010). K-TSTA-50A/ K-TSTA-100A kits from Megazyme International (Bray

International) was used for the analysis. In this method, 0.1 g of sample was used during the assay and each sample run in duplicate.

3.2.4. Functionality

3.2.4.1. Pasting Properties

Pasting profiles of dry pea flour samples was determined using a Rapid Visco Analyzer (RVA) (RVA 4500, Perten Instruments, Springfield, IL) based on the modified AACC International method 61-02.01 (AACCI 2010). Briefly, the modifications included the weight for flour (3.5 g) and water (25 g) adjusted for flour moisture content. Further, the temperature during a run started at 50 °C and then raised to 95 °C over 4 min and 42 seconds followed by a holding period until 7 min and 12 seconds into the run. Then, at 11 minutes the temperature was dropped to 50 °C and remained at 50 °C until the end of 23 min run. Peak time, hot and cold paste viscosities, and breakdown viscosity information was collected from the instrument.

The starch prepared in the RVA was stored at room temperature to cause gelation for 2 hours. The gels formed in the canisters was evaluated for their textural properties using a texture analyzer (Ta.Tx, Texture Technologies Corp, 6 Patton Drive, South Hamilton, MA). Each canister was placed upright on the metal plate and the gel was compressed at a speed of 4 mm/s to a distance of 15 mm and trigger force of 2 g with a cylindrical plunger (diameter= 10 mm). The compression generated a force–time curve from which hardness (height of first peak) was determined.

3.2.4.2. Foaming Capacity and Stability

Foaming capacity and stability was determined as the foaming properties of pea flour (Stone et al. 2015). With a slight modification in the method, 1.00% (w/w) protein

solution (based on weight protein content within the dry powder) was prepared with 10 mM sodium phosphate buffer (pH 7.00), and the resulting solution was stored overnight at 4 °C. Afterward, 15 mL (V_{li}) of the protein solution was transferred into a narrow 400 ml glass beaker and foamed using an Omni Macro homogenizer at the speed of 8000 rpm for 5 min. Immediately following homogenization, the foam was transferred to a 100 mL graduated cylinder. Foam volume was recorded at time zero and after 30 min. Foaming capacity (FC) and foaming stability (FS) were determined using following equations, respectively,

$$\%FC = \frac{v_{fi}}{v_{li}} \times 100\%$$

$$\%FS = \frac{v_{ft}}{v_{fi}} \times 100\%$$

Where, V_{fi} = volume of foam immediately after homogenization and

V_{ft} = volume of foam remaining after 30 minutes.

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

The WAI and WSI of pea flours was determined using a modified method (Simons et al. 2012). Pea flour (2.5 g) was transferred to pre-weighed 50 mL centrifuge tubes and the combined mass was recorded. Water (30 mL) was added and shaken vigorously to break lumps and then stirred with stir bars for 30 min. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant was decanted into pre-weighed beakers, which were placed in an oven at 110 °C for 20 hours followed by storage at 120 °C for 7 hours. The beaker with solids was weighed and difference represents the solids that remained in the supernatant, which is used to calculate WSI. The tubes and the contained wet sediment were weighted to measure WAI. The WAI (g/g) and WSI (%) were calculated using the following formulas:

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

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

3.2.4.4. Water Holding Capacity (WHC)

WHC of pea flour was determined using the method described in AACC Method 56-37.01 (AACCI 2010). Sample (1 g) was placed in a test tube (W_1). The test tube and filter cloth (between test tube and syringe barrel) were placed inside the syringe barrel (W_2). Test tube was removed, and DI water was added slowly (dropwise) to the flour and stirred with a glass rod until wet, stirred for 1 min and glass rod was removed and cleaned with the filter cloth. At the end of the test tube the filter cloth was placed and then kept inside the barrel in upside down direction. This syringe assembly was placed in a 50 ml centrifuge tube and then centrifuged at room temperature at 300 g for 10 min. The final weight was taken after removing the syringe assembly from the centrifuge tube (W_3) and WHC was calculated as:

$$WHC = \frac{(W_3 - W_2) + (W_1 \times mc)}{(1 - mc)W_1}$$

Where, mc= initial moisture content of sample.

3.2.4.5. Oil Absorption Capacity (OAC)

OAC was determined using the method described by Wang et al. (2020). Sample (0.5 g) was transferred into a test tube. The weight of filter paper, test tube with sample and syringe barrel was taken together. Canola oil (1.5 mL) was added to the test tube, and the mixture vortexed for 5 s every 10 min for a total of 20 min. The test tube containing oil and sample was then inverted with the filter paper at the bottom of the test tube into the syringe, then the assembly was immediately placed into a 50 mL centrifuge tube and

centrifuged at 600 x g for 25 min. Upon centrifugation, free oil not bound to the flour passed from the filter paper and collected in the centrifuge tube. The whole assembly, i.e., syringe barrel, filter paper, test tube, sample, and oil absorbed was weighed after centrifugation. A sample blank with filter paper was also included during centrifugation to avoid the problem created by some free oil entrapped in the filter paper and was not collected at the bottom of the conical centrifuge tube. OAC was calculated as:

$$\text{OAC (g oil/g sample)} = \frac{(W_3 - W_2 - W_4)}{(1 - m_c/100)W_1}$$

Where, W_1 = weight of the sample before oil addition (g),

W_2 = weight of the syringe barrel, filter paper, test tube, and sample (g),

W_3 = weight of the syringe barrel, filter paper, test tube, sample, and oil absorbed after centrifugation (g),

W_4 = weight of oil absorbed by the blank filter paper after centrifugation (g),

m_c = initial moisture content of the sample (%).

3.2.4.6. Emulsification

Emulsion activity (EA) and emulsion stability (ES) was determined as the emulsification properties (Yasumatsu et al. 1972). With a slight modification in the method, 1.25 g of stored pea flour was suspended in 48.75 g of 10 mM sodium phosphate buffer (pH 7.00) and kept in fridge for overnight at 4 °C. Protein solution (24.5 mL) was mixed with 24.5 mL of canola oil in a beaker. The solution was then homogenized using Omni Macro homogenizer at the speed of 8000 rpm for 3 min. For EA, 10 mL of the homogenized solution was then transferred to 15 mL centrifuge tubes, height of the entire emulsion was measured, followed by centrifugation at 1315 x g for 5 min. The heights of the emulsified layer were noted after centrifugation. For ES, the remaining portion of the emulsion in

the beaker was heated at 80 °C in a water bath for 30 min and then cooled to room temperature in cold water bath for 15 min. Ten mL of the obtained emulsion was then transferred into a 15 mL centrifuge tube, height of the entire emulsion was taken followed by centrifugation at 1315 x g for 5 min. The heights of emulsified layer were recorded. EA and ES were calculated using the following equations:

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

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

3.2.5. Physical Properties of Whole Seed

3.2.5.1. 1000 Kernel Seed Weight

1000 kernel seed weight was determined based on the modified Hall (2020) method. The modification included counting 50-kernel sample of dry peas weight times 20 gave 1000 seed weight.

3.2.5.2. Water Hydration Capacity (%) and Swelling Capacity (%)

The water hydration capacity (%) evaluation was completed using the AACC method 56-35.01 (AACCI 2010). Hydration capacity of pulses is defined as the amount of water that whole seeds absorb after soaking in excess water for 16 hours at room temperature (22 ± 2 °C) and is expressed as the amount of water absorbed per 100 g of seeds. A modification of the official method included soaking 50 seed of dry pea. Prior to soaking, the seeds were counted, and the mass was taken before soaking. Seeds were then soaked in excess water at room temperature for 16 hours and the final mass of soaked seeds were taken to determine water hydration capacity of seeds. The water hydration capacity was calculated using the following formula:

$$\text{Water Hydration Capacity (\%)} = \frac{\text{Mass of seeds post soak} - \text{Mass of seeds pre soak}}{\text{Mass of seeds pre soak}} \times 100$$

Swelling capacity (%) was determined by measuring the volume before hydration (i.e., soaking) and after hydration as described by Hall (2020). The modified method included 50-kernel of dry pea seeds being counted, and mass and volume being recorded before soaking. Seeds were soaked as described above and the final volume of soaked seeds were taken. The percentage increase was then determined using the formula:

$$\text{Swelling Capacity (\%)} = \frac{\text{volume of seeds post soak} - \text{volume of seeds pre soak}}{\text{volume of seeds pre soak}} \times 100$$

3.2.5.3. Color and Color Difference

Konica Minolta CR-410 Chroma meter was used to determine color and color difference of stored dry peas and soaked peas. The instrument was first calibrated using standard white plate. After calibration, color measurements were randomly taken in duplicates on the peas prior to soaking and then again after soaking. L*, a*, and b* values was recorded to measure color where L* represents lightness/darkness, a* represents 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, and a negative value is greener and a positive b* value is yellower, and a negative value is bluer. Color difference was then calculated through the difference in L*, a*, and b* values for 0 day and 30 days, 0 day and 60 days and so on till 270 days using the following formula:

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

3.2.5.4. Cook Firmness

The method from AACCI international method 56-36.01 (AACCI 2010) was used to complete cook firmness analysis. This method determines the firmness of cooked pulses using a texture analyzer. Firmness of cooked pulses is defined as the maximum force

required to shear the cooked pulses and is expressed as the maximum shear force per gram of cooked sample. In this method, 40 g of stored dry peas were soaked for 22 ± 2 hours at room temperature (22 ± 2 °C). Soaked peas were then cooked for 25 minutes, and 7.5 ± 0.5 g of cooked peas were loaded into a Mini Kramer Shear Cell attached to a texture analyzer (Ta.Tx, Texture Technologies Corp, 6 Patton Drive, South Hamilton, MA). System parameters were set up at a speed of 1.50 mm/s to a distance of 28 mm and trigger force of 50 g with a load cell of 30 kg capacity to determine the firmness of cooked dry peas. The maximum shear force measured was recorded. Firmness was reported as N/g.

3.2.6. Statistical Analysis

All the analysis were performed in duplicate. Principal component analysis (PCA) and Pearson correlation (r) was carried out using R studio software, version 1.4.1717 for determining the relationship between different properties. The PCA results were graphically represented by the projection of the first two principal components. The principal components explaining the highest data variation was selected for further data analysis. Multivariate analysis of variance (MANOVA) for the main effects (variety, days of storage, RH and Temperature) and interactions were determined using R studio software, version 1.4.1717. The statistical comparison within groups were performed using Fisher's Least Significant Difference (LSD) at 5% probability level and presented in the bar graphs and tables as lowercase and uppercase alphabets "a", "b" and so on. Any overlapping error bars in the figures (Standard Error "SE") were not considered for conclusive interpretation (GraphPad 2021). SE in the bar graphs was presented to show the data distribution.

4. RESULTS AND DISCUSSION

4.1. Proximate Composition of Dry Peas

4.1.1. Principle Component Analysis (PCA)

Principal Component Analysis (PCA) on proximate composition i.e., moisture content of flour, protein content (%), starch content (%), fat content (%) and ash content (%) of flour of different varieties of dry pea stored under diverse storage conditions were carried out to study the relationship between various components. PCA on proximate composition of pea flour revealed that PC1 and PC2 accounted for 29.4% and 25.7% of cumulative variance, respectively. The plot shows the observations as a point formed by two principal components and the arrow in the plot showed the original variables as a vector. The longer the vector, the more variability is represented for a variable by the two displayed principal components (Hartmann 2018). Also, the angles between the vectors represent the correlation between the variables.

The biplot (Figure 3), demonstrated that dry pea flour of different varieties, i.e., Agassiz (AG), Salamanca (SA), Arcadia (AR), Ginny (GI), Keystone (KE) and Vail (VA) are different in nutrient composition. Similarly, biplot was plotted for the proximate composition of flour of dry peas with respect to days, temperature, and relative humidity. Increasing days of storage effected the proximate composition of dry peas (Figure 4). However, days 30 and 60 were similar with not much data variation, whereas 90, 180 and 270 days were more variable. Thus, further data analysis was continued only with data from 90, 180, and 270 days being compared with time 0, i.e., the control sample. Temperature biplot (Figure 5), data variation in HT and RT were similar, thus data analysis only for HT were carried out. All the treatment conditions of RH, i.e., LRH,

MRH, HRH produced variation (Figure 6). Thus, further data analysis was proceeded with all the treatment conditions of RH.

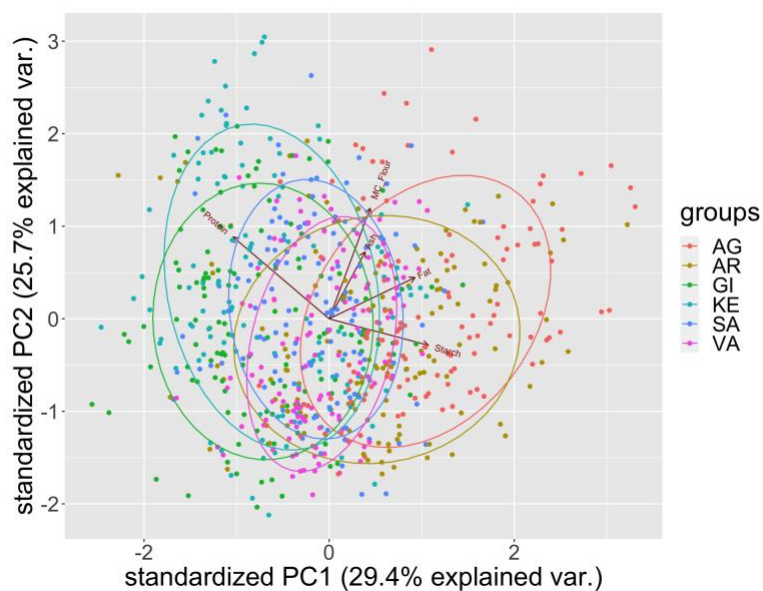


Figure 3: The biplot of two PCs showing proximate composition of different varieties of dry pea flour.

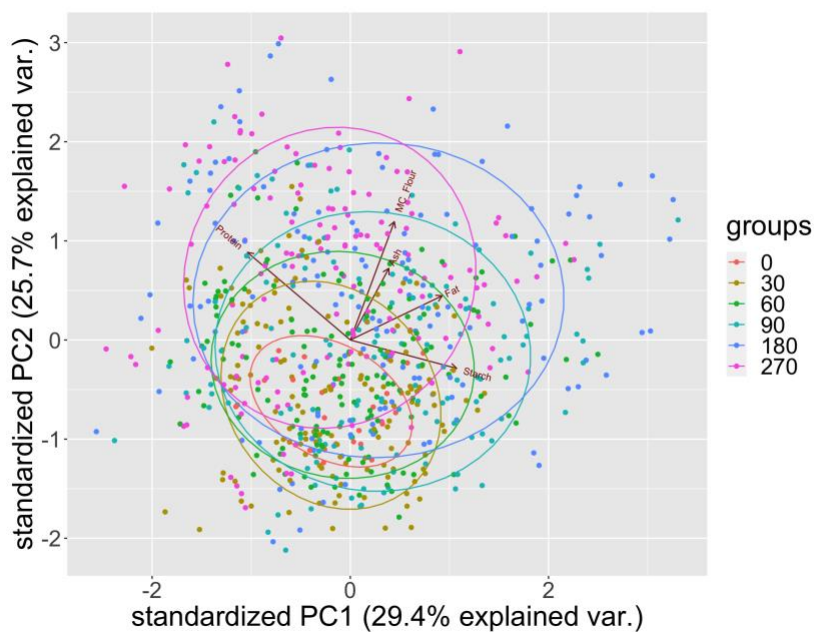


Figure 4: The biplot of two PCs showing proximate composition of different varieties of dry pea flour at different sampling days (0, 30, 60, 90, 180, 270 days).

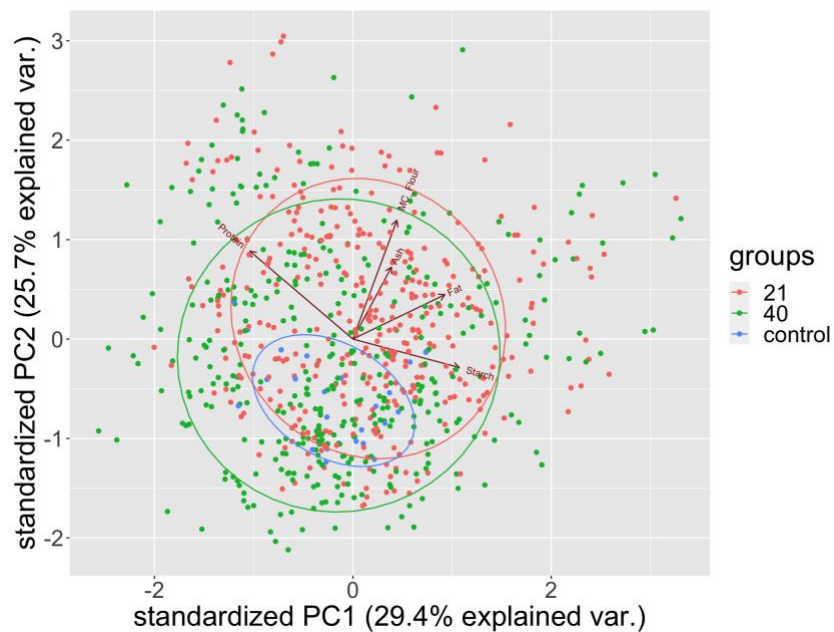


Figure 5: The biplot of two PCs showing proximate composition of different varieties of dry pea flour stored at different temperatures.

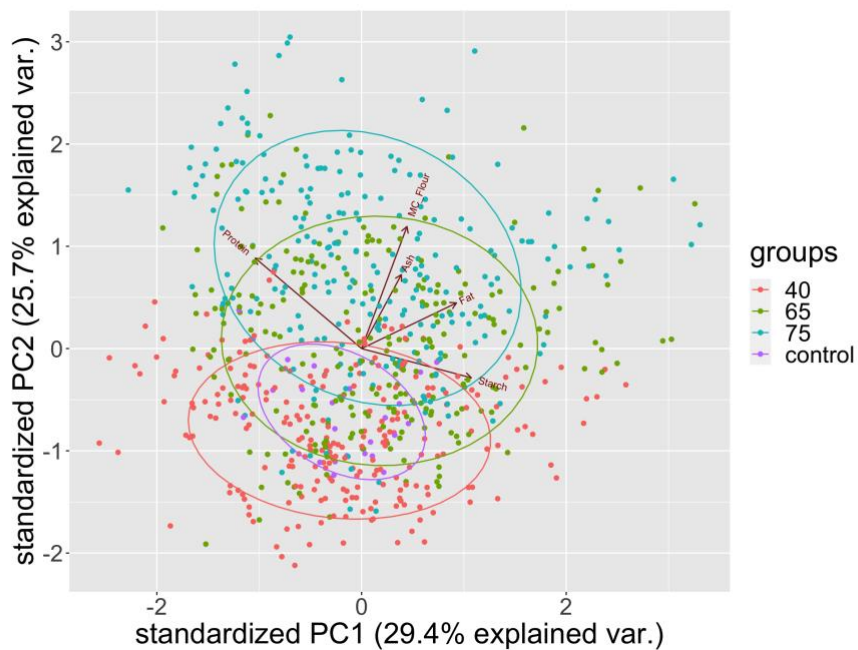


Figure 6: The biplot of two PCs showing proximate composition of different varieties of dry pea flour stored at different RH.

4.1.2. Variable Selection by PCA

The variables that were responsible for the effect on the proximate composition of different varieties of pea flour support moisture content of the flour and protein content as having the most contribution to data variability (Figure 7). However, as starch is one of the important components and is close to the red dash line (i.e., the percent contribution contributing significantly to data variability); thus, further differentiation of the data was completed.

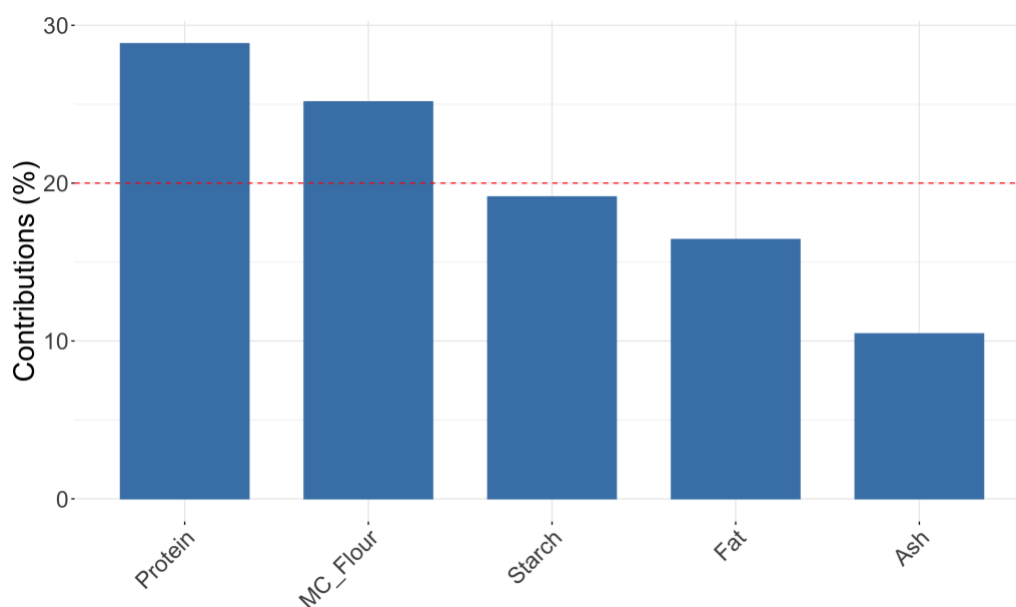


Figure 7: The contribution plot of variables for proximate composition of dry pea flour. The red dash line in the graph represents the average contribution of each variable.

4.1.2.1. Moisture Content of Dry Pea Flour

Storage of dry pea under diverse storage conditions of relative humidity over 270 days at HT resulted in higher moisture content of the different varieties of dry pea seeds, which led to higher moisture content of flour of dry pea of different varieties (Table 4). The interactive effect of different varieties of pea and storage conditions of diverse RH was

significant (p -value <0.05) on moisture content of pea flour. Moisture content of pea flour was significantly higher at HRH of all the varieties when compared with the time 0 (control) samples, i.e., 9.15 to 11.38%, 9.02 to 11.58%, 8.99 to 11.29%, 8.85 to 11.17%, 8.40 to 10.55% and 8.00 to 11.04% for Agassiz, Salamanca, Arcadia, Ginny, Keystone and Vail, respectively. In addition, the interaction effect of days of storage and diverse RH was significant (p -value <0.001) on moisture content of pea flour, regardless of the variety. Storage of dry peas at HRH resulted in higher moisture content of pea flour compared to LRH and MRH. Moisture content of flour of dry peas stored at diverse RH condition increased with the increase in days of storage when compared with the time 0 (control) except for the storage at LRH where a decrease was observed in moisture content, i.e., 8.73 to 7.87% (Table 5). Similar trend of high moisture content was reported for beans and cowpeas when stored at 65% of RH compared to those stored at 30% RH (Hentges et al. 1991).

The increase in moisture content of dry pea seeds due to the physiological activity (i.e., respiration) that causes release of water (Chidananda et al. 2014) can be correlated with the higher moisture content of flour of these stored seeds. Also, moisture content of the seeds change due to its hygroscopic nature (Bradford et al. 2016) where the change in moisture content occurred with respect to certain RH. This change is responsible for the difference in moisture content of flour of different varieties of peas stored at diverse conditions of RH.

Table 4: Moisture content (%) of flour of different varieties of dry pea stored under diverse RH conditions at high temperature (40 °C).

Variety	Control	LRH	MRH	HRH
Agassiz	9.15 ^{ef*}	8.10 ^h	9.97 ^d	11.38 ^{ab}
Salamanca	9.02 ^{ef}	8.41 ^{gh}	10.11 ^d	11.58 ^a
Arcadia	8.99 ^{ef}	8.45 ^{gh}	10.28 ^{cd}	11.29 ^{ab}
Ginny	8.85 ^{fg}	7.72 ^{ij}	9.33 ^{ef}	11.17 ^b
Keystone	8.40 ^{gh}	7.27 ^k	9.36 ^{ef}	10.55 ^c
Vail	8.00 ^{hi}	7.35 ^{jk}	9.38 ^e	11.04 ^b

*Different lowercase letters represent significant difference among the varieties when stored at diverse storage conditions of RH based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

Table 5: Moisture content (%) of flour of dry peas stored over 270 days and different RH conditions at high temperature (40 °C).

Days of Storage	LRH	MRH	HRH
0 (control)	8.73 ^{e*}	8.73 ^e	8.73 ^e
90	7.90 ^f	9.51 ^d	10.84 ^b
180	7.88 ^f	9.45 ^d	11.25 ^a
270	7.87 ^f	10.26 ^c	11.41 ^a

*Different lowercase letters represent significant difference due to the diverse storage conditions of RH and different sampling days based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

4.1.2.2. Protein Content of Dry Pea Flour

Protein content (%) of different varieties of dry peas were observed to be significantly different (p -value <0.001). The highest protein content was observed in Keystone variety (24.86%) followed by Ginny (24.78%), Salamanca (24.50%), Vail (23.92%), Arcadia (23.10%) and Agassiz (21.58%) when stored at HT (Table 6). Noticeable influence of days of storage was observed as the protein content was significantly (p -value <0.001) higher after 270 days of storage than the time 0 (control) samples (Table 6). In addition, RH also had significant effect (p -value <0.001) on the protein content of pea flour regardless of the variety, i.e., storage at HRH resulted in high protein content (24.83%) than at LRH (23.07%) and MRH (23.54%) (Table 6). Five different varieties of common beans stored for 5 years identified as hard to cook beans, and freshly harvested beans was compared for their protein content (Martín-Cabrejas et al. 1997). The stored beans were reported to have higher protein content than the fresh beans and this was true for all the varieties. Our results agree with this research where higher protein content in the flours of peas stored over 270 days as compared to time 0 was observed.

However, different results were reported by several other authors. For instance, (El-Refai et al. 1988) documented significant reduction in the protein content of the Faba beans stored over 9 months and the decrease was explained as the activity of proteolytic enzymes. In addition, slight decrease in protein content in pinto beans stored at different temperatures and moisture contents was observed along with an increase in moisture content and temperature (Rani et al. 2013). In contrast, no significant effect was observed on protein content of common beans due to storage (Berrios et al. 1999; Nyakuni et al. 2008).

Table 6: Protein content (%) of different varieties of dry peas stored over 270 days and different RH conditions at high temperature (40 °C).

Varieties	Protein (%)
Agassiz	21.58 ^{d*}
Salamanca	24.50 ^{ab}
Arcadia	23.10 ^c
Ginny	24.78 ^a
Keystone	24.86 ^a
Vail	23.92 ^b
p-value	<0.001
Days of Storage	Protein (%)
0 (control)	23.61 ^b
90	23.29 ^b
180	23.60 ^b
270	24.54 ^a
p-value	<0.001
Relative Humidity (RH)	Protein (%)
0 (control)	23.61 ^b
LRH	23.07 ^b
MRH	23.54 ^b
HRH	24.83 ^a
p-value	<0.001

*Different lowercase letters in a column represent significant difference within each variety, storage days, and different RH conditions, respectively based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH-High Relative Humidity (75%).

4.1.2.3. Starch Content of Dry Pea Flour

Different varieties of dry pea flour, days of storage and diverse RH condition had the significant interaction effect (p -value <0.005) on the starch content of pea flour.

Noticeable decrease in the starch content (%) of flour of different varieties of dry peas stored at HT and diverse RH over 270 days was observed (Table 7). When compared with time 0 (control) the starch content was lower within each variety, i.e., 46.6 to 43.6%, 47.37 to 44.9%, 46.3 to 44.6%, 46.3 to 44.6%, 46.1 to 44.9% and 47.7 to 45.1% for Agassiz, Salamanca, Arcadia, Ginny, Keystone and Vail, respectively. Significant differences in the starch content of flour of the different varieties of peas stored at LRH and HRH condition for 90 and 180 days were observed. While storage of dry peas of different varieties at MRH caused significant difference in starch content among varieties only after 180 days of storage.

Reduction in starch content was observed in faba beans stored over 9 months was reported by El-Refai et al. (1988). The reduction in starch digestibility of the different varieties of common beans stored for 6 months was reported (Nyakuni et al. 2008) and this reduction was attributed to high amount of dietary fibre and also the presence of amylase inhibitors in the beans.

Table 7: Starch content (%) of different varieties of dry peas stored under diverse storage conditions at high temperature (40 °C).

Storage Conditions (Days-RH)	Agassiz	Salamanca	Arcadia	Ginny	Keystone	Vail	p-value
0 (control)	46.6 ^{d*}	47.4	46.3	46.3	46.2	47.7	0.053
90-LRH	47.1 ^{cd, AB}	47.0 ^{AB}	47.5 ^A	45.9 ^C	46.1 ^{BC}	46.8 ^{ABC}	0.022
180-LRH	47.6 ^{bc, A}	45.8 ^{BC}	47.7 ^A	45.5 ^{BC}	45.2 ^C	46.8 ^{AB}	0.011
270-LRH	45.8 ^e	45.3	46.1	45.3	44.7	45.0	0.138
90-MRH	47.5 ^{bc}	47.0	47.2	46.9	46.4	47.3	0.438
180-MRH	48.3 ^{ab, A}	45.4 ^D	46.6 ^{BC}	44.5 ^D	45.5 ^{CD}	47.6 ^{AB}	<0.001
270-MRH	47.2 ^{cd, A}	45.5 ^{BC}	46.1 ^B	44.7 ^C	45.5 ^{BC}	46.1 ^B	<0.001
90-HRH	48.5 ^{a, A}	47.0 ^B	45.7 ^C	46.4 ^{BC}	45.7 ^C	47.1 ^B	<0.001
180-HRH	48.7 ^{a, A}	44.7 ^D	46.8 ^{BC}	45.6 ^{CD}	46.1 ^{BCD}	47.6 ^{AB}	<0.001
270-HRH	43.6 ^f	45.0	44.7	44.6	44.9	45.1	0.116
p-value	<0.001	0.741	0.395	0.059	0.675	0.374	

*Different lowercase letters in a column represent significant difference across storage days and RH within each variety. Different uppercase letters in a row represent significant difference across varieties in that storage condition of days and RH. No letters represent no significant difference based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

Reduction in the starch digestibility was associated with hard to cook phenomenon, due partly by the interaction between starch and other cellular compound such as tannins which inhibits water uptake by starch and increases the cooking time for HTC beans.

Ash content in the pea flour was slightly higher after 270 days of storage than compared to control samples. Whereas studies by El-Refai et al. (1988) demonstrated that storage of faba bean for 9 months led to a gradual reduction in ash content with no loss of phosphorus, calcium, iron, or magnesium contents. Higher fat content in flour of dry peas stored at HRH over 270 days was observed. This result was different than the (Chen 1991), where author reported significant loss in fat of beans due to the storage. Also, Berrios et al. (1999) documented no changes in the fat content in black bean after 2 years of storage. The general changes in the proximate composition of the flours of different varieties of peas stored at the accelerated condition might be due to changes on mass balance among components rather than the true increase or decrease of the value, for example, starch content was lower which caused other constituents to be higher. Regardless, change in composition were observed and the impact of HT, HRH, and days of storage on composition was clearly observed.

4.2. Functional Properties

Storage of dry peas regardless of the varieties affected some functional properties of flour when stored at harsh conditions. The functional properties evaluated were foaming capacity (FC), foaming stability (FS), emulsion activity (EA), emulsion stability (ES), water absorption index (WAI), water solubility index (WSI), water holding capacity (WHC) and oil absorption capacity (OAC). Principal component analysis (PCA) on

functional properties of different varieties of dry pea flour were carried out to study the relationship between these properties. The properties most affected by the storage were established by the PCA and those seemed to have the most contribution to variation were subsequently compare in greater detail.

4.2.1. Principle Component Analysis (PCA)

The PCA on the functional properties of stored dry pea flour did not show much variation in the overall data; however, it was revealed that PC1 and PC2 accounted for 29.1% and 16.3% of cumulative variance, respectively. The biplot (Figure 8) illustrates that functional property of different varieties of dry pea flour were similar. The varieties were clearly overlapped revealing that varieties did not have much effect in the data variation. Biplot (Figure 9) illustrates the functional properties of dry peas with respect to days. Not much data variation was observed in 30, 60 and 90 days; however, data from 180 and 270 days were more diverse and contributes to data variation. Thus, further analysis with data from time 0 supported that HT and RT contributed to data variation of the functional properties of dry pea flour (Figure 10). With RH, not much data variation was observed in LRH and MRH as illustrated by their overlap (Figure 11). Therefore, further data analysis was proceeded with all the treatment levels of temperature (RT and HT) and only MRH and HRH conditions.

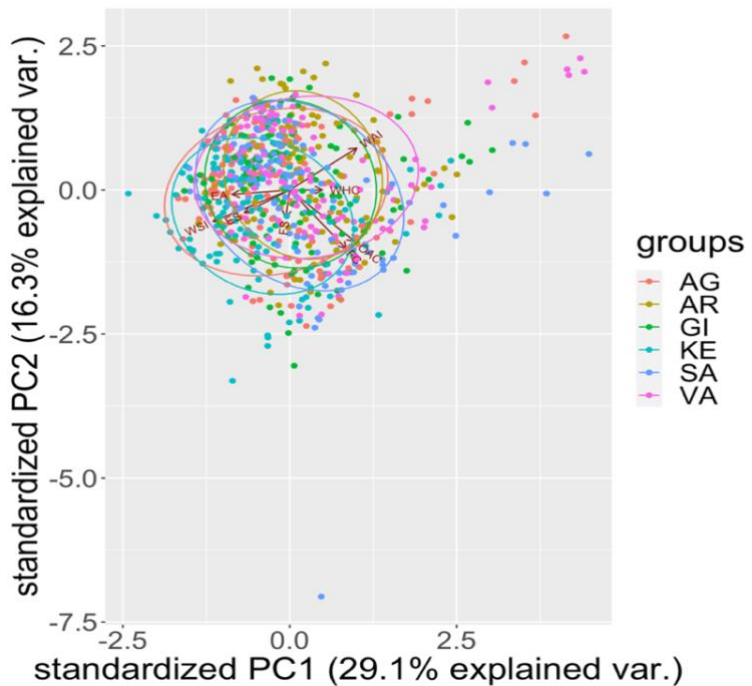


Figure 8: The biplot of two PCs showing functional properties of different varieties of dry pea flour.

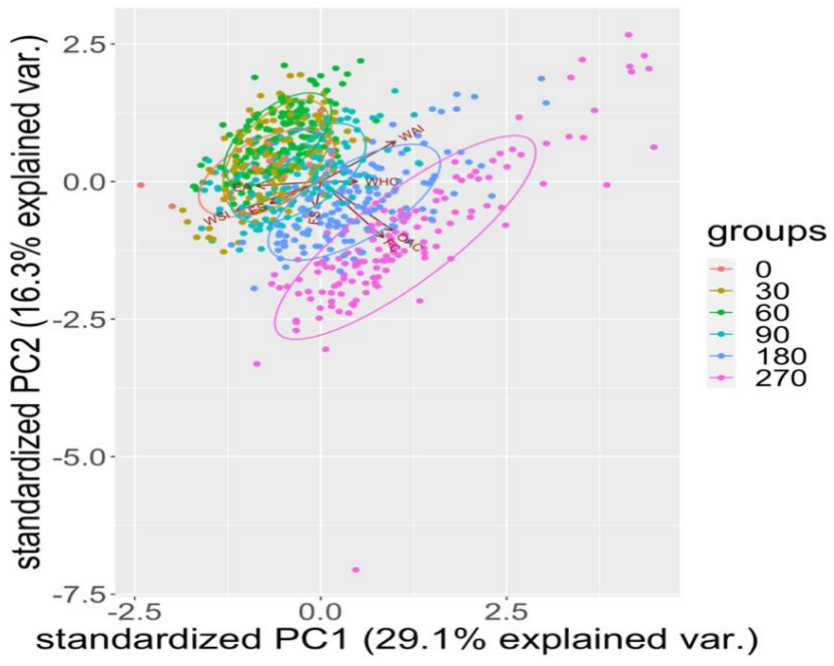


Figure 9: The biplot of two PCs showing functional properties of dry pea flour at different sampling days (0, 30, 60, 90, 180, 270 days).

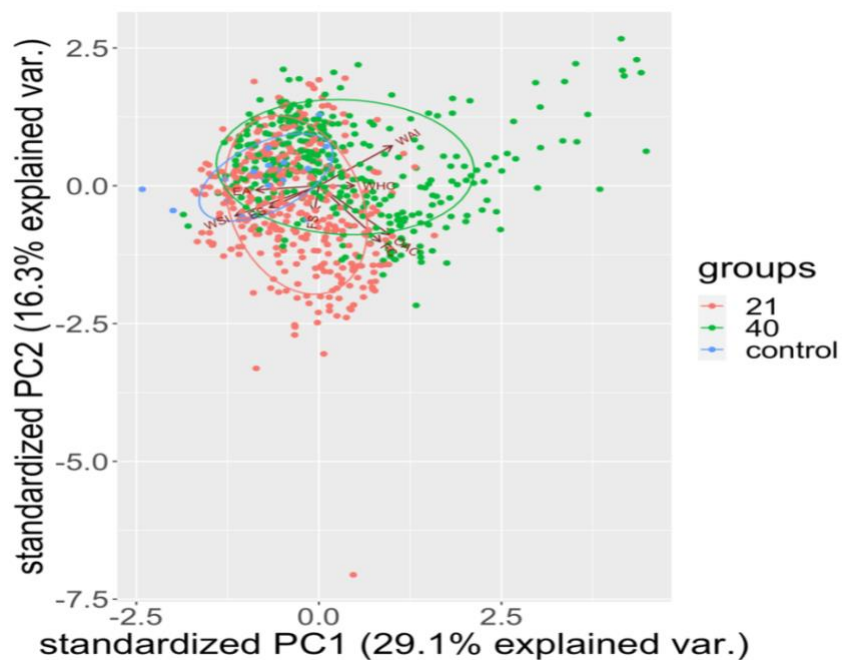


Figure 10: The biplot of two PCs showing functional properties of dry pea flour stored at different temperatures.

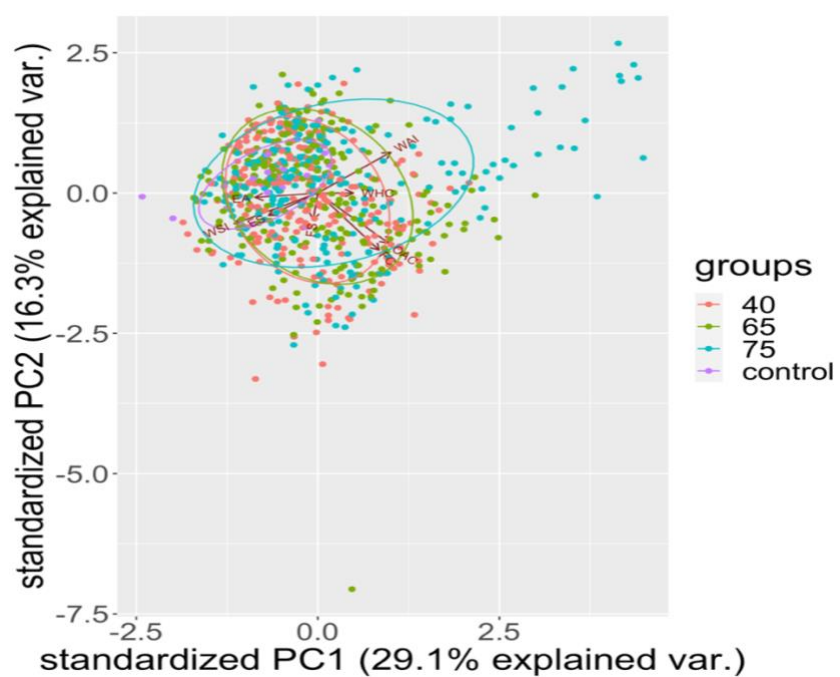


Figure 11: The biplot of two PCs showing functional properties of dry pea flour stored at different RH.

4.2.2. Variable Selection by PCA

The contribution plot (Figure 12) illustrated the variables that were responsible for the effect on the functional properties of dry pea flour. The variables having the contributions value higher than the red dash lines, i.e., oil absorption capacity (OAC), foaming capacity (FC), water absorption index (WAI), and water solubility index (WSI) were further differentiated.

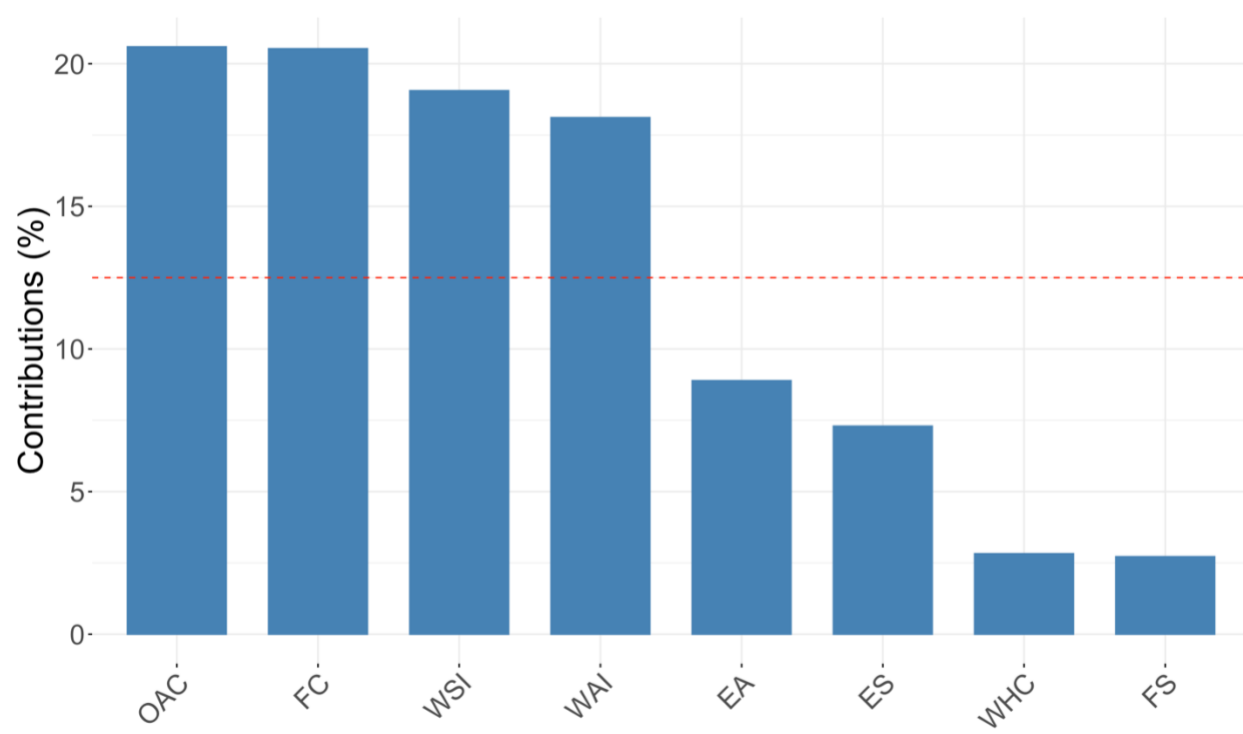


Figure 12: The contribution plot of variables for functional properties of dry pea flour. The red dash line in the graph represents the average contribution of each variable.

4.2.2.1. Oil Absorption Capacity (OAC)

The OAC of different varieties of dry pea flour was determined. No significant difference in OAC among the varieties of dry pea was observed. Furthermore, OAC among samples was not impacted significantly by temperature and RH. Thus, the varieties are responding

in the same manner to the storage conditions used in the study. In contrast to variety and storage conditions, the OAC increased as the storage time (days) increased significantly (Figure 13). The interaction effect of days and varieties was significant (p -value <0.001) for the OAC. Regardless of the variety, the OAC of pea flour increased steadily over time when compared with the time 0 (control) samples, i.e., Agassiz (0.35 to 0.53 g/g, day 0 to day 270), Salamanca (0.35 to 0.53 g/g), Arcadia (0.34 to 0.53 g/g), Ginny (0.35 to 0.53 g/g), Keystone (0.34 to 0.51 g/g) and Vail (0.34 to 0.51 g/g).

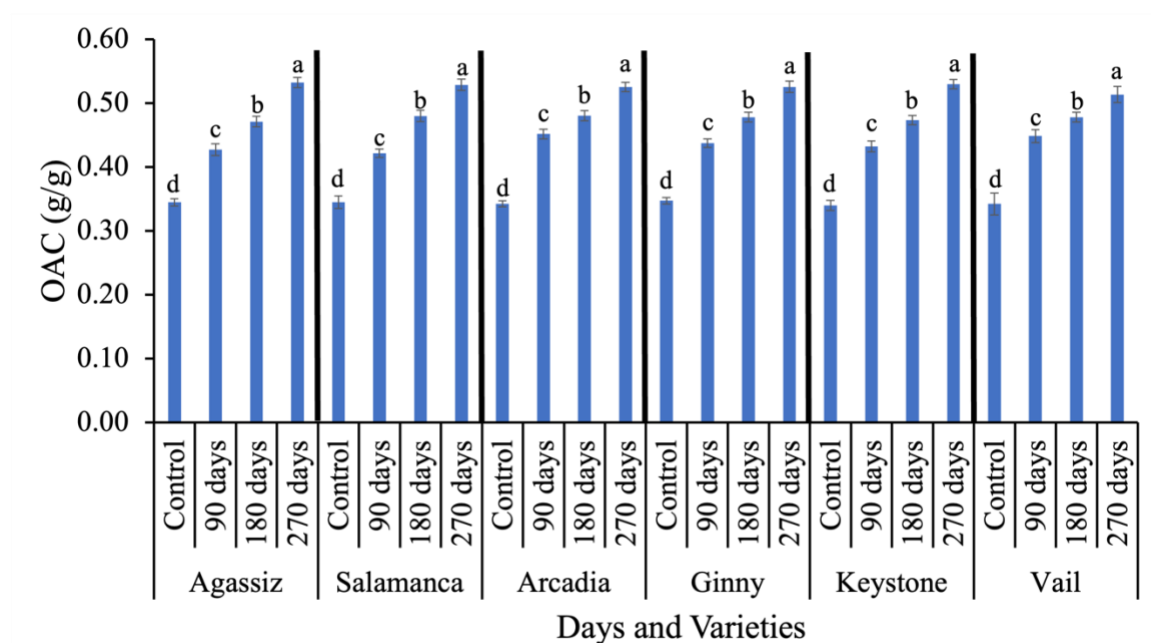


Figure 13: Oil absorption capacity (g/g) of flours from different varieties of dry pea stored over 270 days. Different lowercase letters represent significant difference across storage days within each variety based on 5% lsd values. Error bars represent standard error.

The OAC of the protein isolated from long-term stored black beans was evaluated (Ferreira et al. 2018). Similar trend of higher OAC of the protein isolated from beans after storage was reported compared to freshly harvested beans. This higher OAC was

attributed to the hydrolysis of proteins and exposure of internal hydrophobic sites. OAC of flour is primarily affected by the protein content and the distribution of hydrophilic and hydrophobic segments, as dictated by amino acid sequence, which can interact with water and oil. Non-polar amino acid side chains can form hydrophobic interactions and hydrocarbon interactions with hydrocarbon chains of lipid (Jitngarmkusol et al. 2008). As a result, the greater these interactions, the greater will be the OAC. Although not determined in the current research, the observed changes in OAC of stored peas might relate to changes in protein structure.

4.2.2.2. Foaming Capacity (FC) of dry pea flour

The FC of different varieties of dry pea flour was determined and observed to be influenced by the long-term storage. The interaction effect of days of storage and different varieties was significant ($p\text{-value} < 0.05$) on the FC of flour from dry peas stored at accelerated condition. With the increasing days of storage, FC of the pea flour, regardless of the varieties, increased (Figure 14). The FC followed a general downward trend for the samples stored for 90 days then by an upward trend until 270 days of storage. The trend in FC was consistent across variety and overall, no significant differences were observed in FC between varieties. When compared to the time 0 (control), FC of pea flour increased drastically ($p\text{-value} < 0.001$) over 270 days of storage from 145 to 188%, 143 to 207%, 160 to 195%, 132 to 192%, 143 to 195%, and 140 to 201% for Agassiz, Salamanca, Arcadia, Ginny, Keystone and Vail, respectively. Similarly, FC for the flour of peas stored at HT, regardless of variety, was higher than RT after 270 days of the storage ($p\text{-value} < 0.005$) (Figure 15). However, the storage temperature appears to impact FC to a lesser degree than storage day.

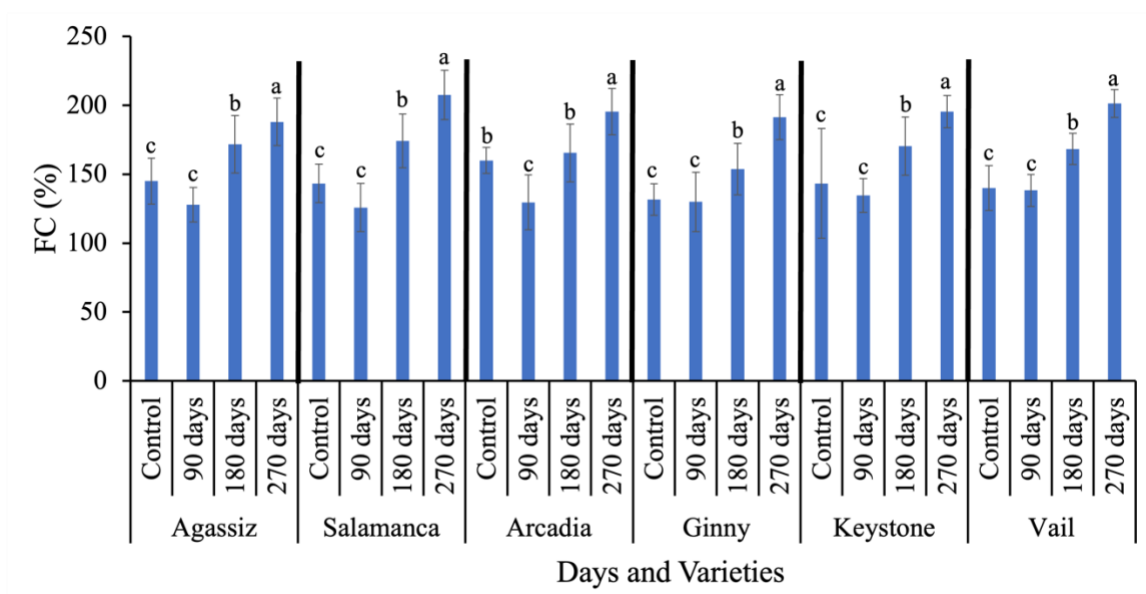


Figure 14: Foaming capacity (%) of flour from different varieties of dry pea stored over 270 days. Different lowercase letters represent significant difference across storage days within each variety based on 5% lsd values. Error bars represent standard error.

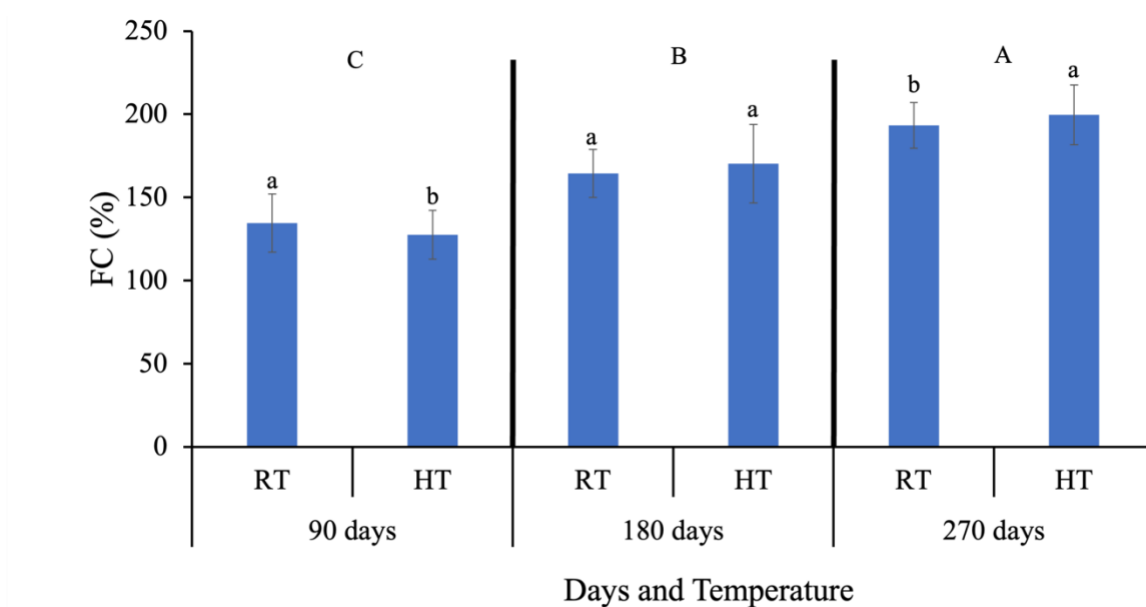


Figure 15: Foaming capacity (%) of flour from dry pea stored over 270 days at RT (21 °C) and HT (40 °C). Different lowercase and uppercase letters represent significant difference between storage temperature within each sampling day and across days of storage, respectively based on 5% lsd values. Error bars represent standard error.

Within each variety, significant differences (p -value <0.05) in FC were observed for samples stored under different conditions (Table 8). Our result agrees with the result reported by Ferreira et al. (2018). In their research, significantly higher FC was observed for protein isolates of black bean stored at 17% moisture and 32 °C (412%) and 14% moisture content and 32 °C (340%) compared to FC of the freshly harvested beans (272%). Overall, storage promoted or enhanced pea flour FC regardless of variety.

Table 8: Foaming Capacity (%) of flour from different varieties of dry pea stored at diverse storage conditions.

Storage Conditions (Temperature-RH)	Agassiz	Salamanca	Arcadia	Ginny	Keystone	Vail
RT-MRH	163 ^{a*}	157 ^b	160 ^a	167 ^a	166 ^a	173 ^a
RT-HRH	167 ^a	159 ^b	169 ^a	157 ^{ab}	162 ^{ab}	168 ^{ab}
HT-MRH	171 ^a	181 ^a	166 ^a	152 ^b	172 ^a	173 ^a
HT-HRH	148 ^b	180 ^a	159 ^a	157 ^{ab}	167 ^a	163 ^b

*Different lowercase letters in a column represent significant difference within each variety based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%) RT-Room temperature (21 °C), HT-High temperature (40 °C).

The increase in FC was attributed to the changes in the structure of bean protein (Ferreira et al. 2018). Globulins are the major proteins found in beans and in dry peas. Globular proteins are rigid, folded and compact in structure that is stabilized by both polar and non-polar interactions. The change in the structure of globulins during the storage was likely a conformation change or relaxation of globulins as the possible cause for the increase in FC (Ferreira et al. 2018; Sathe 2002). The general trend of higher protein observed in the pea samples supports that a degradation of structure did not occur

through hydrolysis. However, the change in globulins might be the basis for the higher FC in stored samples. This assumption is based on the observation that protein content (Table 6) and FC (Table 8) were not correlated ($r=0.19$, $p\text{-value}<0.001$).

4.2.2.3. Water Absorption Index and Water Solubility Index

Water Absorption Index (WAI) of flour from dry pea samples stored at RT and HT were significantly different ($p\text{-value}<0.001$), thus for the ease of further analysis, data were divided into RT and HT. The main effect of days of storage, diverse RH and different varieties were observed in the WAI of flour obtained from pea samples stored at RT (Table 9). The effect of increasing days of storage and storage at HRH at RT resulted in significantly lower WAI in pea flours. The two-way interaction effect of days of storage and different varieties; and days of storage and different RH on the WAI of flour from pea stored at HT was significant ($p\text{-value}<0.001$). Storing samples at HT caused significantly higher WAI of the dry pea flour with the increasing number of days of storage (Figure 16). WAI was higher when compared with the time 0 (control) samples, i.e., 2.22 to 2.47 g/g, 2.22 to 2.50 g/g, 2.26 to 2.44 g/g, 2.24 to 2.35 g/g, 2.11 to 2.39 g/g and 2.24 to 2.49 g/g for Agassiz, Salamanca, Arcadia, Ginny, Keystone and Vail, respectively.

Table 9: Water Absorption Index (g/g) of flour of peas stored at room temperature (21 °C).

Days of Storage	Water Absorption Index (g/g)
0 (control)	2.21 ^{a*}
90	2.16 ^b
180	2.15 ^b
270	2.12 ^c
p-value	<0.001
Varieties	Water Absorption Index (g/g)
Agassiz	2.15 ^b
Salamanca	2.15 ^b
Arcadia	2.20 ^a
Ginny	2.18 ^{ab}
Keystone	2.10 ^c
Vail	2.16 ^{ab}
p-value	<0.001
Relative Humidity (RH)	Water Absorption Index (g/g)
MRH	2.17 ^a
HRH	2.12 ^b
p-value	<0.001

*Different lowercase letters represent significant difference with the days of storage, among varieties and RH based on 5% lsd values.

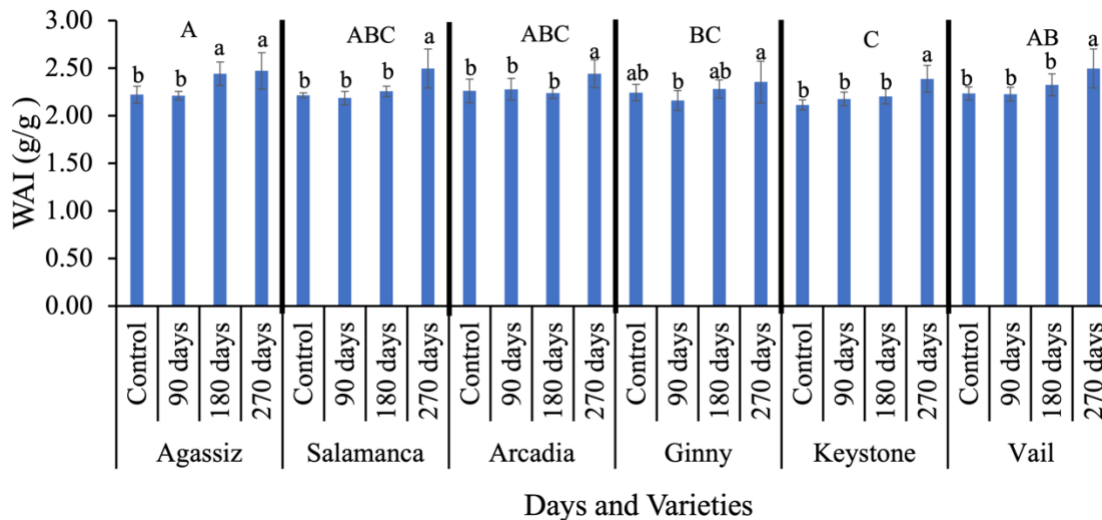


Figure 16: WAI (g/g) of flour from different varieties of dry pea stored over 270 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across storage days within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error.

The interactive effect of different varieties and different RH on the WAI of flour from pea stored at HT was significant (p -value <0.05). WAI of flour obtained from peas stored at HT and HRH was higher than WAI of flour from peas stored at HT and MRH, i.e., 2.30 and 2.44 g/g, 2.27 and 2.36 g/g, 2.32 and 2.32 g/g, 2.20 and 2.33 g/g, 2.23 and 2.28 g/g and 2.28 and 2.42 g/g for Agassiz, Salamanca, Arcadia, Ginny, Keystone and Vail, respectively (Figure 17). These results were, however, not significant for all the varieties of dry pea. In addition, the interactive effect of days of storage and different RH on the WAI of flour from pea stored at HT was significant (p -value <0.001). Higher WAI was observed in the flour from peas stored at HRH within each sampling days however, this was not true for 90 days sampling, i.e., storage at MRH resulted in higher WAI of pea flour than at HRH (Figure 18).

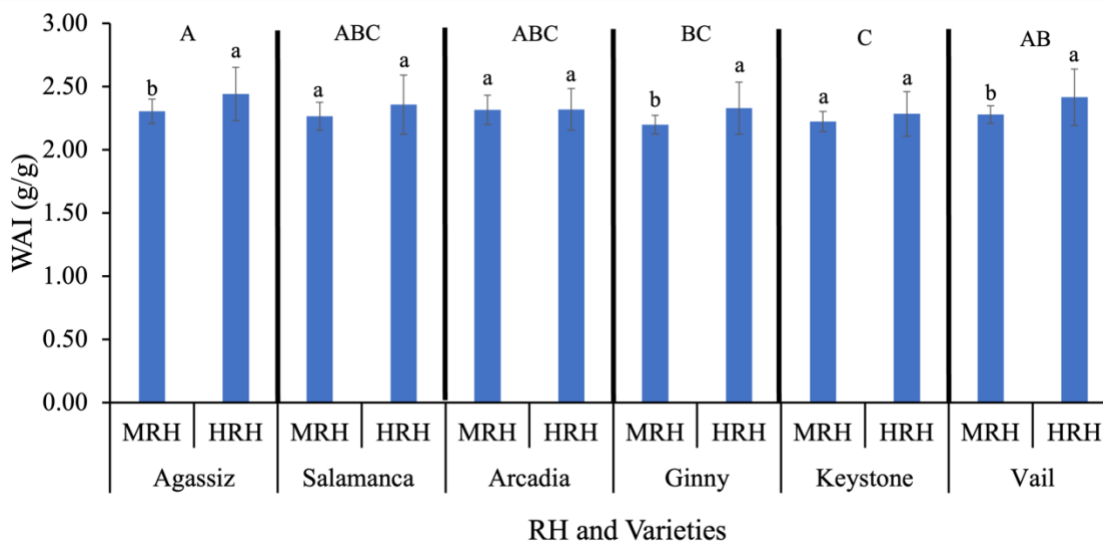


Figure 17: WAI (g/g) of flour from different varieties of dry pea stored under MRH and HRH at temperature (40 °C). Different lowercase and uppercase letters represent significant difference across storage days within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error. MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

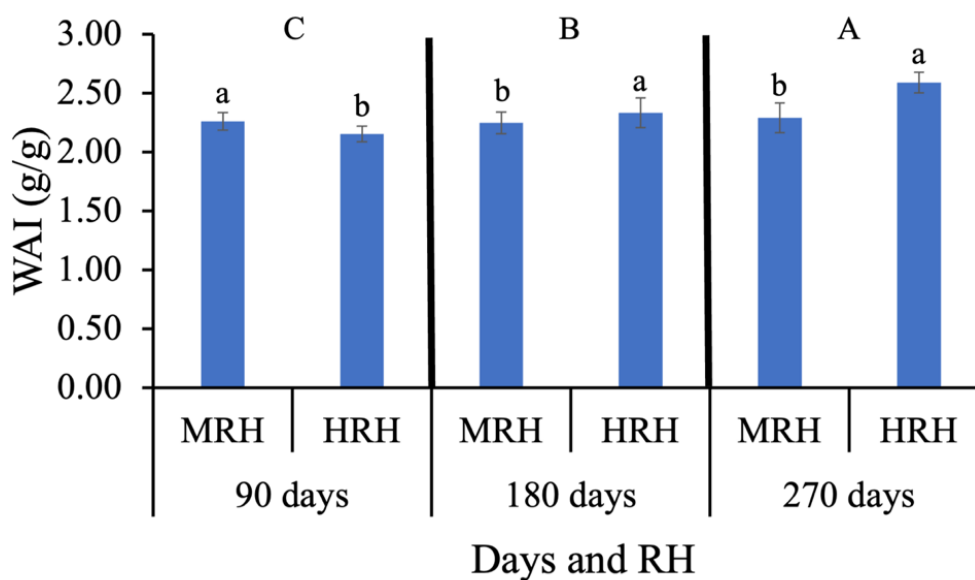


Figure 18: WAI (g/g) of flour from dry peas stores at different RH over 270 days of storage at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference within days across diverse RH condition and across days of storage, respectively based on 5% lsd values. Error bars represent standard error. MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

Water solubility index (WSI) of dry pea samples stored at RT and HT were significantly different (p -value <0.001); thus, data analysis was divided into RT and HT for the ease of analysis. The main effect of days of storage, diverse RH and different varieties were observed in the WSI of flour obtained from pea samples stored at RT (Table 10). The effect of increasing days of storage and storage under HRH at RT caused decrease in WSI significantly. The two-way interaction effect of days of storage and different RH on the WSI of flour from pea stored at HT was significant (p -value <0.001). The general trend of decrease in WSI value was observed with increasing days of storage under diverse RH condition. The WSI value was significantly lower in the flour obtained from peas stored under HRH than MRH (Figure 19).

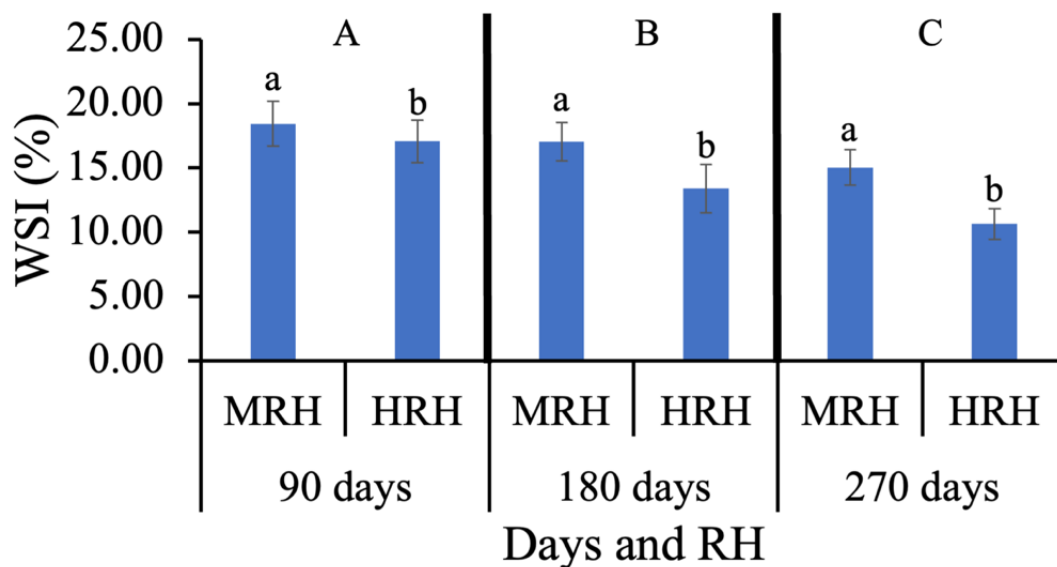


Figure 19: WSI (%) of flour from of dry peas stores at different RH over 270 days of storage at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference within days across diverse RH condition and across days of storage, respectively based on 5% lsd values. Error bars represent standard error. MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

Table 10: Water Solubility Index (%) of flour obtained from peas stored at room temperature (21 °C).

Days of Storage	Water Solubility Index (%)
0 (control)	22.28 ^{a*}
90	21.30 ^b
180	21.10 ^{bc}
270	20.45 ^c
p-value	<0.005
Varieties	Water Solubility Index (%)
Agassiz	21.69 ^b
Salamanca	20.54 ^c
Arcadia	19.27 ^d
Ginny	20.57 ^c
Keystone	24.06 ^a
Vail	20.73 ^{bc}
p-value	<0.001
Relative Humidity (RH)	Water Solubility Index (%)
MRH	21.33 ^a
HRH	20.57 ^b
p-value	<0.05

*Different lowercase letters represent significant difference with the days of storage, among varieties and RH based on 5% lsd values.

The WAI and WSI values of the pea flour were highly influenced by the storage conditions. The WAI is the measure of capacity of flour to absorb water that is dependent on the availability of hydrophilic groups that binds to the water molecules. The WSI index relates to the solubilization of flour components. Thus, protein degradation into peptides or free amino acids and degradation of starch are the two most likely candidates for lower WSI in samples. Lower WSI value indicates less soluble starch (González-Soto et al. 2007; Hernandez-Diaz et al. 2007). In addition, polymerization of protein and degraded starch could lead to the low WSI since these polymers will less likely be soluble in water. It is most likely that less starch degradation or possible polymerization was linked to the lower WSI values over the course of the study. The significant increase in the protein content (Table 6) and reduction in the starch values (Table 7) of the pea flour was observed showing starch degradation in the flour. Thus, changes in the WAI and WSI of the flour due to storage at diverse conditions could be related to the changes in the starch composition and degradation and possible polymerization.

4.3. Starch Functionality

4.3.1. Principle Component Analysis (PCA)

Principal Component Analysis (PCA) on starch properties of different varieties of dry pea flour were carried out to study the relationship between various properties. The starch properties analyzed were peak viscosity (PV), hot paste viscosity, final viscosity (FV), setback viscosity (SV), and gel strength of the paste formed. The RVA profiles (Figures 1-6, appendix) shows differences in pasting behavior for some flours from stored pea on adverse conditions). PCA on the starch properties of stored dry pea flour revealed that

PC1 and PC2 accounted for 56.1% and 37.1% of cumulative variance, respectively (Figure 20).

The biplot (Figure 20) illustrated that functional properties of starch of dry pea flour of different varieties were similar. The varieties were clearly overlapped revealing that, varieties did not show much difference in data variation. Similarly, biplot were plotted for the starch properties of dry peas with respect to days (Figure 21), temperature (Figure 22), and relative humidity (Figure 23). Long term storage affected the starch properties of dry pea flour. However, not much data variation was observed at 30, 60 and 90 days while data for 180 days and 270 days were more diverse. Thus, further analysis was proceeded only with data from 90, 180, and 270 days being compared with time 0 days (control). In addition, data variation of temperature, and RH were observed on the starch properties of dry pea flour. Hence, further data analysis was continued with all the treatment levels of temperature (RT and HT) and RH (LRH, MRH, and HRH). Dry pea samples stored at RT and HT were significantly different (p -value <0.001), thus for the ease of further analysis, data were divided into RT and HT.

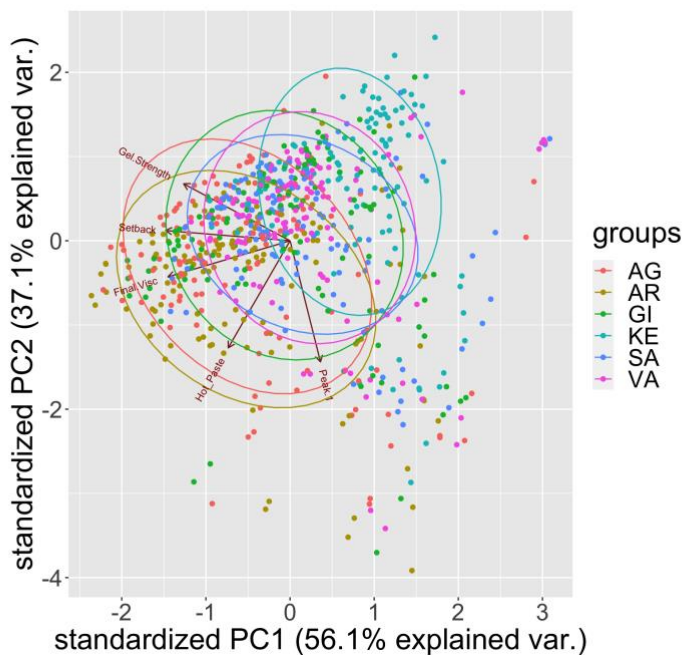


Figure 20: The biplot of two PCs showing starch properties of different varieties of dry pea flour.

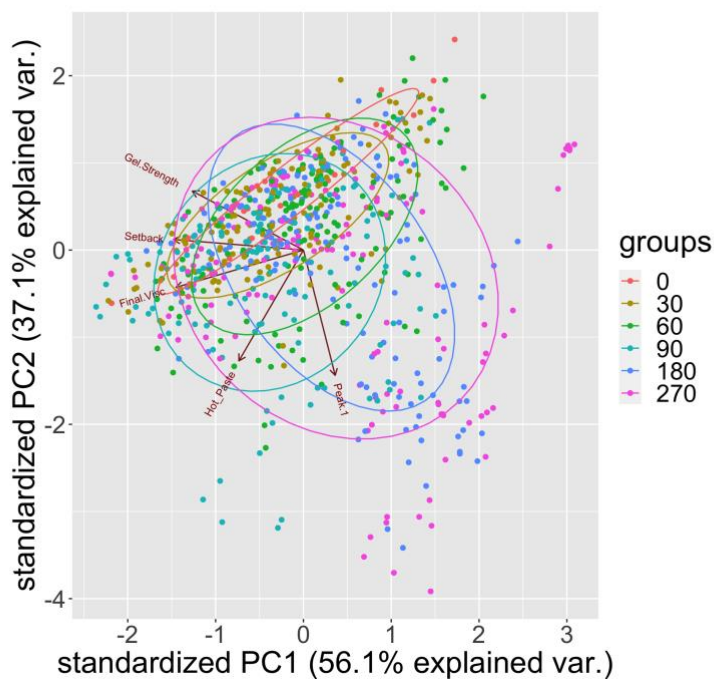


Figure 21: The biplot of two PCs showing starch properties of dry pea flour at different sampling days (0, 30, 60, 90, 180, 270 days).

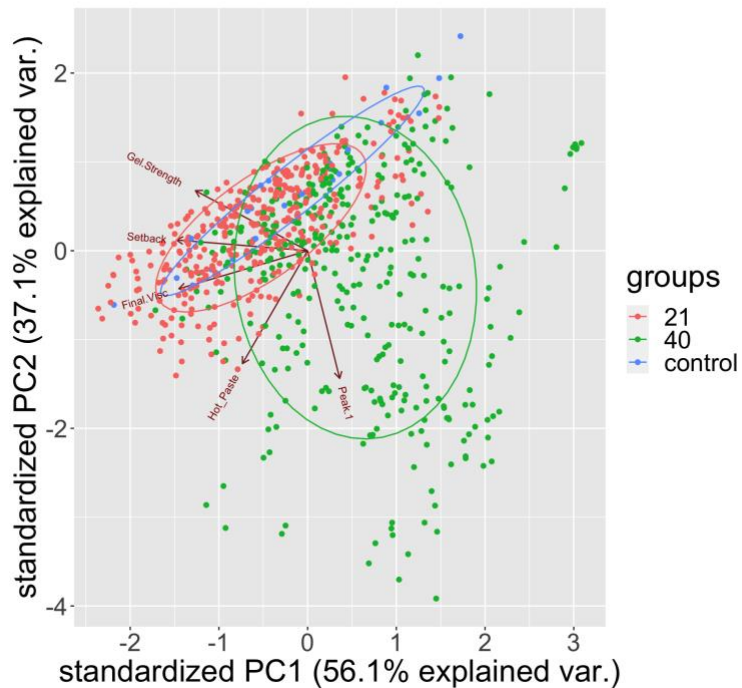


Figure 22: The biplot of two PCs showing starch properties of dry pea flour stored at different temperatures.

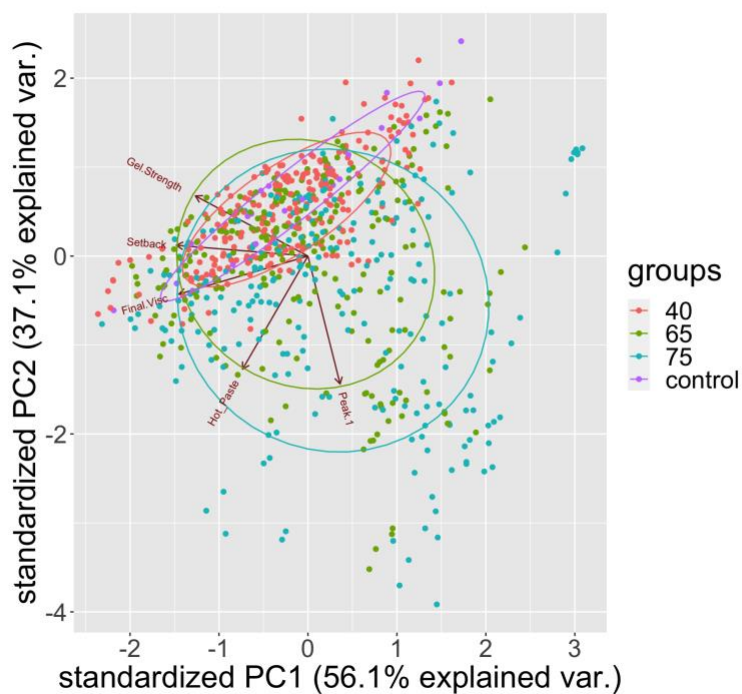


Figure 23: The biplot of two PCs showing starch properties of dry pea flour stored at different RH.

4.3.2. Variable Selection by PCA

The contribution plot (Figure 24) illustrates the variables that were responsible for the effect on the starch properties of dry pea flour. The variables having the contributions value higher than the red dash lines, i.e., final viscosity, setback viscosity and peak viscosity were selected for further analysis.

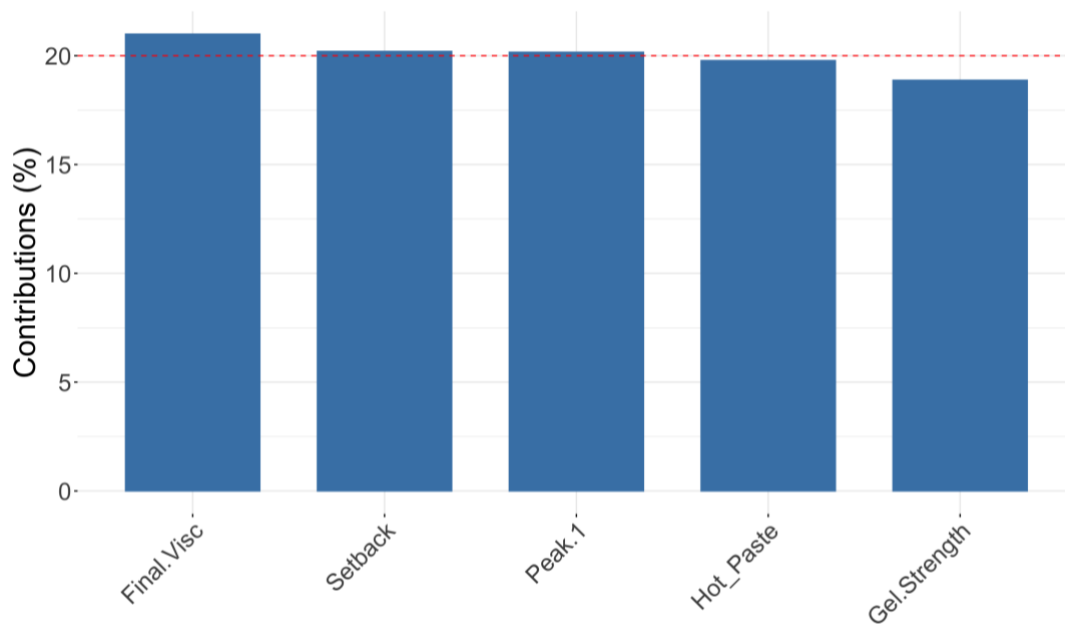


Figure 24: The contribution plot of variables for starch functionality of dry pea flour. The red dash line in the graph represents the average contribution of each variable.

4.3.2.1. Final viscosity (FV) of Dry Pea Flour

Differences among the varieties and changes in the FV of the different varieties of pea flour due to diverse storage conditions was observed. The main effect of days of storage, and different varieties were observed in final viscosity of flour obtained from the pea samples stored at RT (Table 11). The FV for the time 0 (control) samples was the highest for Arcadia (2914 cP), followed by Vail (2421 cP), Agassiz (2708 cP), Salamanca (2316 cP), Ginny (2199 cP) and Keystone (1838 cP).

Table 11: Final viscosity (cP) of flour of peas stored at room temperature (21 °C).

Days of Storage	Final Viscosity (cP)
0 (control)	2399 ^{c*}
90	2735 ^a
180	2507 ^{bc}
270	2588 ^b
p-value	<0.001
Varieties	Final Viscosity (cP)
Agassiz	2895 ^b
Salamanca	2539 ^c
Arcadia	3011 ^a
Ginny	2457 ^c
Keystone	2141 ^d
Vail	2490 ^c
p-value	<0.001

*Different lowercase letters represent significant difference with the days of storage, among varieties and RH based on 5% lsd values.

The two-way interactive effect of the days of storage and different varieties, days of storage and RH, and different varieties and diverse RH were significant (p-value<0.001) for final viscosity of pea flour when stored at HT. Final viscosity (FV) of dry pea samples stored at HT for 270 days at diverse RH showed significant differences among the varieties as well as within the varieties (Figures 25 and 26). Similarly, final viscosity decreased with increasing days of storage at diverse RH condition, HRH having

the lower value within each sampling days (Figure 27). Significant reduction in FV of stored dry pea samples was observed while compared with the control dry pea samples. The reduction observed in the FV agreed with the findings of lower viscosity in FV of starch isolated from black beans stored at higher moisture content and temperature, i.e., 17% moisture and 32 °C temperature, for 360 days compared to the starch isolated from freshly harvested black beans (Ferreira et al. 2017). In a follow up study, beans stored at 14% and 17% moisture content and 32 °C for 12 months had significantly lower FV compared to freshly harvested beans (Ferreira et al. 2018). In addition, similar reduction in FV was reported in carioca beans when stored at 25 °C for 360 days (Rupollo et al. 2011). Storage of whole yellow pea flour resulted in a significant reduction in the FV from 193 RVU to 146 RVU after 24 months of storage (Sopiwnyk et al. 2020). These results support the result of our present study.

Ferreira et al. (2017) reported no change in amylose content, a reduction in relative crystallinity and peak intensities. The changes in the molecular structure of the crystalline region due to the amylose content and the reduction in relative crystallinity and peak intensities were theorized as the contributing parameters for the reduction in FV of stored black bean starch. Similar changes may have occurred in the pea starch to account for reduction in FV observed in the pea stored at high temperature and RH in the current study.

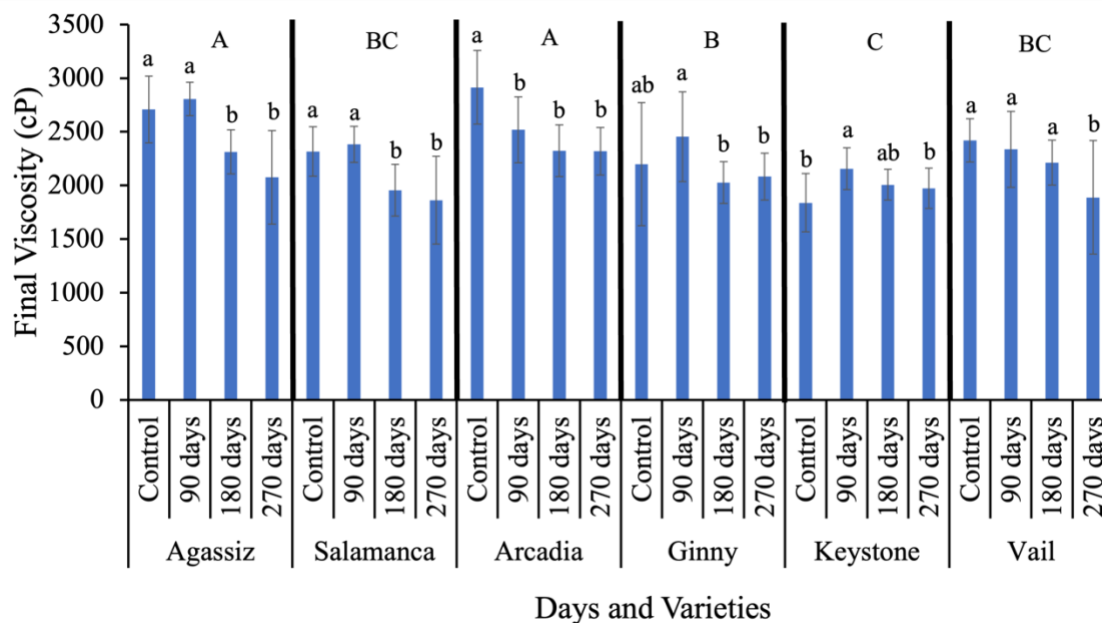


Figure 25: Final viscosity of flour from different varieties of dry peas stored under diverse storage conditions over 270 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across storage days within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error.

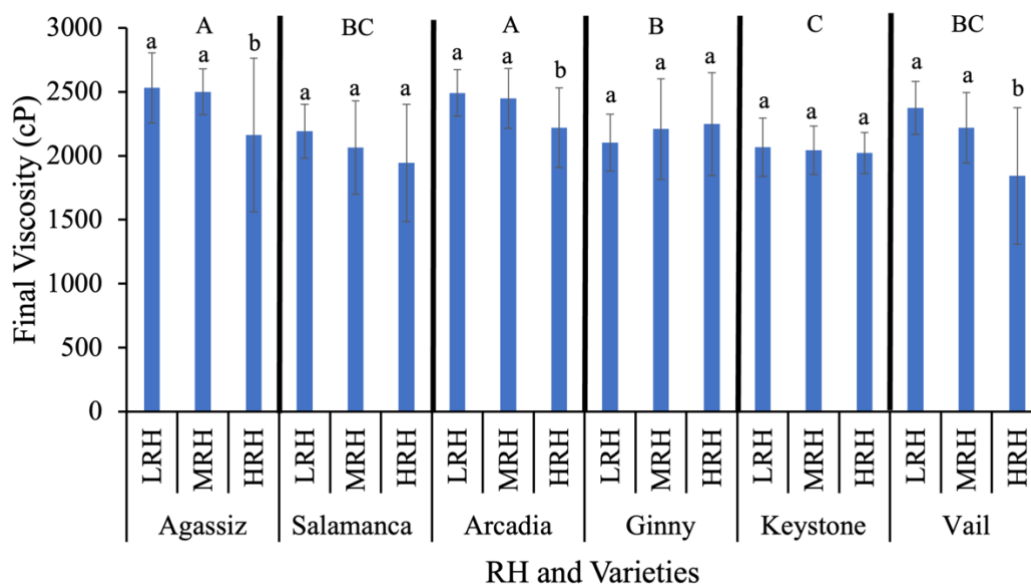


Figure 26: Final viscosity of flour from different varieties of dry peas stored at different RH and at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across different RH within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error. LRH- Low Relative Humidity, MRH- Medium Relative Humidity, HRH- High Relative Humidity.

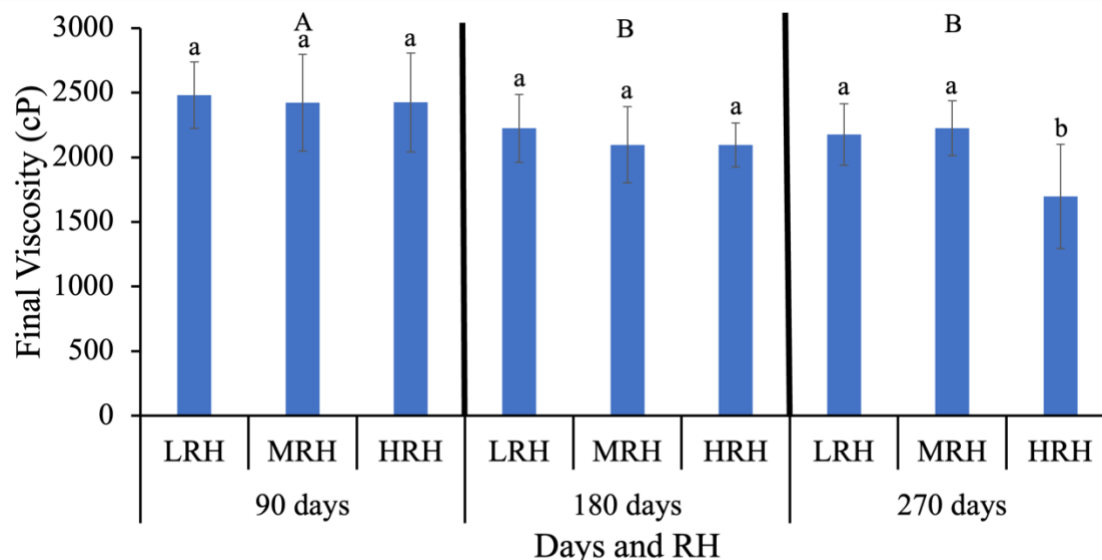


Figure 27: Final viscosity of flour from dry peas stores at different RH over 270 days of storage at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference within days across diverse RH condition and across days of storage, respectively based on 5% lsd values. Error bars represent standard error. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

4.3.2.2. Setback Viscosity (SV) of Dry Pea Flour

Diverse storage conditions impacted setback viscosity (SV) of starch of dry pea flour.

The main effect of days of storage, diverse RH and different varieties were observed in the SV of flour obtained from pea samples stored at RT (Table 12). The interaction effect of days of storage and different varieties on the viscosity of flour from pea stored at HT was significant (p -value<0.005). The SV of pea flour among the varieties decreased significantly after 270 days of storage at HT (Figure 28) in comparison to control samples, i.e., Agassiz (1256 to 657 cP), Salamanca (1020 to 548 cP), Arcadia (1368 to 764 cP), Ginny (930 to 613 cP), Keystone (731 to 592 cP) and Vail (1089 to 614 cP).

Table 12: Setback viscosity (cP) of flour of peas stored at room temperature (21 °C).

Days of Storage	Setback Viscosity (cP)
0 (control)	1066 ^{b*}
90	1097 ^a
180	1146 ^b
270	1254 ^b
p-value	<0.001
Varieties	Setback Viscosity (cP)
Agassiz	1409 ^a
Salamanca	1100 ^b
Arcadia	1423 ^a
Ginny	1046 ^b
Keystone	853 ^c
Vail	1104 ^b
p-value	<0.001
Relative Humidity (RH)	Setback Viscosity (cP)
LRH	1229 ^a
MRH	1193 ^a
HRH	1076 ^b
p-value	<0.001

* Different lowercase letters represent significant difference with the days of storage, among varieties and RH based on 5% lsd values.

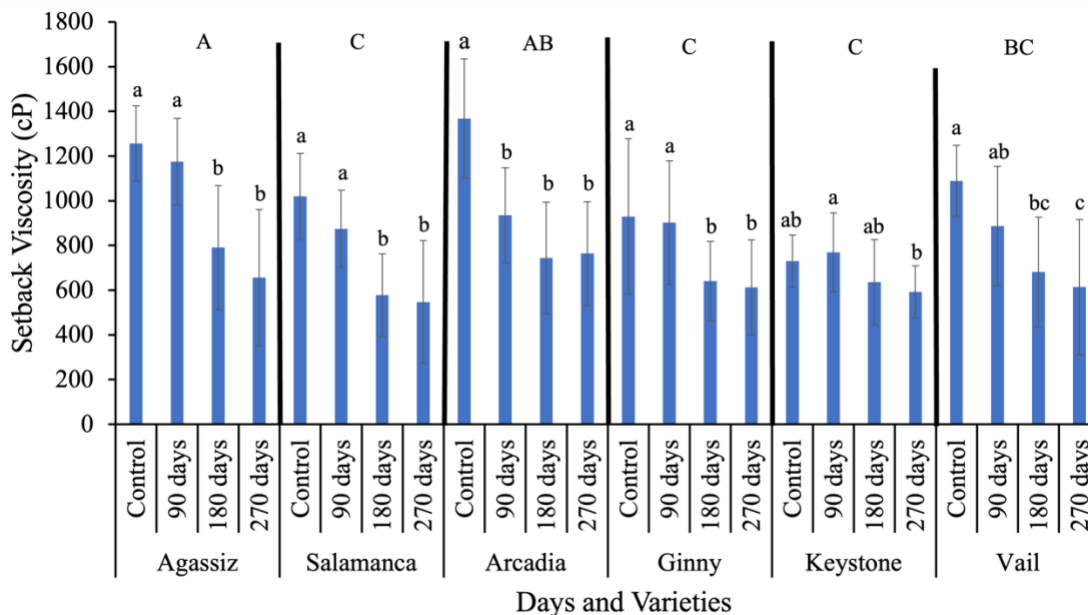


Figure 28: Setback viscosity of flour from different varieties of dry peas stored under diverse storage conditions over 270 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across storage days within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error.

The interactive effect of diverse RH and different varieties on the viscosity of flour from pea stored at HT was significant (p -value<0.001). The setback viscosity (SV) of flour from stored dry peas of different varieties at HRH was lower upon storage at HT (Figure 29). Storage at HT and HRH mostly influenced SV of dry pea flour. Furthermore, the two-way interaction effect of days of storage and different RH on the viscosity of flour from pea stored at HT was significant (p -value<0.005). Trend of decreasing SV was observed with increasing days of storage at diverse RH condition, HRH having the lower values within each sampling day (Figure 30). The study of stored carioca beans at different temperature (5 °C, 15 °C and 25°C) for 360 days by Rupollo et al. (2011),

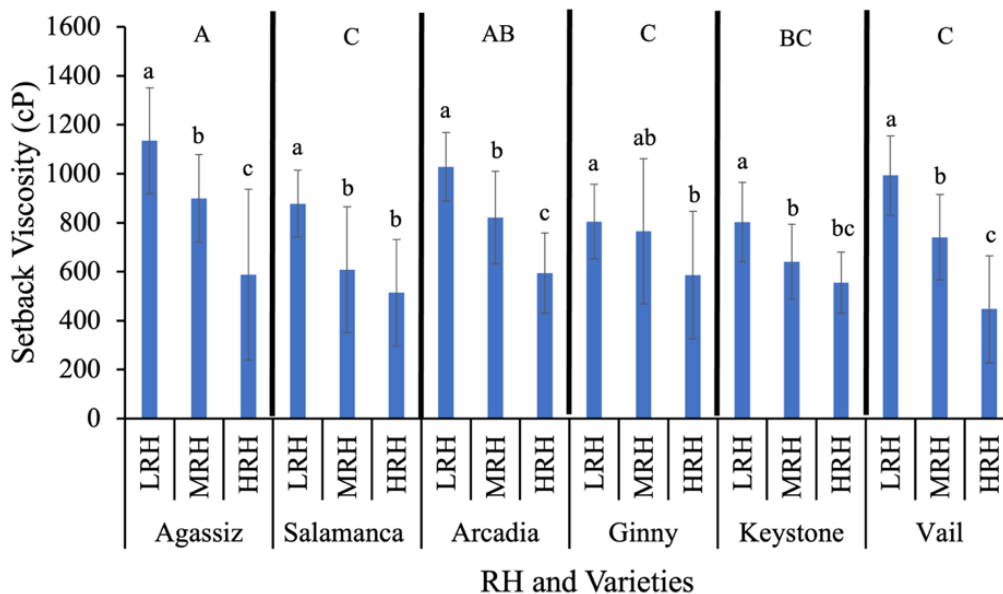


Figure 29: Setback viscosity of flour from different varieties of dry peas stored at different RH and at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across different RH within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error. LRH- Low Relative Humidity, MRH- Medium Relative Humidity, HRH- High Relative Humidity.

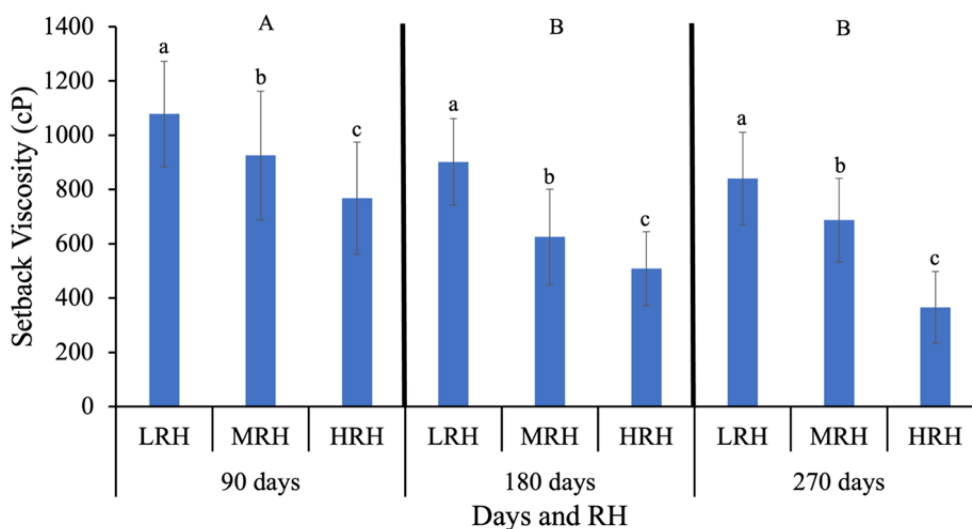


Figure 30: Setback viscosity of flour from dry peas stores at different RH over 270 days of storage at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference within days across diverse RH condition and across days of storage, respectively based on 5% lsd values. Error bars represent standard error. LRH- Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

indicated that SV was mostly impacted and reduced in the bean flour stored at 25 °C. The values of SV of beans stored at 5 °C, 15 °C and 25°C were 2425, 2207 and 2194 cP, respectively. In addition, similar results of SV were reported on beans by Ferreira et al. (2018) and Ferreira et al. (2017). As discussed prior, the changes occurred in the molecular structure of the crystalline region due to the amylose content and the reduction in relative crystallinity and peak intensities was documented as the important factor causing lower SV. Alterations occurring on the amylopectin chains (e.g., breakage and release of amylopectin small chains) of starch from the stored beans was reported to be the reason behind the formation of weaker gels that resulted from reduction in FV and SV of starch (Ferreira et al. 2017). Less gel strength of the flour from pea stored at HT and HRH over 270 days was observed in the peas regardless of variety, i.e., 290 to 34 g, 330 to 25 g, 382 to 44 g, 356 to 35 g, 242 to 26 g and 301 to 18 g for Agassiz, Salamanca, Arcadia, Ginny, Keystone and Vail, respectively. Whereas storage at RT over 270 days under diverse RH caused only slight reduction in the gel strength of pea flour.

4.3.2.3. Peak Viscosity (PV) of Dry Pea Flour

In contrast to final viscosity (FV) and setback viscosity (SV), peak viscosity (PV) of stored dry pea flour increased during storage at accelerated conditions. The main effect of days of storage, diverse RH and different varieties were observed in the PV of flour obtained from pea samples stored at RT (Table 13). The two-way interaction of days of storage and different varieties was significant (p -value<0.001) on the PV of flour obtained from different varieties of pea when stored at HT. Increasing number of days of storage at HT resulted in significantly higher PV of pea flour for most, but not all,

varieties. The Vail variety had PV tended to increase up to 180 days; however, a significant drop in PV occurred with continued (270 days) storage at HT (Figure 31).

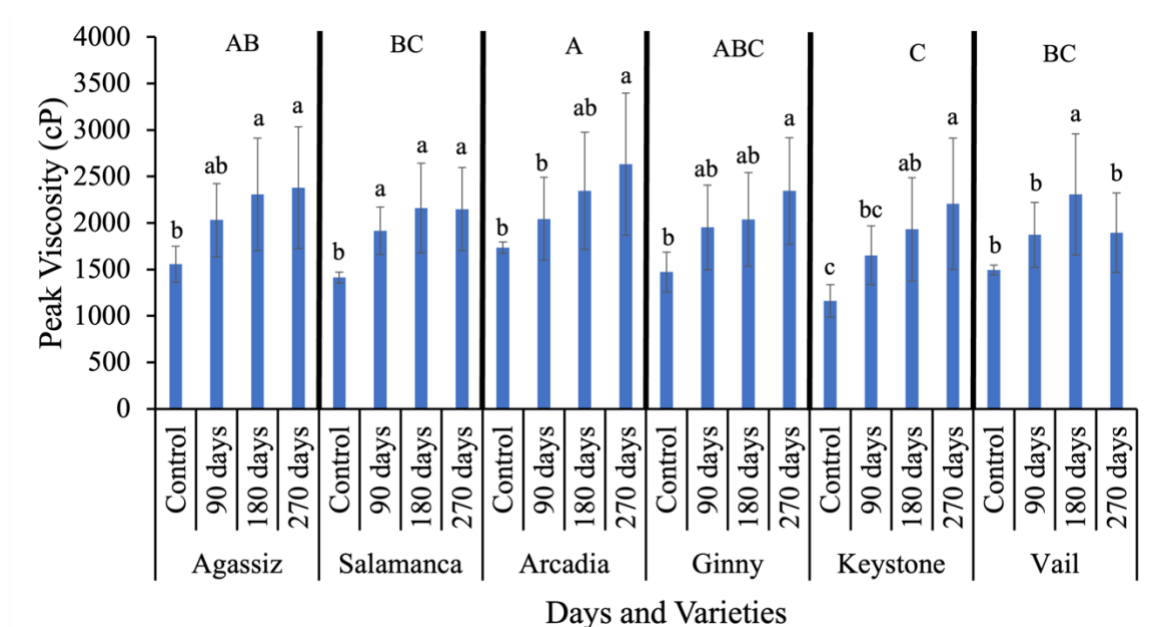


Figure 31: Peak viscosity of flour from different varieties of dry peas stored under diverse storage conditions over 270 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across storage days within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error.

The two-way interaction of days of storage and different varieties; and days of storage and RH was significant (p -value<0.001) for the PV of flour obtained from different varieties of pea when stored at HT. Storing dry peas at HRH was found to be more impactful, having high PV than the LRH and MRH on storability of peas, at HT (Figures 32 and 33). In all varieties except Salamanca stored at HT, significantly higher PV were observed for samples stored at HRH. Our results are in the agreement with the results reported by Ferreira et al. (2017), where these authors reported that the highest PV

Table 13: Peak viscosity (cP) of flour of peas stored at room temperature (21 °C).

Days of Storage	Peak Viscosity (cP)
0 (control)	1473 ^{c*}
90	1608 ^a
180	1554 ^b
270	1582 ^{ab}
p-value	<0.001
Varieties	Peak Viscosity (cP)
Agassiz	1588 ^b
Salamanca	1570 ^b
Arcadia	1786 ^a
Ginny	1585 ^b
Keystone	1345 ^c
Vail	1551 ^b
p-value	<0.001
Relative Humidity (RH)	Peak Viscosity (cP)
LRH	1496 ^c
MRH	1549 ^b
HRH	1699 ^a
p-value	<0.001

*Different lowercase letters represent significant difference with the days of storage, among varieties and RH based on 5% lsd values.

value (291 RVU compared to 276 and 277, respectively) was in the starch isolated from beans stored at 17% moisture and 32 °C for 12 months, than at 14% moisture and 32 °C, and freshly harvested beans. Comparing these results to ours in the present study, high RH leading to high moisture content influenced PV of the starch. Swelling power of the starch was determined to be high and was documented that the high swelling power could be the possible reason for the increase in PV of the starch isolated from the beans (Ferreira et al. 2017).

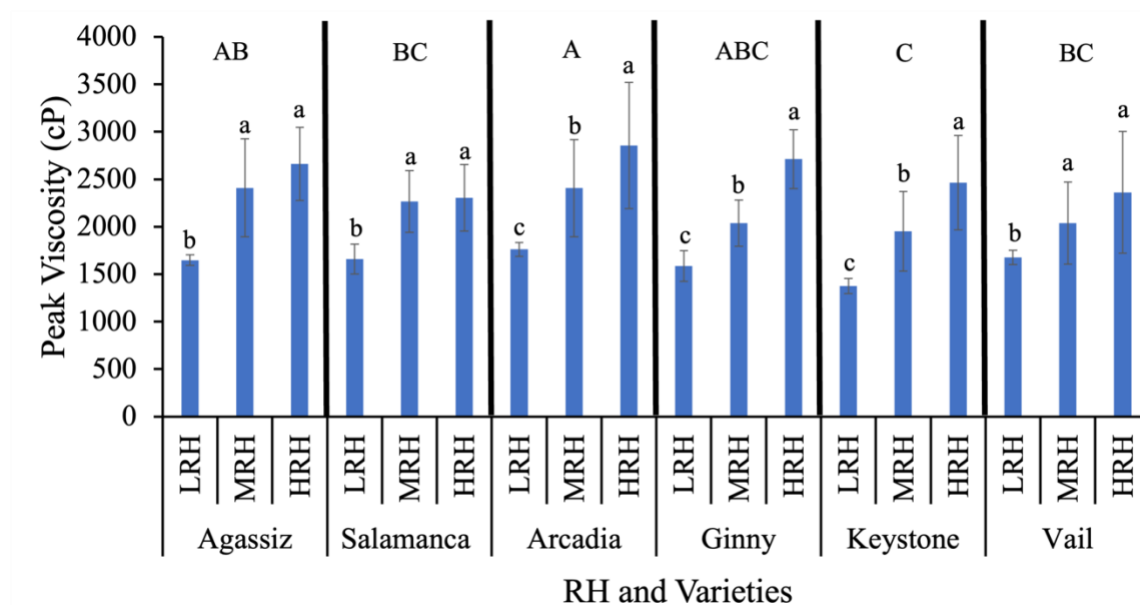


Figure 32: Peak viscosity of flour from different varieties of dry peas stored at different RH and at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across different RH within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error. LRH-Low Relative Humidity, MRH- Medium Relative Humidity, HRH- High Relative Humidity.

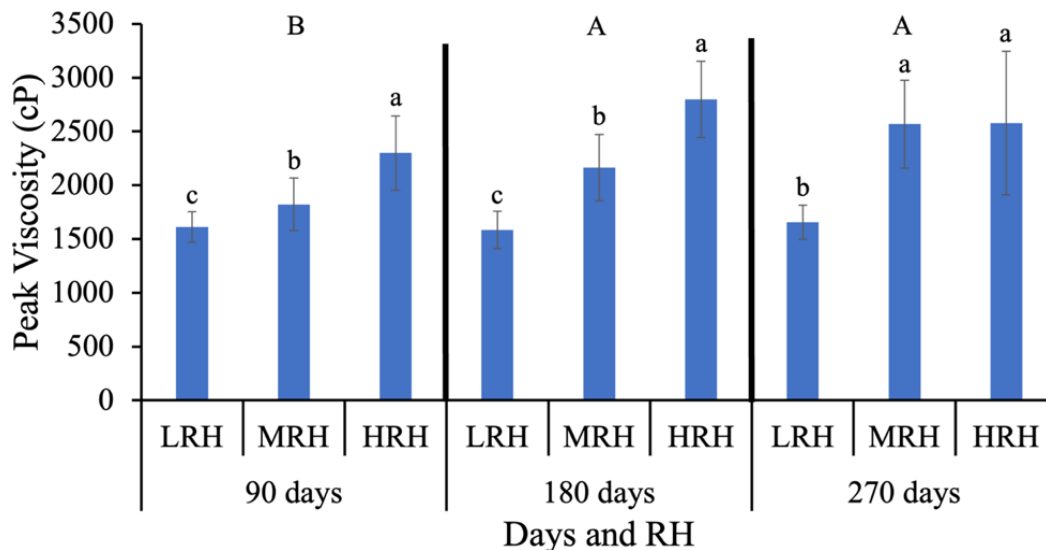


Figure 33: Peak viscosity of flour from dry peas stores at different RH over 270 days of storage at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference within days across diverse RH condition and across days of storage, respectively based on 5% lsd values. Error bars represent standard error. LRH- Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

4.4. Physical Properties of Peas

4.4.1. Principle Component Analysis (PCA)

Principal Component Analysis (PCA) on physical properties, i.e., moisture content, color and color difference, 1000 kernel seed weight, swelling capacity and hydration capacity of different varieties of dry pea seeds were carried out to study the relationship between various properties. PCA on physical properties of seed revealed that PC1 and PC2 accounted for 53.5% and 20.3% of cumulative variance, respectively. The biplot (Figure 34), demonstrated that dry pea varieties differed in physical properties. The data representing yellow pea varieties Agassiz (AG) and Salamanca (SA) were clearly overlapping indicating minimal data variation. Similarly, green and winter green pea varieties Arcadia (AR), Ginny (GI), Keystone (KE) and Vail (VA) overlapped revealing

that not much difference in data variation between these varieties were observed. The plot showed that the moisture content and 1000 kernel seed weight were highly correlated. Also, hydration capacity and swelling capacity was observed to be correlated. Figure 35 shows that increasing days of storage affected the physical properties of dry peas. However, days 60 and 90 days were similar, whereas 180 and 270 days were more diverse. Thus, further data analysis was continued only with data from 30, 90, 180, and 270 days being compared with time 0, i.e., the control sample. Data variation in HT and RT were observed from the biplot, this was true for RH as well for all the treatment conditions, i.e., LRH, MRH, HRH (Figures 36 and 37). Thus, further data analysis was proceeded with all the treatment conditions of temperature and RH. Dry pea samples stored at RT and HT were significantly different ($p\text{-value} < 0.001$), thus for the ease of further analysis, data analysis was divided into RT and HT.

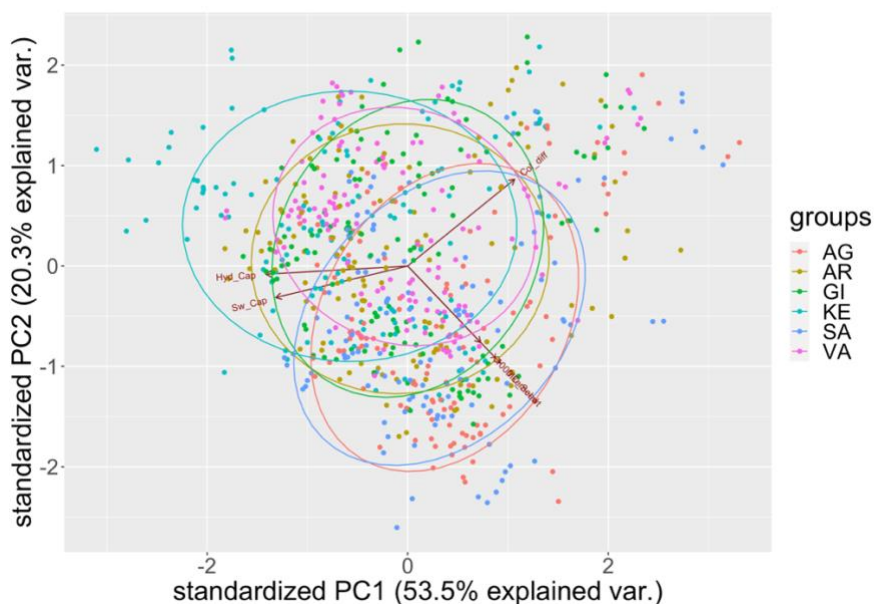


Figure 34: The biplot of two PCs showing physical properties of different varieties of dry peas.

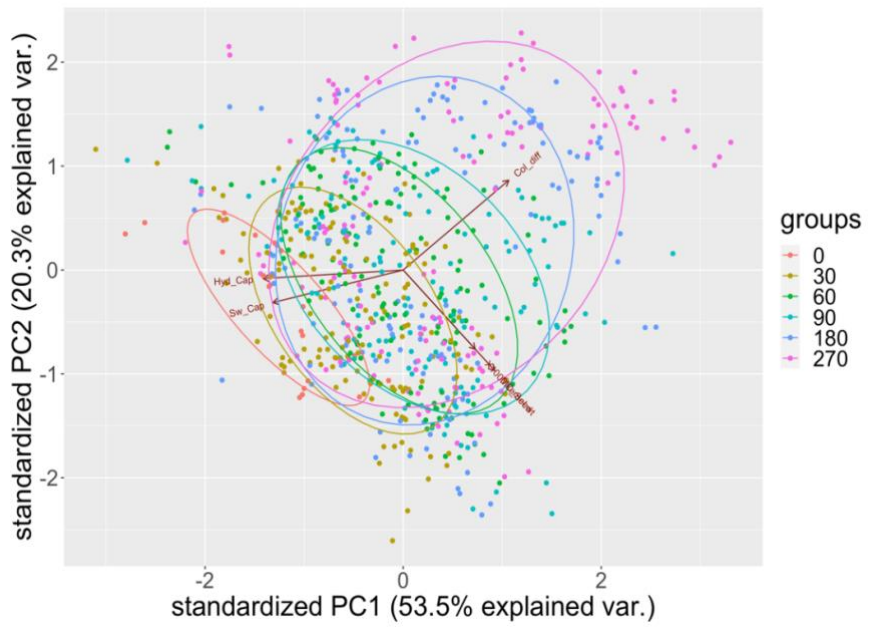


Figure 35: The biplot of two PCs showing physical properties of dry peas at different sampling days (0, 30, 60, 90, 180, 270 days).

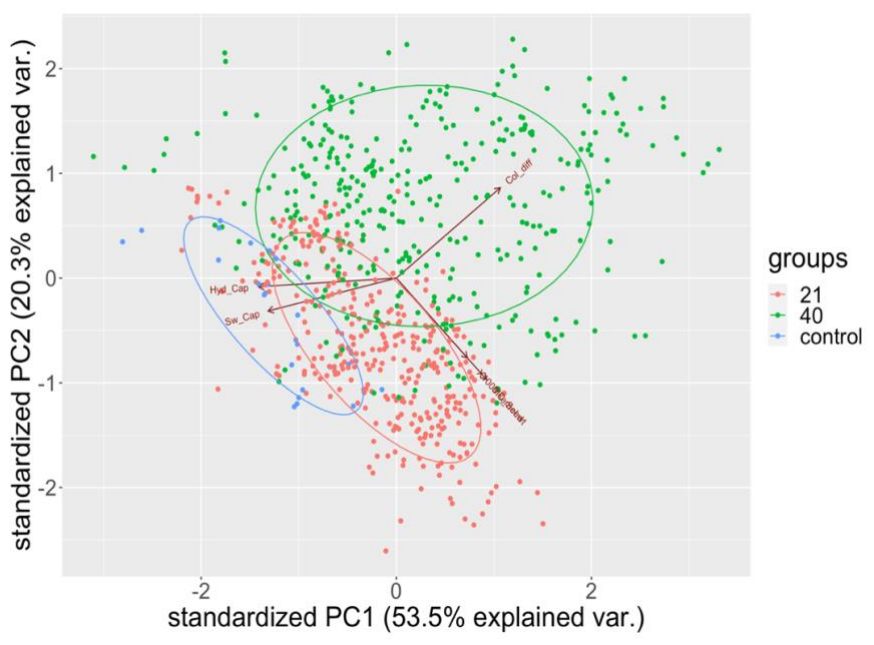


Figure 36: The biplot of two PCs showing physical properties dry peas stored at different temperature.

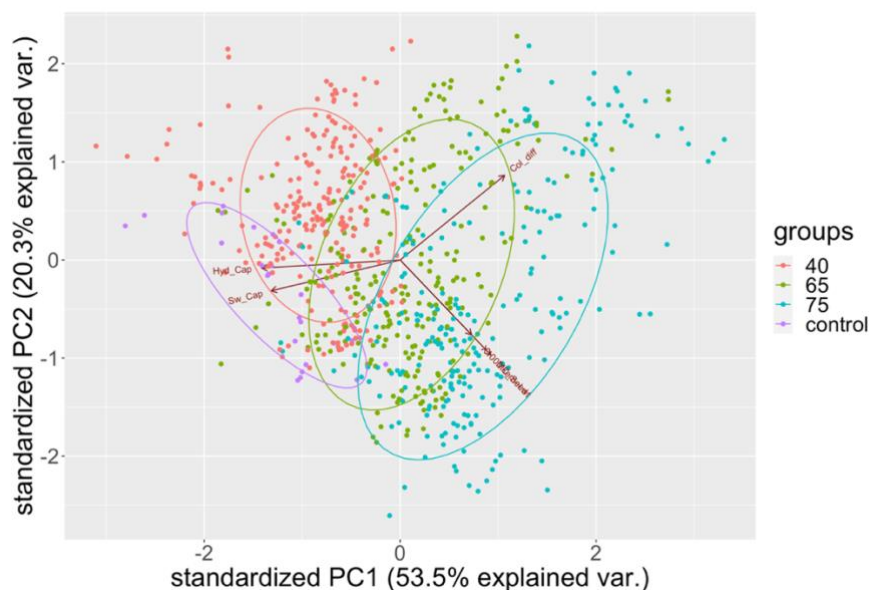


Figure 37: The biplot of two PCs showing physical properties dry peas stored at different RH.

4.4.2. Variable Selection by PCA

The variables that were responsible for the effect on the physical characteristics of dry peas accounted for at least 20% to the variability (Figure 38). The variables that contribute most to variation include moisture content, color difference, water hydration capacity and swelling capacity of dry pea seed.

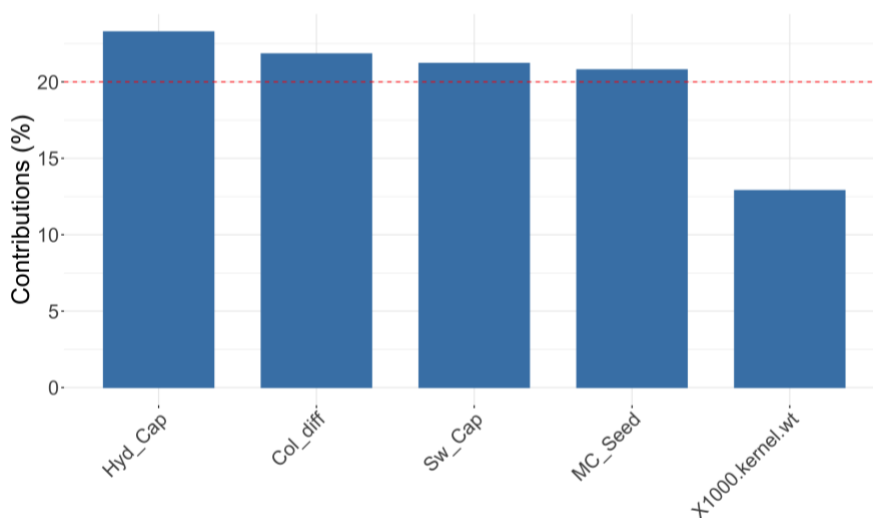


Figure 38: The contribution plot of variables for physical properties of whole dry pea seed. The red dash line in the graph represents the average contribution of each variable.

4.4.2.1. Moisture Content of Dry Pea

Moisture contents among varieties was different. At time 0 (control), moisture content of Agassiz (9.11%) was the highest followed by Arcadia (8.36%), Salamanca (8.12%), Ginny (7.28%), Keystone (6.73%) and Vail (6.54%). There was an increase in moisture content over the storage time, the interactive effect of days and varieties was significant (p -value <0.005) when stored at RT (Figure 39); however, it was not significant at HT condition. Increase in moisture content in the dry pea seeds is pronounced due to the physiological activity called respiration. Chidananda et al. (2014), reported that the moisture content and respiration rate of the pulses (i.e., chickpea, green lentil and pinto bean) were positively correlated; thus, moisture content increased significantly over time could possibly be due to the release of water during respiration. However, Nyakuni et al. (2008), reported significant reduction in the moisture content of common beans with the storage period.

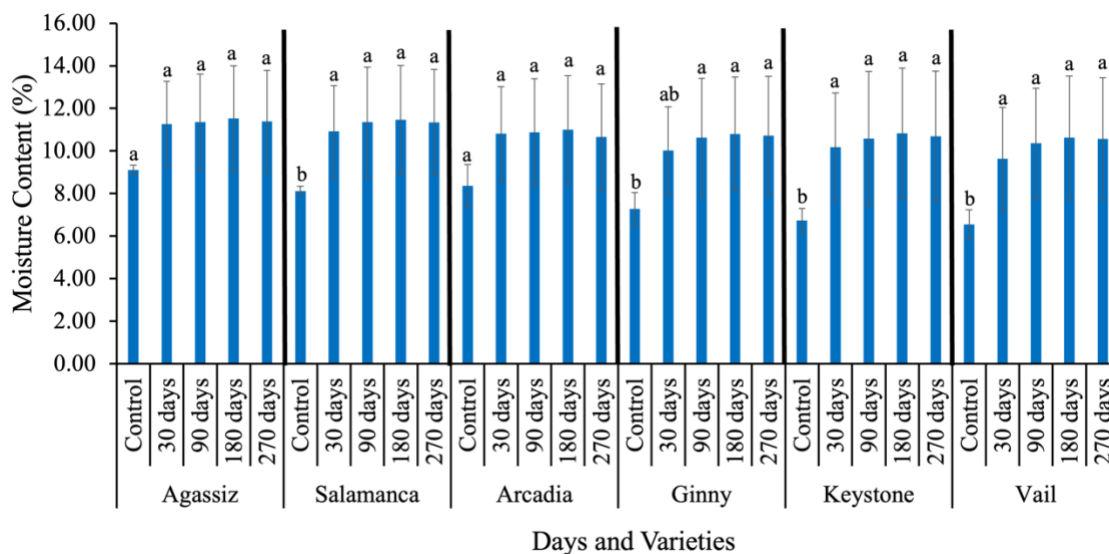


Figure 39: Moisture content of different varieties of dry peas stored over 270 days at room temperature (21 °C). Different lowercase letters represent significant difference across storage days and within each variety based on 5% lsd values. Error bars represent standard error.

The interactive effect of RH and varieties was significant (p -value <0.05) when stored at RT and HT condition. The moisture content of all the varieties of dry peas stored at HRH were significantly higher than those stored at LRH regardless of the variety when stored at HT and RT (Figures 40 and 41). The days and RH interaction effect was also significant (p -value <0.001) for both RT and HT storage conditions. Moisture content of the dry pea seeds, regardless of the varieties was higher in each sampling day when stored at HRH at RT as well as HT (Figures 42 and 43). Seeds are hygroscopic in nature, thus when they are exposed to the air of a certain RH, their moisture content will change with respect to RH of the air (Bradford et al. 2016). This could be the possible reason for the difference in moisture content of the dry pea seeds stored at different RH.

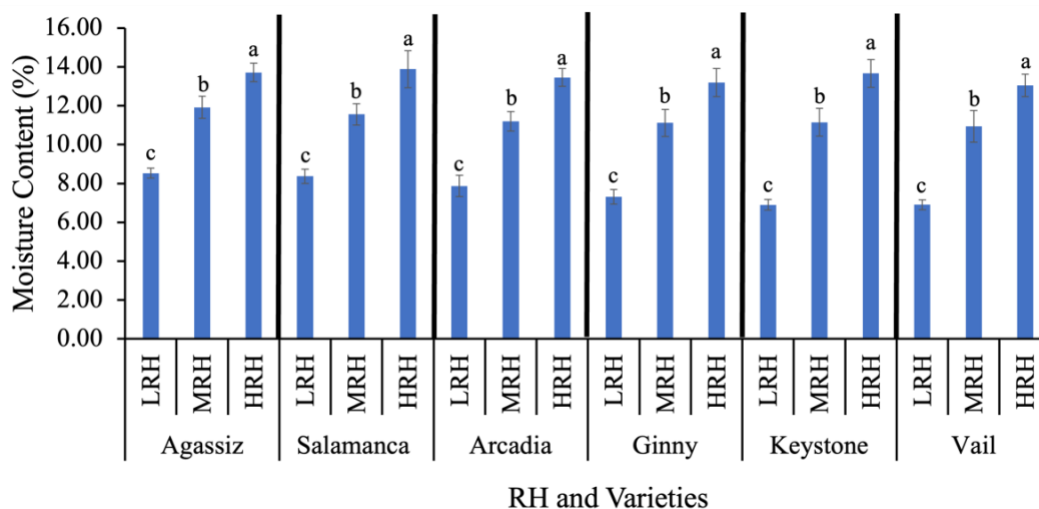


Figure 40: Moisture content of different varieties of dry peas stored at different RH and at room temperature (21 °C). Different lowercase letters represent significant difference across different RH within each variety based on 5% lsd values. Error bars represent standard error. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

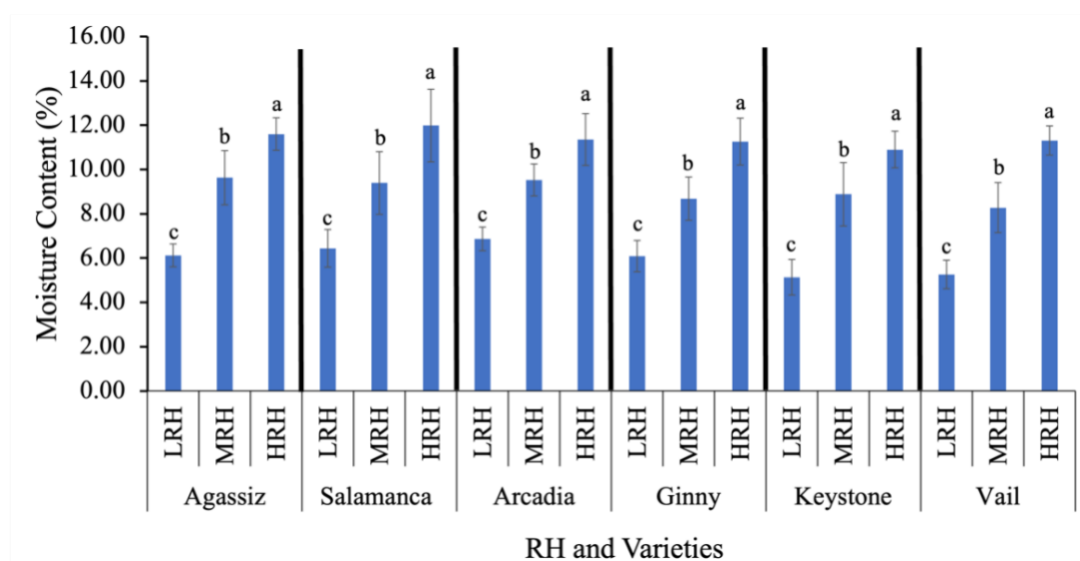


Figure 41: Moisture content of different varieties of dry peas stored at different RH and at high temperature (40 °C). Different lowercase letters represent significant difference across different RH within each variety based on 5% lsd values. Error bars represent standard error. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

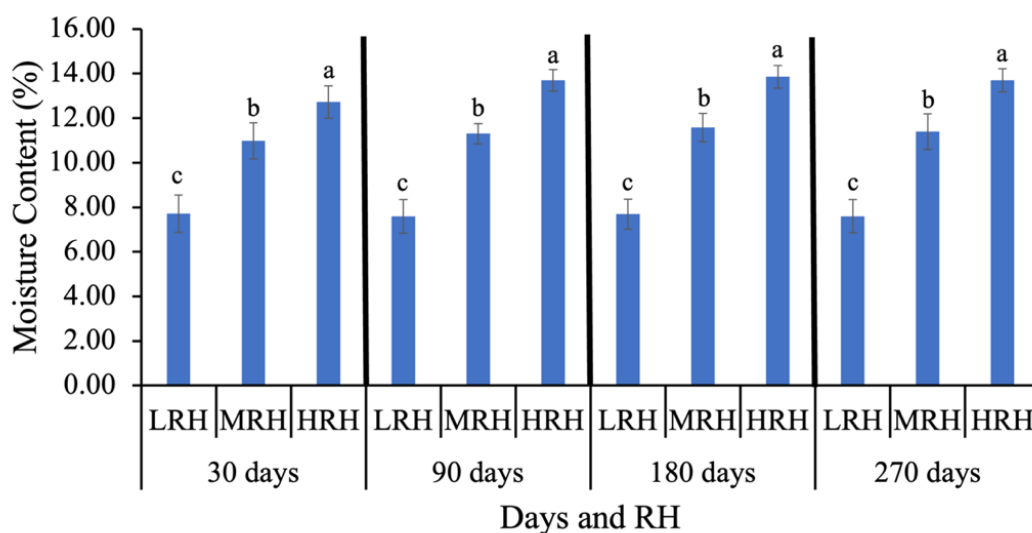


Figure 42: Moisture content of dry peas stored at different RH over 270 days of storage and at room temperature (21 °C). Different lowercase letters represent significant difference within days across diverse RH condition based on 5% lsd values. Error bars represent standard error. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

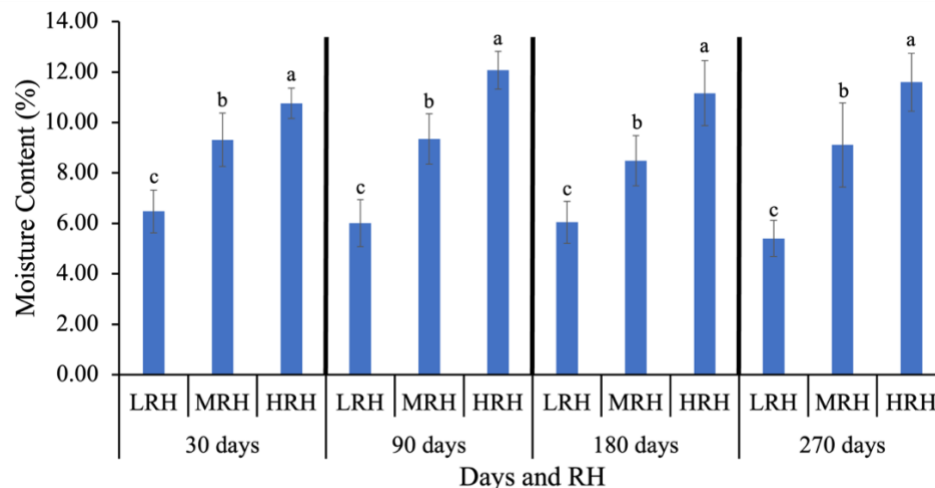


Figure 43: Moisture content of dry peas stored at different RH over 270 days of storage and at high temperature (40 °C). Different lowercase letters represent significant difference within days across diverse RH condition based on 5% lsd values. Error bars represent standard error. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

4.4.2.2. Color and Color Difference of Dry Pea

An increasing trend of color difference was observed for each variety of dry peas stored at HT and RT. However, HT had a greater impact on color than RT storage. Furthermore, the highest color difference occurred at 270 days of storage. The color of pea became darker for yellow varieties and creamy yellow for green varieties upon 270 days of storage at HT and HRH (Figure 44). The visual color difference in the different varieties of dry pea seeds stored under diverse storage conditions also was significant after soaking (Figure 45). Salamanca variety stored at RT for 270 days did not show significant color difference with the days of storage. This in part may be due to the high color differences among the data. The interactive effect of days of storage and varieties was significant (p -value<0.001) when stored at RT and HT. Other varieties had significant color change from time 0 to 270 days of storage at RT with the average color difference value of 2.54-1.50. In contrast, Salamanca had the highest color difference

(10.57) followed by Ginny (10.01), Agassiz (9.66), Arcadia (8.14), Keystone (7.34) and Vail (6.08) when stored at HT for 270 days (Figures 46 and 47).

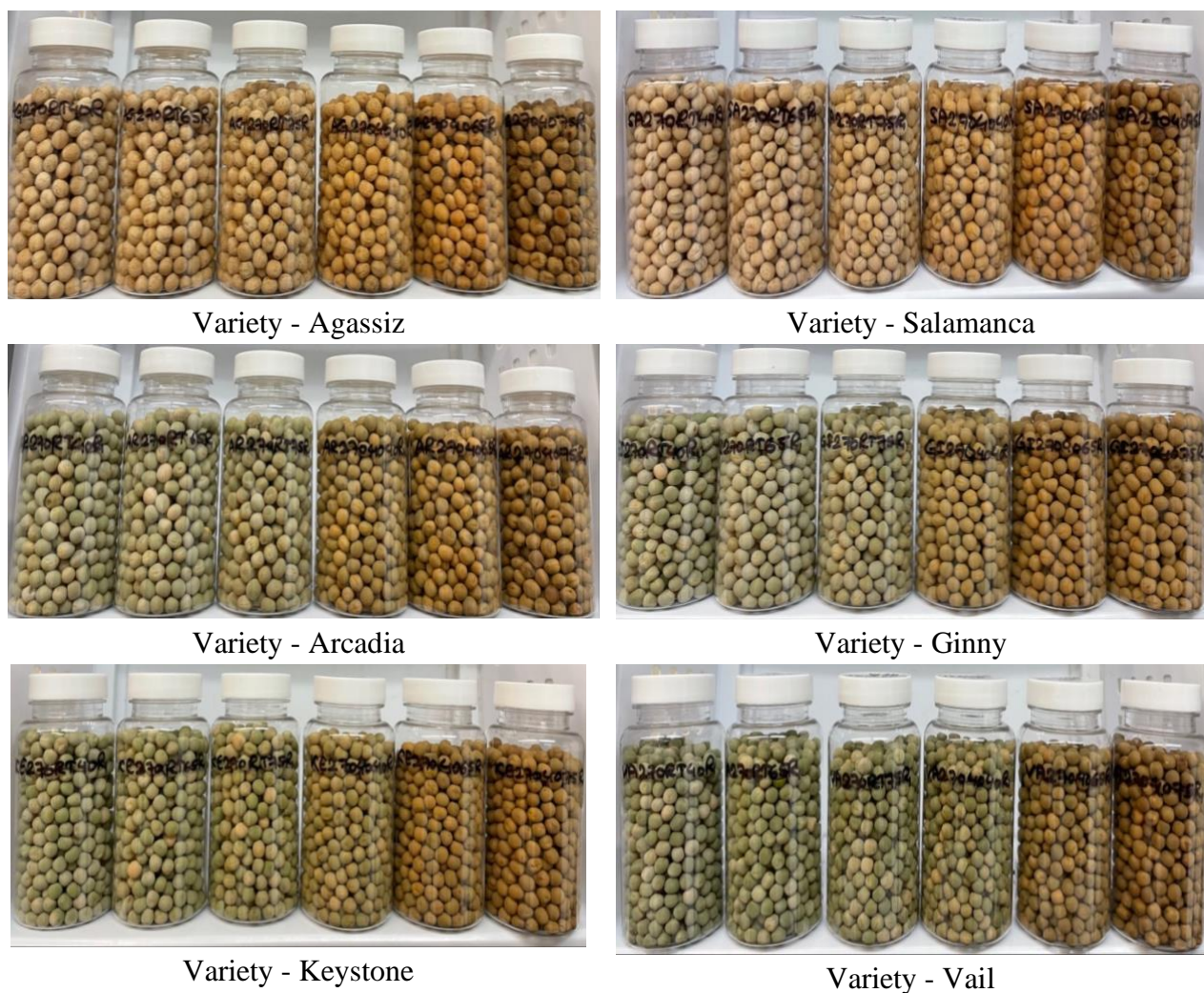


Figure 44: Discoloration occurred in dry pea seeds due to long term storage under harsh conditions. **Storage Conditions: RT-LRH, RT-MRH, RT-HRH, HT-LRH, HT-MRH, HT-HRH from left to right; RT- Room Temperature (21 °C) HT- High Temperature (40 °C); LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).



Figure 45: Differences in color of dry peas stored under diverse storage conditions for 270 days after soaking. **Storage Conditions: RT- Room Temperature (21 °C) HT- High Temperature (40 °C); LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

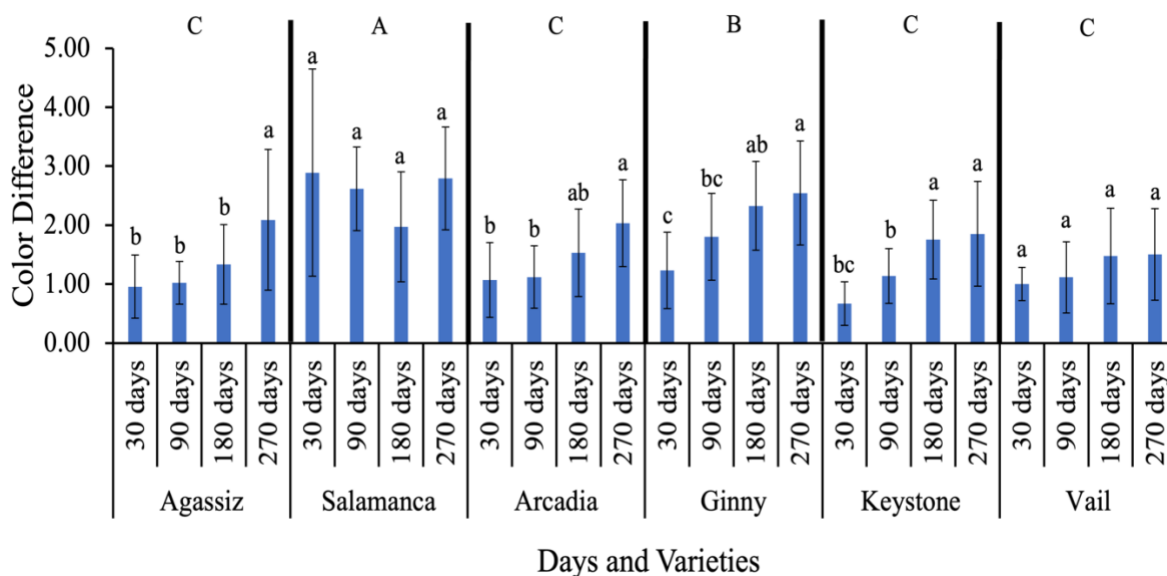


Figure 46: Color difference of different varieties of dry peas stored over 270 days at room temperature (21 °C). Different lowercase and uppercase letters represent significant difference across storage days within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error.

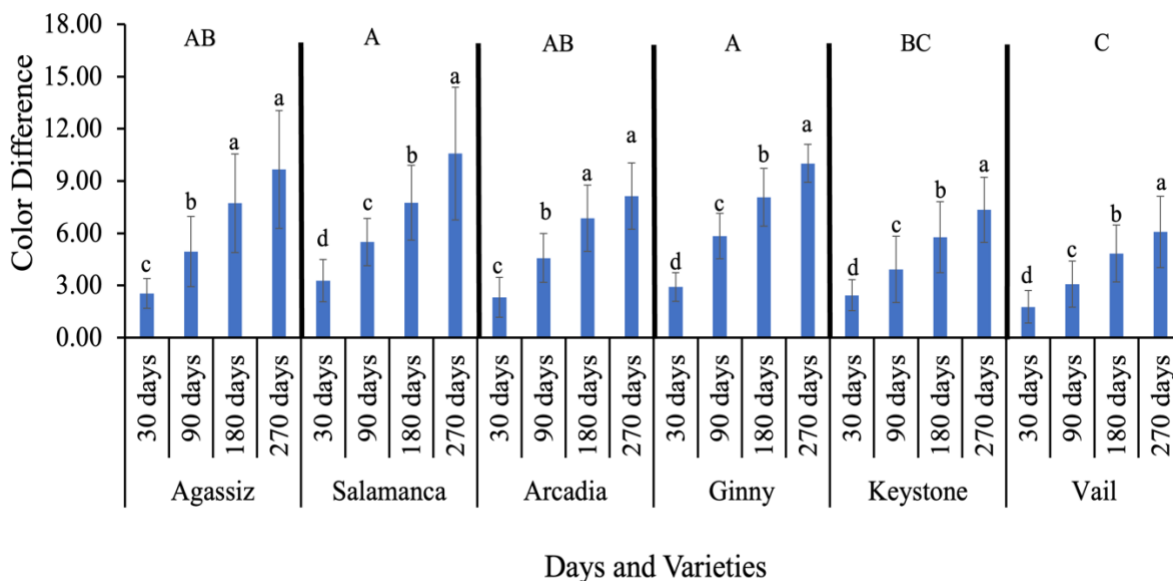


Figure 47: Color difference of different varieties of dry peas stored over 270 days at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across storage days within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error.

The interaction effect of RH and varieties when stored at RT and HT was significant (p -value <0.05) for color difference. With the increase in RH, color difference also increased for all the varieties. Color difference was not significantly different for samples stored under MRH and HRH, regardless of temperature (Figures 48 and 49). Salamanca variety was the most affected by HRH showing the color difference of 8.48 followed by Agassiz (8.16), Ginny (7.65), Arcadia (6.48), Keystone (6.18) and Vail (5.46). There was significant (p -value <0.001) effect for the days of storage and diverse RH condition on color difference. The color difference value was higher for the peas stored under HRH for 270 days at both the temperatures. Also, the trend of increasing color difference value was observed with the increasing days of storage (Figures 50 and 51).

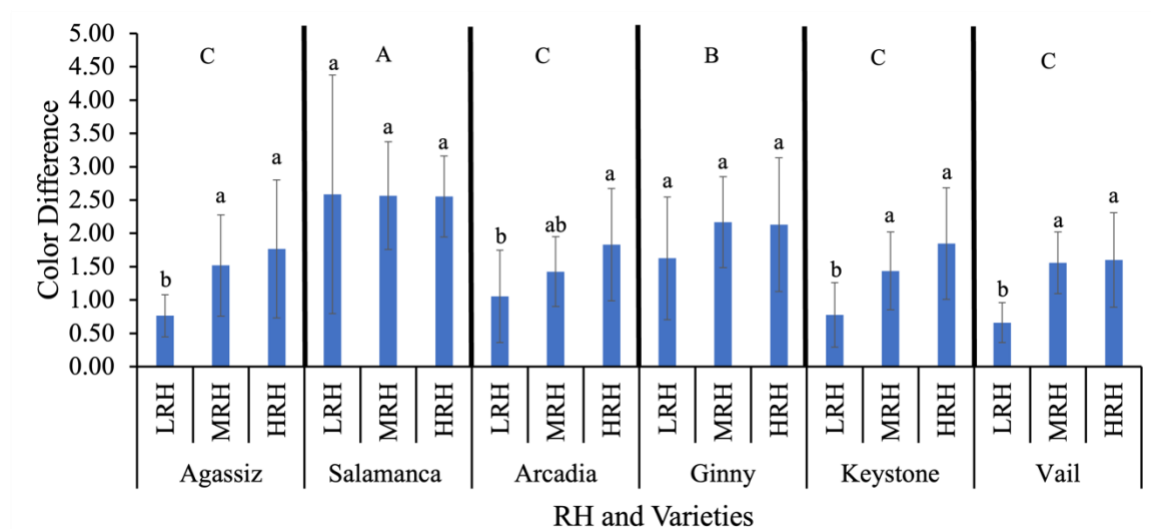


Figure 48: Color difference dry peas stored at different RH and at room temperature (21 °C). Different lowercase and uppercase letters represent significant difference across different RH within each variety and across varieties, respectively based on 5% lsd values. Error bars represent standard error. LRH-Low Relative Humidity (40%), MRH-Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

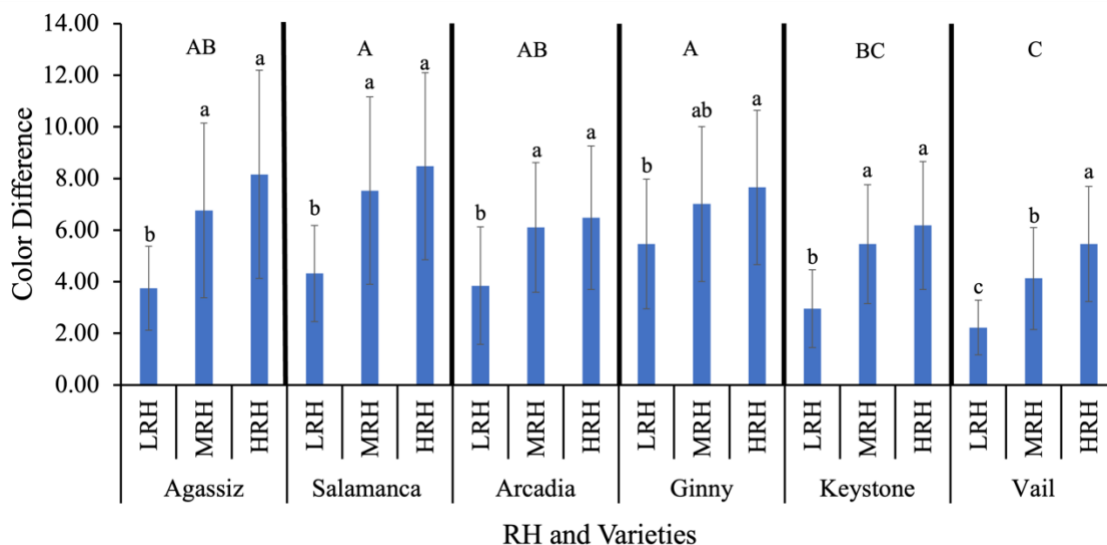


Figure 49: Color difference of dry peas stored at different RH and at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across different RH within each variety and across varieties, respectively based on 5% Lsd values. Error bars represent standard error. LRH- Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

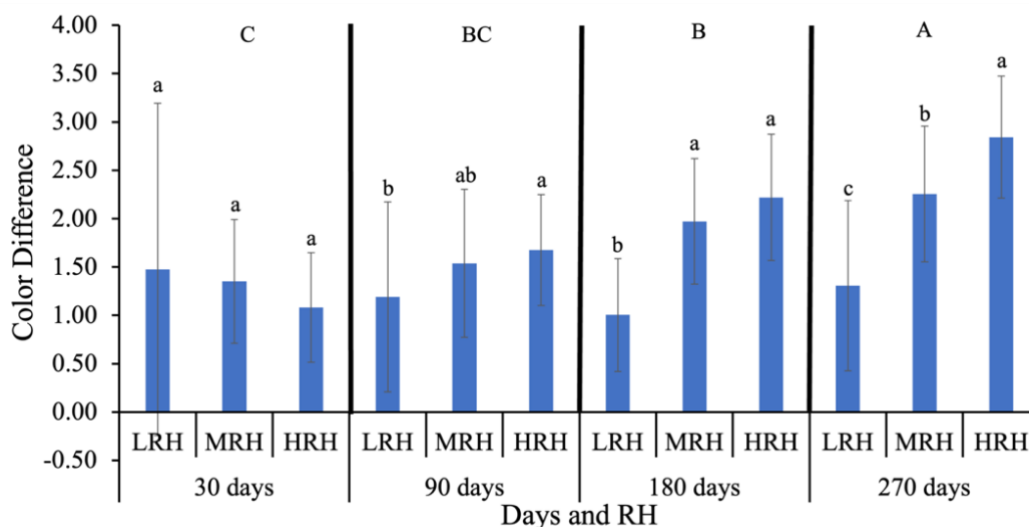


Figure 50: Color difference of dry peas stored at diverse RH over 270 days of storage at room temperature (21 °C). Different lowercase and uppercase letters represent significant difference across different RH within each day of storage and across days of storage, respectively based on 5% Lsd values. Error bars represent standard error. LRH- Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

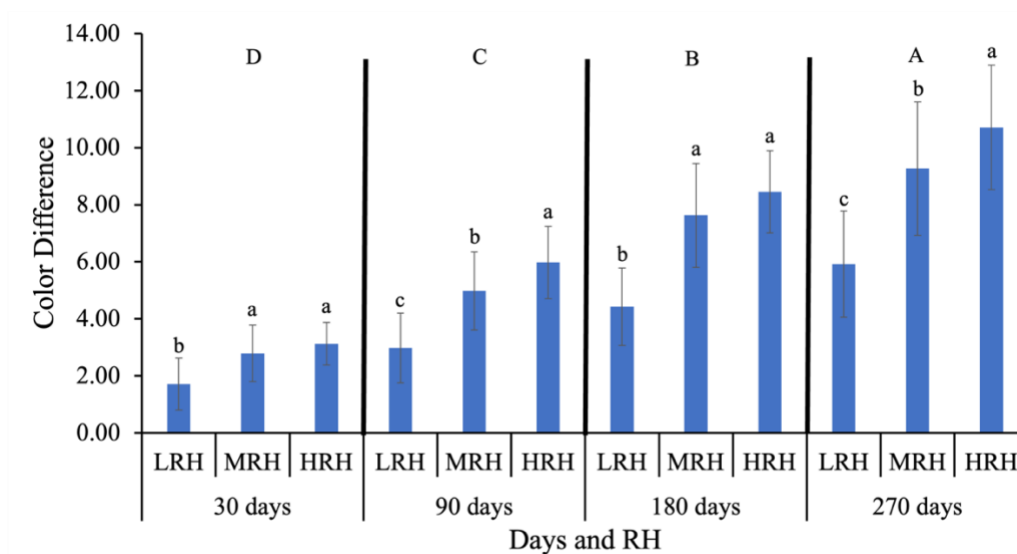


Figure 51: Color difference of dry peas stored at diverse RH over 270 days of storage at high temperature (40 °C). Different lowercase and uppercase letters represent significant difference across different RH within each day of storage and across days of storage, respectively based on 5% lsd values. Error bars represent standard error. LRH- Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

The difference in the color was observed due to the difference in L^* , a^* and b^* values of dry peas. Under the diverse storage condition, L^* values (i.e., indicator of lightness) of all the varieties of dry peas decreased, a^* (i.e., indicator of red/green coordinate) and b^* (i.e., indicator of yellow/blue coordinate values) increased from time 0 to 270 days. The a^* values became more positive for green peas, which theoretical indicates less greenness in a sample, and visually changed from dark green to creamy yellow.

The change in color in the green dry peas is particularly due to the color degradation called bleaching. Bleaching occurs by the combination of genetic and environmental factors during the post-harvest storage that will aid in the discoloration of dry pea seeds from green to creamy yellow due to the degradation of chlorophyll pigment (McDonald et al. 2019). Research conducted on green dry peas (Cheng et al. 2004),

indicated that bleaching faded the green color of dry peas in the presence of moisture. Furthermore, the increase in a^* value was observed to be the highest with the seeds under the highest RH. In general, color changes of legumes are due to temperature, seed moisture and light during storage. Darkening in the testa color of chickpea stored for 160 days at 33-35 °C and 75% RH was observed visually by a decrease in L value and was attributed to the non-enzymatic darkening due to the polymerization reaction of phenolic compounds (Reyes-Moreno et al. 2000). In addition, color of lentils stored at HT (20-30 °C) and HRH (100%) turned brown in 3 weeks; whereas, at 5 °C and HRH (100%) browning did not occur before 5 weeks of storage, supporting temperature effect on lentils. This was explained as the bleaching of chlorophyll in cotyledons and seed coats and also due to the browning; the result of polymerization of low molecular weight phenolic precursors to brown-colored high molecular weight tannins (Nozzolillo and Bezada 1984). Although not identified in this study, the color change observed in dry peas stored at HRH and HT is likely due to chlorophyll degradation and non-enzymatic browning.

4.4.2.3. Water Hydration Capacity and Swelling Capacity of Dry Pea

Effect of diverse storage conditions on the water hydration capacity (%) and the swelling capacity (%) of different varieties of dry peas were evaluated. The hydration capacity among the different varieties of seed was significantly different when stored at RT (p-value<0.001) and HT (p-value<0.005). However, the swelling capacity was only significantly different among the varieties stored at RT (p-value<0.001). No significant difference in swelling capacity was observed in the samples stored at HT but interaction effect of storage days and RH was observed in some varieties.

Under diverse storage condition, trend of decrease in water hydration capacity was significant for all the varieties. When dry peas of different varieties were stored at RT over 270 days under diverse storage conditions of RH, no significant reduction in water hydration capacity within the varieties was observed except for Vail (Table 14). Difference in water hydration capacity among the varieties was significant after 180 days of storage at LRH, and only at 90 days at HRH storage conditions. Whereas MRH influenced water hydration capacity of all the varieties of dry peas significantly over 270 days of storage. Reduction in water hydration capacity was significant within each variety except for Arcadia and Keystone (Table 15) when stored at HT for 270 days. The storage of dry peas at HT at LRH caused no significant reduction in water hydration capacity over 270 days among the varieties. This reduction was significant only at MRH and HRH after 180 days of storage.

Table 14: Hydration Capacity (%) of different varieties of dry peas stored under diverse storage conditions at room temperature (21 °C).

Storage Conditions (Days-RH)	Agassiz	Salamanca	Arcadia	Ginny	Keystone	Vail	p-value
0 (control)	94 ^C	99 ^{BC}	102 ^A	103 ^{AB}	107 ^A	100 ^{a, BC*}	0.017
30-LRH	97	100	100	104	105	96 ^b	0.114
90-LRH	96	98	98	103	107	97 ^{ab}	0.114
180-LRH	98 ^{BC}	100 ^{BC}	98 ^{BC}	103 ^{AB}	106 ^A	96 ^{b, C}	0.026
270-LRH	97 ^{BC}	99 ^{AB}	99 ^{AB}	101 ^{AB}	105 ^A	91 ^{c, C}	0.012
30-MRH	93 ^B	97 ^{AB}	96 ^{AB}	100 ^A	100 ^A	91 ^{c, B}	0.023
90-MRH	89 ^B	96 ^A	94 ^A	96 ^A	98 ^A	88 ^{de, B}	0.001
180-MRH	92 ^{AB}	95 ^A	93 ^A	96 ^A	96 ^A	87 ^{ef, B}	0.027
270-MRH	90 ^{BC}	95 ^{AB}	95 ^{AB}	97 ^A	96 ^A	85 ^{fg, C}	0.001
30-HRH	90	96	93	95	96	90 ^{cd}	0.092
90-HRH	82 ^B	91 ^A	88 ^{AB}	90 ^A	92 ^A	86 ^{efg, AB}	0.049
180-HRH	87	89	88	88	88	84 ^{gh}	0.128
270-HRH	85	86	85	87	85	83 ^h	0.646
p-value	0.207	0.076	0.744	0.666	0.781	0.011	

*Different lowercase letters in a column represent significant difference across storage days and RH within each variety. Different uppercase letters in a row represent significant difference across varieties. No letters represent no significant difference based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%)

Table 15: Hydration Capacity (%) of different varieties of dry peas stored under diverse storage conditions at high temperature (40 °C).

Storage Conditions (Days-RH)	Agassiz	Salamanca	Arcadia	Ginny	Keystone	Vail	p-value
0 (control)	94 ^{bc, C*}	99 ^{abc, BC}	102 ^{AB}	103 ^{ab, AB}	107 ^A	100 ^{a, BC}	0.017
30-LRH	98 ^{ab}	102 ^a	103	104 ^a	109	97 ^b	0.159
90-LRH	102 ^a	101 ^{ab}	98	98 ^c	105	98 ^{ab}	0.394
180-LRH	97 ^b	100 ^{abc}	100	99 ^c	102	96 ^{bc}	0.304
270-LRH	96 ^b	100 ^{abc}	97	97 ^c	102	95 ^{cd}	0.242
30-MRH	87 ^d	95 ^{bcd}	95	99 ^{bc}	101	93 ^{de}	0.151
90-MRH	90 ^{cd}	90 ^d	90	97 ^c	97	91 ^e	0.130
180-MRH	81 ^{e, DE}	81 ^{ef, E}	84 ^{CD}	88 ^{d, AB}	90 ^A	85 ^{f, BC}	<0.001
270-MRH	78 ^{ef, CD}	80 ^{ef, BC}	82 ^{AB}	82 ^{e, AB}	85 ^A	75 ^{h, D}	<0.001
30-HRH	87 ^d	94 ^{cd}	86	95 ^c	95	86 ^f	0.129
90-HRH	81 ^e	82 ^e	72	79 ^{ef}	87	77 ^g	0.102
180-HRH	73 ^{fg, A}	76 ^{f, A}	75 ^A	75 ^{fg, A}	75 ^A	69 ^{i, B}	0.032
270-HRH	69 ^{g, BC}	69 ^{g, BC}	73 ^{AB}	73 ^{g, AB}	77 ^A	67 ^{i, C}	0.008
p-value	<0.001	<0.001	0.258	<0.001	0.731	<0.001	

*Different lowercase letters in a column represent significant difference across storage days and RH within each variety. Different uppercase letters in a row represent significant difference across varieties. No letters represent no significant difference based on 5% lsd values. LRH- Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

Similarly, swelling capacity of peas of different varieties was significantly affected by the storage at RT under diverse storage conditions of RH over 270 days (Table 16). Reduction in swelling capacity within each variety was significant only for Salamanca and Keystone when compared to time 0 (control) and 270 days of storage at diverse RH conditions. However, among the varieties this change was significant only after 270 days at LRH conditions, 270 days at MRH conditions and under HRH the difference among the varieties was significant only in the initial days, i.e., 30 and 90 days. Storage at HT under diverse RH condition over 270 days reduced swelling capacity of all the varieties of dry pea, however this reduction was not significant for Arcadia and Keystone. Difference in the reduction in swelling capacity among the varieties was significant after 90 days of storage at MRH and only at 90 days at HRH storage conditions (Table 17).

Water hydration capacity, i.e., properties of the seed to imbibe water after soaking, was affected by the storage conditions. Variability in the seed size, seed coat thickness and water absorption properties of seed could be the reasons for the differences in the hydration capacity of dry peas (Yadav et al. 2018). Adzuki beans stored at 30 °C for 6 months absorbed less water in comparison to those stored at 20 °C and 10 °C (Yousif et al. 2002). Water absorption capacity of the seeds is affected by the structure of cell wall, composition of the seeds, and compactness of the cells in the seed. Swelling capacity of the beans was documented to be positively correlated with the L* values of seeds indicating more swelling of lighter accessions than darker seeds during hydration (Yadav et al. 2018). This result supports our result that the seeds with high L* value in the initial days had higher swelling capacity than after storing for longer periods that led

to decrease in L* value and swelling capacity. Structural and chemical changes in the testa of seeds will make it harder and less permeable to water so that it acts as a barrier preventing water from reaching the cotyledons, which aids in the low hydration and swelling coefficients after the storage at high temperature (Liu et al. 1992).

Table 16: Swelling Capacity (%) of different varieties of dry peas stored under diverse storage conditions at room temperature (21 °C).

Storage Conditions (Days-RH)	Agassiz	Salamanca	Arcadia	Ginny	Keystone	Vail	p-value
0 (control)	122 ^C	138 ^{a, AB*}	134 ^B	128 ^{BC}	148 ^{a, A}	137 ^B	0.002
30-LRH	123	135 ^{ab}	137	127	133 ^{bcd}	124	0.051
90-LRH	117	120 ^{efgh}	126	115	125 ^{de}	111	0.067
180-LRH	131	122 ^{defg}	122	124	131 ^{cd}	129	0.495
270-LRH	125 ^{BC}	129 ^{bcd, B}	133 ^B	117 ^C	145 ^{ab, A}	118 ^C	<0.001
30-MRH	118	125 ^{cdef}	131	133	140 ^{abc}	119	0.051
90-MRH	106	112 ^{hi}	113	112	115 ^{ef}	102	0.105
180-MRH	122	127 ^{cde}	115	118	116 ^{ef}	115	0.066
270-MRH	119 ^{AB}	118 ^{fgh, AB}	123 ^A	114 ^{BC}	126 ^{de, A}	107 ^C	0.005
30-HRH	115 ^B	131 ^{abc, A}	129 ^A	115 ^B	133 ^{bcd, A}	118 ^B	0.006
90-HRH	104 ^B	114 ^{hi, A}	103 ^B	103 ^B	107 ^{f, AB}	102 ^B	0.023
180-HRH	115	117 ^{gh}	111	110	107 ^f	110	0.142
270-HRH	114	106 ⁱ	110	106	112 ^f	110	0.301
p-value	0.882	0.002	0.168	0.669	0.013	0.397	

*Different lowercase letters in a column represent significant difference across storage days and RH within each variety. Different uppercase letters in a row represent significant difference across varieties. No letters represent no significant difference based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%)

Table 17: Swelling Capacity (%) of different varieties of dry peas stored under diverse storage conditions at high temperature (40 °C).

Storage Conditions (Days-RH)	Agassiz	Salamanca	Arcadia	Ginny	Keystone	Vail	p-value
0 (control)	122 ^{ab, C*}	138 ^{a, AB}	134 ^B	128 ^{ab, BC}	148 ^A	137 ^{a, B}	0.002
30-LRH	122 ^{ab}	123 ^{bc}	131	132 ^a	141	123 ^{bc}	0.260
90-LRH	118 ^{abc}	119 ^{bc}	119	117 ^c	129	105 ^{fg}	0.101
180-LRH	119 ^{ab}	124 ^b	124	122 ^{bc}	122	111 ^{ef}	0.149
270-LRH	123 ^a	127 ^{ab}	131	120 ^{bc}	127	114 ^{de}	0.059
30-MRH	113 ^{abc}	121 ^{bc}	113	122 ^{bc}	128	129 ^b	0.172
90-MRH	107 ^{cde, B}	111 ^{c, AB}	102 ^B	113 ^{c, AB}	123 ^A	102 ^{g, B}	0.059
180-MRH	100 ^{def, B}	98 ^{d, B}	105 ^{AB}	115 ^{c, A}	105 ^{AB}	107 ^{efg, AB}	0.055
270-MRH	96 ^{ef, BC}	91 ^{de, C}	102 ^B	102 ^{d, B}	105 ^B	120 ^{cd, A}	<0.001
30-HRH	111 ^{bcd}	122 ^{bc}	109	116 ^c	119	114 ^{de}	0.398
90-HRH	99 ^{ef, AB}	98 ^{d, AB}	86 ^C	87 ^{e, C}	101 ^A	92 ^{h, BC}	0.008
180-HRH	96 ^f	95 ^{de}	88	91 ^e	93	87 ^h	0.189
270-HRH	79 ^g	85 ^e	88	84 ^e	82	73 ⁱ	0.101
p-value	0.009	<0.001	0.281	0.013	0.509	<0.001	

*Different lowercase letters in a column represent significant difference across storage days and RH within each variety. Different uppercase letters in a row represent significant difference across varieties. No letters represent no significant difference based on 5% lsd values. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%).

4.4.3.4. Cook Firmness

The cook firmness of time 0 (Control) samples was observed to be the highest for Arcadia (30.50 N/g), followed by Salamanca (26.56 N/g), Agassiz (26.41 N/g), Ginny (21.47 N/g), Vail (22.87 N/g), and Keystone (17.07 N/g) (Table 1. Appendix). After 90 days of storage at HT and MRH-HRH, dry peas did not soften and gave overload issues while running texture analysis. However, no overload effect was observed in the peas stored at RT and LRH. With the increasing days of storage at 270 days, dry peas stored at HT and LRH, MRH and HRH showed hard to cook effect. Again, no such effect was observed in the peas stored at RT. Hardness of pulses is generally the problem of texture in which pulses do not soften enough while cooking. Storage of beans at high temperature was documented as the cause for hardening of faba beans. Storage at temperature $>30^{\circ}\text{C}$ was observed to develop harder texture than those stored at $<25^{\circ}\text{C}$ for 1 year. Hardening of seeds due to storage in the accelerated temperature likely contributed to decreased hydration and swelling capacity (Nasar-Abbas et al. 2008). Similarly, cooking time for chickpeas stored at $33\text{-}35^{\circ}\text{C}$ increased as compared to lower temperature (Reyes-Moreno et al. 2000).

The hard to cook (HTC) defect in the pulses were attributed to the physical and chemical changes during storage. It has been reported that the insolubilization of the pectic substances due to the enzyme phytase results in hard texture of cooked pulses. Removal of methyl groups from pectins by pectin esterases, hydrolysis of storage proteins by proteases, oxidation of polyphenols by peroxidases and oxidation of lipids by lipoxygenases are some of the enzymatic reactions reported to be responsible for the HTC defect (Hohlberg and Stanley 1987). Nyakuni et al. (2008) reported that the HTC

defect in common beans was related to a decrease in protein and starch digestibility, and the reduction in phytic acid content that led to increase in cooking time. Furthermore, in the study of physiochemical changes of long-term stored beans, the authors reported higher protein content in HTC beans than compared to the fresh beans which supports our result of higher protein in the peas stored over 270 under HTH and HT, and the development of hardening in those peas (Martín-Cabrejas et al. 1997).

5. CONCLUSION

Storage of dry peas of different varieties under diverse storage conditions of temperature and RH over the extended period influenced the nutrient composition, functionality, and physical characteristics. Nutrient composition of flour of different varieties of dry pea when stored under diverse storage conditions of RH and temperature over 270 days were evaluated. The moisture and protein content of pea flour was observed to be higher with increasing days of storage and HRH. Whereas starch content of pea flour decreased significantly due to the accelerated storage conditions. Ash content in the pea flour was slightly higher after 270 days of storage than compared to control samples. Similarly, higher fat content in flour of dry peas stored at HRH over 270 days was observed. This outcome suggested that the long-term storage of dry peas at 40 °C (HT) and diverse RH condition of 40%, 65% and 75% (i.e., LRH, MRH and HRH) can alter the nutrient composition of flour, with starch being most affected.

Functional properties of dry pea flour were influenced by the long-term storage of dry peas under diverse storage conditions of temperature and RH, regardless of the varieties. Oil absorption capacities, one of the important functional properties of flour that influences the mouthfeel, flavor, texture, and yield of the final product, increased

with increasing days of storage as the higher OAC. Similarly, FC of flours was higher for peas stored over 270 days; however, the FS of the flour decreased. Water based functionalities such as WAI and WSI was impacted due to the HT and HRH storage conditions. Minimal effects due to the storage was observed in WHC, EA and ES of the pea flour.

Starch functionalities were influenced by the long-term storage of different varieties of dry peas in the accelerated conditions of temperature and RH. The HT and HRH conditions of storage were the harshest based on having the most significant impact on starch properties. In general, FV and SV decreased with the diverse storage conditions, whereas PV and hot paste viscosity increased. The weaker gels of the starch were formed after the 270 days of storage at HT and HRH resulting in a very low gel firmness value. Although, this change could serve as an approach to modify starch functionality, the observations suggested that dry peas should be stored at RT and LRH conditions for safe and long-term storage.

Different varieties of dry peas have different physical properties; however, when they were stored under the similar storage conditions, they were affected in similar way due to the long-term storage at accelerated temperature and RH. Dry peas stored at 40 °C (HT) and 75% (HRH) resulted in the decreased swelling and hydration capacity that will ultimately lead to hard to cook defect in the dry pea seeds. Due to this, dry peas stored at 40 °C (HT) and 75% (HRH) were not cooked after 90 days of storage. The storage of dry peas in the accelerated temperature and RH caused bleaching and browning effect in the dry pea seeds that can impact the visual quality, which is a major contributor to decrease market value of dry peas. Higher RH storage led to peas with higher moisture contents

and 1000 kernel seed weight of dry peas. Minimal effect was observed in the seed weight due to the temperature and days of storage; however, varieties differed in the 1000 kernel weight due to the size of the seeds. Physical properties of dry peas of different varieties were highly influenced by the diverse storage condition.

In summary, extended period of storage at 75% (HRH) and 40 °C (HT) were observed to be the harshest storage conditions for dry peas, with non-significant variety impact for most functional properties. These findings could be of interest to the plant-based food industries looking for the alternatives of meat products as well as to replace other flour with high gluten content. In addition, dry pea growers and handlers also benefit with the knowledge of varietal behavior of dry peas. Furthermore, food industries processing pea into flour benefit because the proper storage can facility uniformity in pea ingredients since the 21 °C (RT) and LRH (40%) did not cause significant changes over extended storage. The outcome of this research provides proper storage guidelines for the dry peas to maintain the quality and enhance their value as productiveness.

6. FUTURE WORK RECOMMENDATION

Compositional, functional, and physical changes occurred in dry peas due to adverse storage conditions suggests that storage might affect phytochemical composition of peas. So, impact of the storage conditions on the phytochemical composition and bioactive compounds of different varieties of dry peas needs to be further elucidated. Changes in the starch and protein structure/composition contributing functionality changes are needs to be studied. Color difference in the pea flour and its impact while incorporating in the food product can be studied to see how color changes impact final product. Furthermore, HTC phenomenon in the dry peas needs to be studied in detail that would provide better understanding of the development of the defect and help in search for appropriate methods to prevent it.

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Appendix

Table 1: Cook Firmness (N/g) of the dry peas stored under diverse storage conditions

Storage Conditions (Days-Temp-RH)	Agassiz	Salamanca	Arcadia	Ginny	Keystone	Vail
Control	26.41	26.56	30.50	21.47	17.07	22.87
90-RT-LRH	27.00	30.01	32.12	22.75	15.35	21.36
180- RT-LRH	37.38	40.02	40.03	43.67	25.70	23.93
270- RT-LRH	29.26	23.53	30.89	25.95	12.95	24.08
90-RT-MRH	24.56	28.43	28.37	20.24	15.21	20.64
180- RT-MRH	41.72	42.92	47.78	40.75	32.74	22.73
270- RT-MRH	34.49	28.97	30.89	30.76	17.71	24.56
90-RT-HRH	26.75	27.45	34.73	24.56	20.15	28.98
180- RT-HRH	27.62	28.45	31.57	24.56	19.60	25.33
270- RT-HRH	38.39	32.03	38.02	40.10*	17.84	29.40
90-HT-LRH	37.18	44.42	39.48	28.77	16.04	25.89
180- HT-LRH	37.38	38.48	48.64	31.42	21.65	27.30
270- HT-LRH	NR	NR	NR	NR	25.42	26.68*
90-HT-MRH	44.94	39.10	42.34	39.94	26.87	45.96
180- HT-MRH	NR	NR	NR	NR	52.26	NR
270- HT-MRH	NR	NR	NR	NR	NR	NR
90-HT-HRH	49.40*	NR	45.57	42.14*	26.86*	NR
180- HT-HRH	NR	NR	NR	NR	NR	NR
270- HT-HRH	NR	NR	NR	NR	NR	NR

Note: NR means no reading was taken as the samples exceeded lower limit of Texture Analyzer.

* Means reading of only one of the replications of the same variety and treatment conditions was taken. LRH-Low Relative Humidity (40%), MRH- Medium Relative Humidity (65%), HRH- High Relative Humidity (75%)
RT-Room temperature (21 °C), HT-High temperature (40 °C)

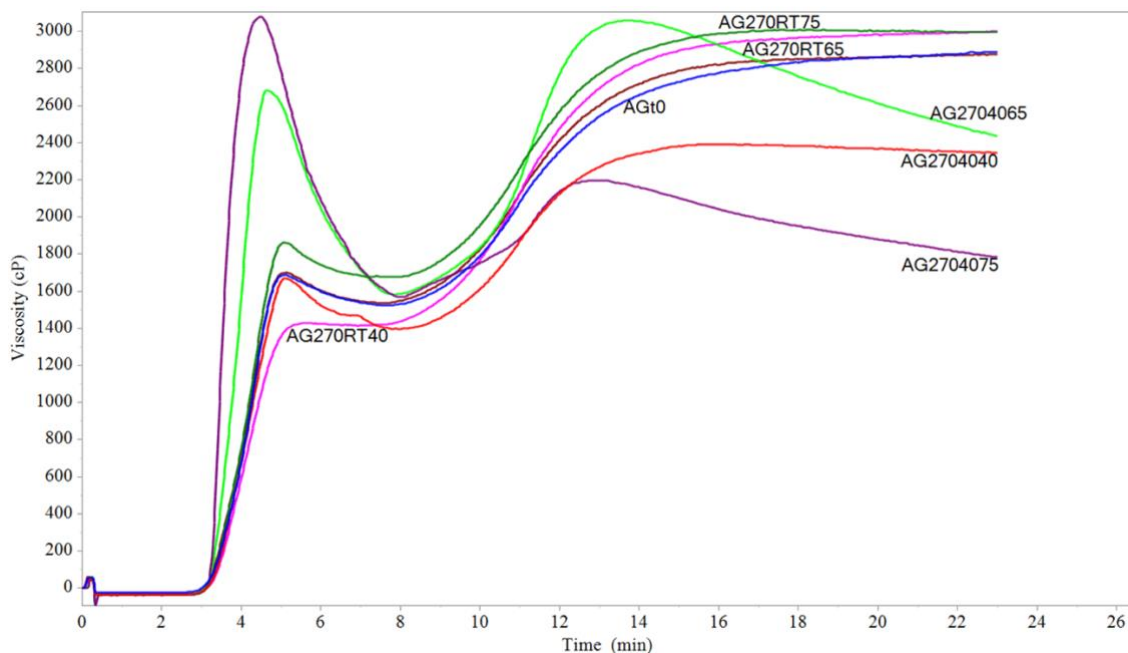


Figure 1: Rapid visco analyzer (RVA) plot of flour from Agassiz variety, time 0 and 270 days at 21 °C and 40 °C under 40%, 65% and 75% RH.

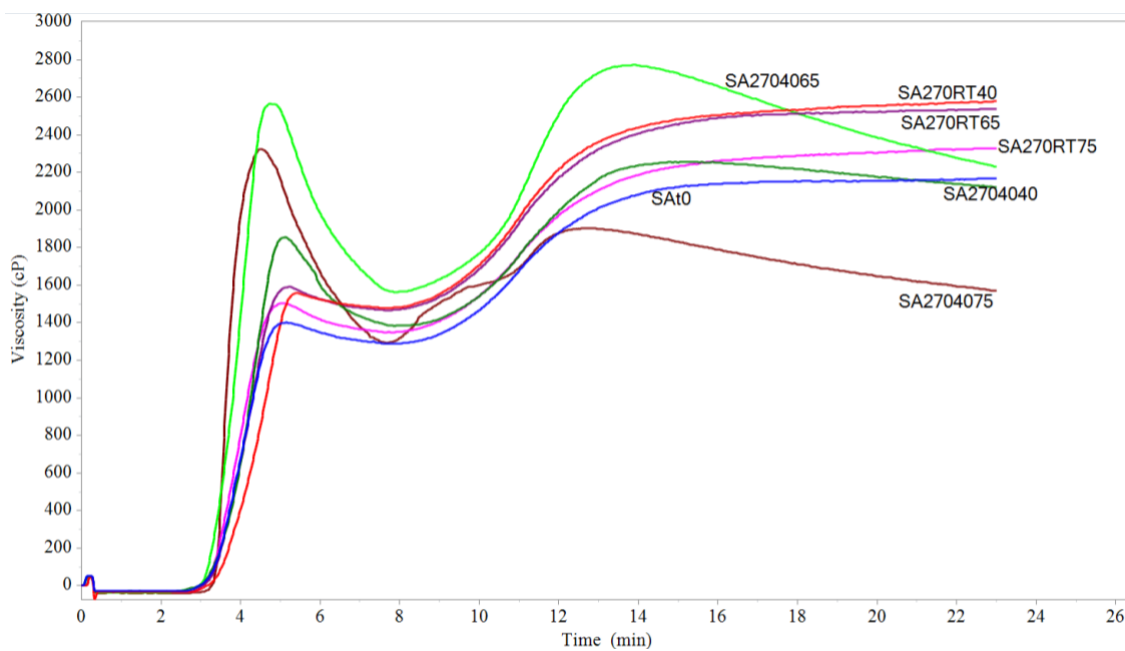


Figure 2: Rapid visco analyzer (RVA) plot of flour from Salamanca variety, time 0 and 270 days at 21 °C and 40 °C under 40%, 65% and 75% RH.

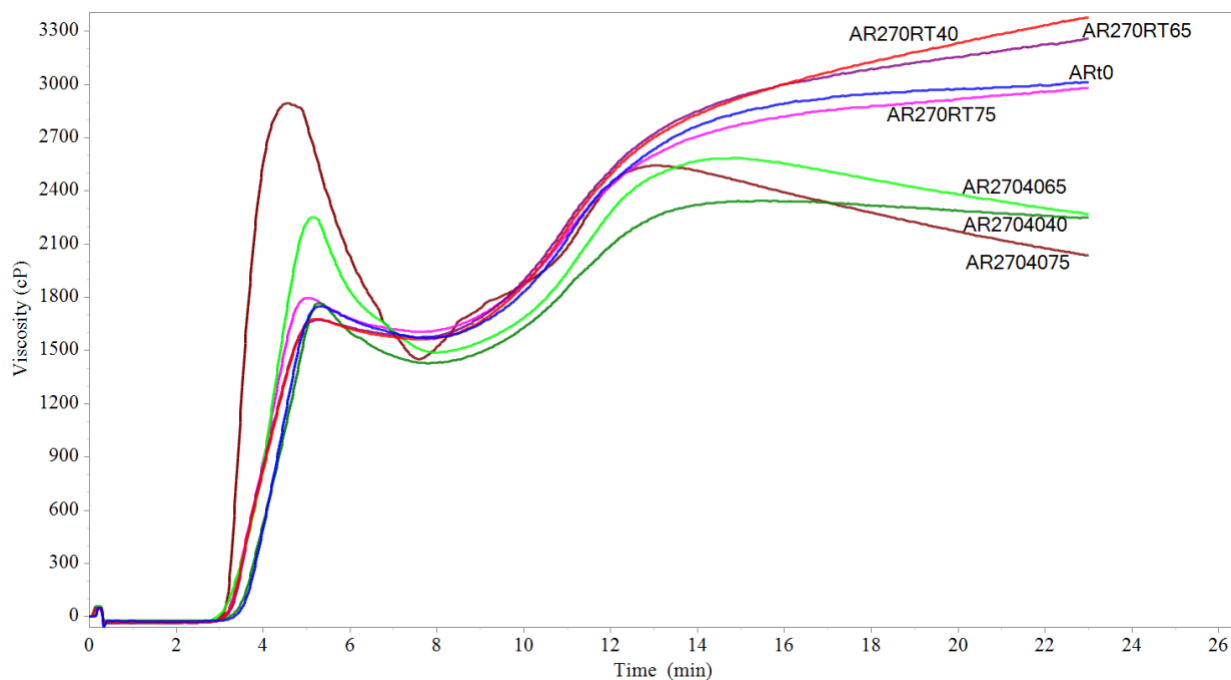


Figure 3: Rapid visco analyzer (RVA) plot of flour from Arcadia variety, time 0 and 270 days at 21 °C and 40 °C under 40%, 65% and 75% RH.

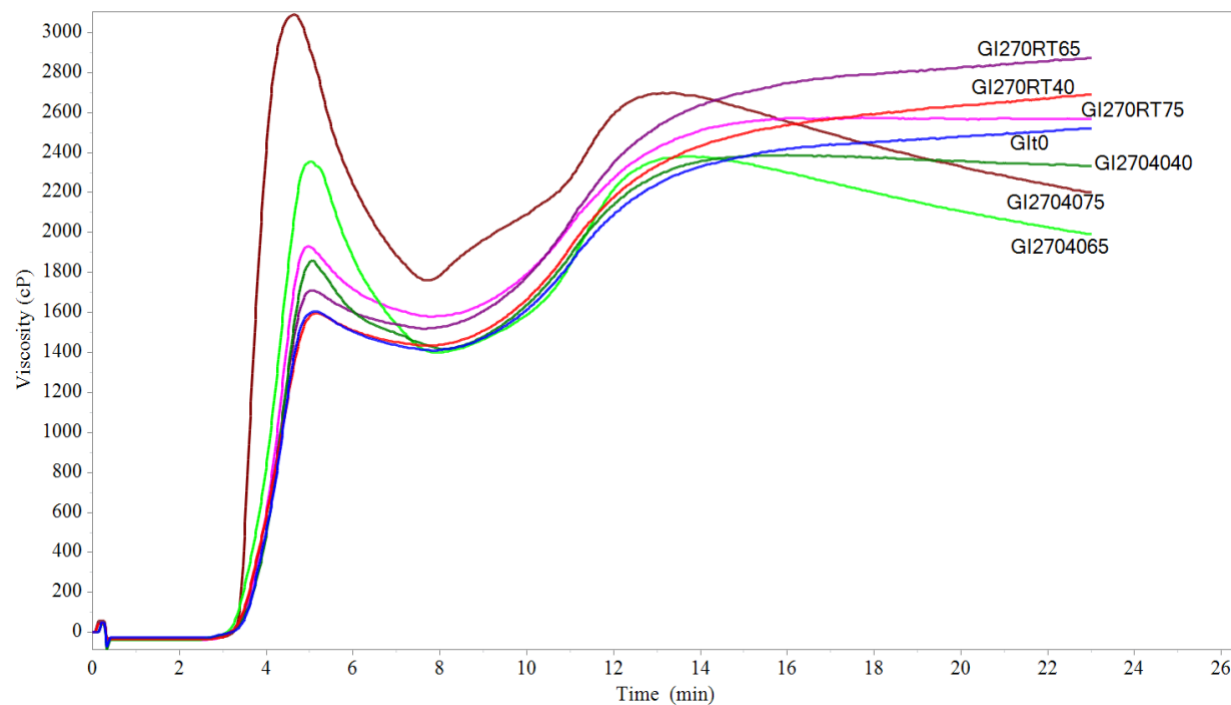


Figure 4: Rapid visco analyzer (RVA) plot of flour from Ginny variety, time 0 and 270 days at 21 °C and 40 °C under 40%, 65% and 75% RH.

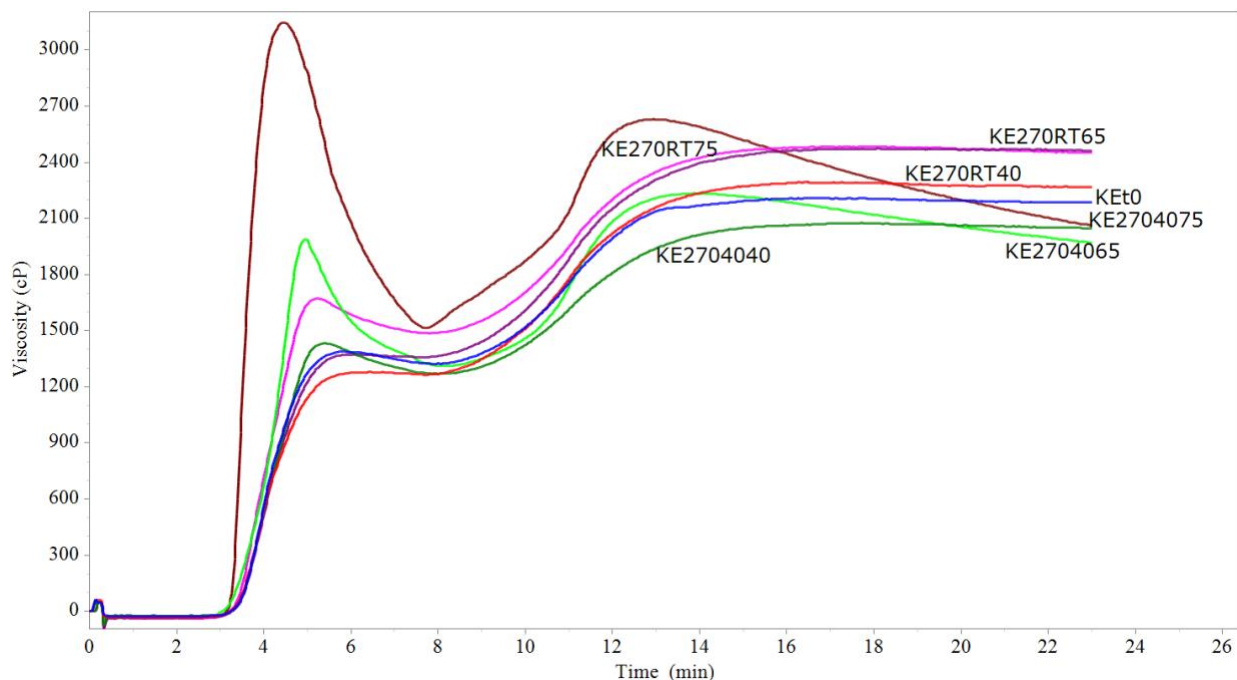


Figure 5: Rapid visco analyzer (RVA) plot of flour from Keystone variety, time 0 and 270 days at 21 °C and 40 °C under 40%, 65% and 75% RH.

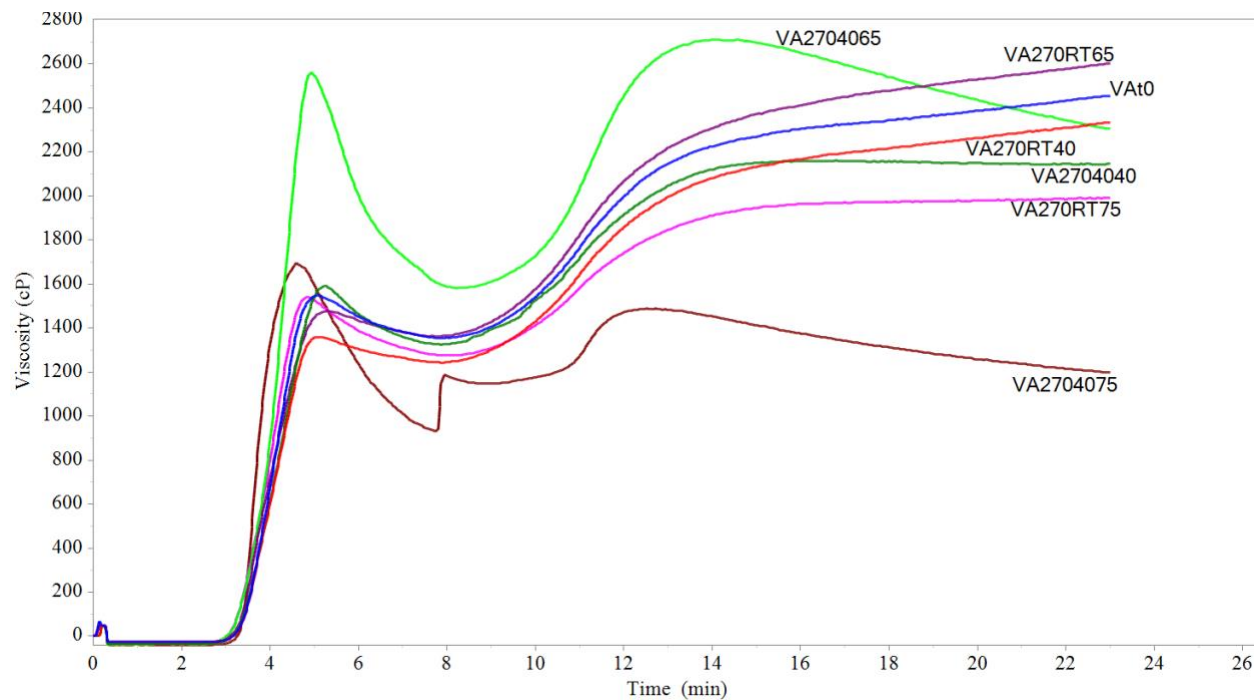


Figure 6: Rapid visco analyzer (RVA) plot of flour from Vail variety, time 0 and 270 days at 21 °C and 40 °C under 40%, 65% and 75% RH.