

Investigating dough rheology and texture properties of bakery products containing soy or pulse flours

by

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Abstract

Soybean and pulse flours are excellent source of various macro- and micro-nutrients, such as proteins, dietary fibers, minerals, and bioactive compounds associated with health benefits. They are becoming increasingly important to the food industry as product formulators and consumers are seeking nutritionally attractive ingredients. Fortification of cereal foods with pulse or soybean flour represents a promising approach to enhance their nutritional value, health benefits, and flavor profiles. However, the lack of gluten protein in these flours results in undesirable dough rheological properties and baked products with poor texture and other quality defects. This thesis is comprised of two studies that aimed to investigate and understand the effect of incorporating soybean or pulse flours on the dough properties and bakery product quality.

In the first study, we investigated the effect of formulation (e.g., shortening, sugar, and water) and pretreatment of soy flour on dough properties and cookie texture in a gluten-free (GF) system. Cookies were formulated with composite flours containing commercial GF flour and original or pretreated soy flour (70/30). Reducing agents (cysteine, glutathione, and sodium sulfite) and protease (Flavourzyme) were used for soy flour pretreatment, respectively. The modified proteins were characterized by analyzing contents of free sulfhydryl and free amino group and SDS-PAGE profiles. Dough properties were measured using mixograph and texture analyzer. Cookie quality was determined by analyzing spread ratio, texture, color, and moisture content. Results showed that adding relatively higher amount of sugar, shortening, and water greatly increased cookie spread and improved cookie cracking pattern, though it also increased the stickiness of the dough. Pretreatment of soy flour with reducing agent or protease reduced protein molecular size, and thus, weakened the GF dough, which also led to increased cookie spread and better cracking pattern.

In the second study, we investigated the effect of different types (yellow pea, green pea, red lentil, and chickpea) and amounts (0-25%) of whole pulse flours on whole wheat flour-based dough properties and bread quality. Pulse grains were ground using a Wiley mill to obtain whole pulse flour. Mixolab was used to analyze mixing and pasting properties of the whole wheat/pulse composite flours, and bread was characterized. Results showed that increasing the amount of pulse flour in the composite significantly weakened the dough and decreased dough viscosity due to a dilution of gluten proteins. Bread specific volume decreased, and hardness increased with higher amount of pulse flours. Among all the tested pulse flours, the composite flour containing yellow pea flour had overall better baking performances.

This research demonstrated the potential of utilizing soybean and pulse flours for bakery applications. The knowledge will be useful for the industry in developing new gluten-free or fortified bakery products.

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Chapter 1 - Soy applications in bakery products: a review

Abstracts

Soybean protein is considered as a complete protein source. There has been a growing interest in formulating soy in bakery products to improve food nutritional values. Different types of soy products are used to improve the quality and nutritional value of bakery goods, such as soy flour, soy protein, soy lecithin, soy milk, and okara. Approximately 0.5-1.0% of enzyme-active soy flour is recommended to add in wheat dough formulation as a whitening agent. Adding soy products increases water absorption of the composite flour and viscosity of batter and dough. Incorporating relatively lower amount of soy flour improves crumb cell distribution and uniformity of wheat bread. Adding soy protein improves cake batter consistency, which helps incorporate more air during batter mixing and retain air bubbles during baking, resulting in better cake quality. Wheat cookies with soy protein isolate have better properties in color, crispness, and flavor compared to that with soy flour or soy protein concentrate. Soy has more profound effects on gluten-free products than on wheat-based bakery products. There has been a strong demand for gluten-free products, which tend to have lower nutritional value, especially lower protein content. Adding soy products help build a protein viscoelastic network and can improve the quality and nutrition of gluten-free bread. The addition of soy products in gluten-free cookie formulation decreases hardness of the cookie dough and spread ratio of cookies. Further research is needed to modify soy products and enhance their performance for bakery applications.

Keywords: Bakery products; gluten-free; soy flour; bread; cookie

1.1 Introduction

Different types of protein are added into bakery products to improve the quality, palatability, and nutritional value. Soybean is rich in protein, and soy protein possesses high nutritional value and some desirable functional properties. Consumption of soy products may reduce the risk of cardiovascular diseases (Food and Drug Administration, 1999; Wang and Johnson, 2001). Different forms of soy products have been used to improve bakery product quality, such as soy flour, soy protein, soy lecithin, soymilk, and okara. Soy protein mainly consists of 7S globulin (β -conglycinin) and 11S globulin (glycinin) (Petrucelli and Añón, 1994). Okara is a byproduct generated during soy milk and tofu processing, presenting yellowish-white color with a neutral, smooth flavor (Park *et al.*, 2015). Okara contains approximately 34.6% protein and 20.4% fiber (Campderrós, 2017). It has high nutritional values, good water holding properties, and emulsifying characteristics, and is commonly added in snack foods (Katayama and Wilson, 2008).

Soy protein has several advantages for food uses, such as good digestibility, flavor retention ability, and functional and health benefits (Singh *et al.*, 2008) (Tömösközi *et al.*, 2001). The protein content in different soy products (e.g., defatted flour, protein concentrate, protein isolate) can range from 50 to 90%. Soy protein contains all the nine essential amino acids with a high amount of lysine, and it can be comparable to egg protein (Gorissen *et al.*, 2018). Texturized soy products can be used as meat substitutes with a lower cost to realize economic efficiency (Wolf, 1970). For vegetarians, soy products provide essential minerals and vitamins such as zinc, iodine, calcium, vitamin D, and vitamin B-12 in the diet, and also may help reduce the risk of chronic disease (Craig and Mangels, 2009). In 1999, FDA authorized the claim that soy (6.25 g soy protein per serving) may reduce the risk of heart disease (Food and Drug

Administration, 1999). In 2017, the Agency reevaluated the soy protein health claim, called off the previous health claim, and finally revoked it due to a lack of significant evidence. However, using soy products for food still remains attractive to the food industry. Genistein, daidzein, and glycitein in soy isoflavones are reported to decrease the risk of cardiovascular disease, improve bone health, lower menopausal symptoms, and restrict blood sugar (Marcus, 2013).

Soy proteins have good gelling, emulsification, water-holding, and oil-holding ability. Protein functional properties influence food processing, storage, preparation, and consumption (Malhotra and Coupland, 2004). Protein solubility is commonly investigated to evaluate protein denaturation, aggregation, and modification, and it is related to the amino acid composition, sequence, and molecular weight (Zayas and Zayas, 1997). Protein gel formation can be affected by protein denaturation, dissociation-association, and aggregation, and both glycinin (stable structure) and conglycinin (irregular structure) in soy protein can benefit the formation of gel structure (Hermansson, 1986). Soy protein was reported to be less efficient compared to whey protein isolate and casein in stabilizing corn oil-in-water emulsions measured through lipid hydroperoxide and headspace hexanal (Hu *et al.*, 2003). Compared with unheated soy protein emulsions, heating improved protein-protein interaction and stabilized oil-in-water emulsions (Keerati-u-rai and Corredig, 2009). Soy protein presents higher viscosity properties than milk and lupine gels due to higher solid content, self-aggregation of soy globulins, and the intermolecular hydrophobic interactions between protein-protein and starch-protein (Lim *et al.*, 2006; Ribotta *et al.*, 2007). Soy protein gels have a more condensable continuous phase than starch gels (Ribotta *et al.*, 2007).

Soy protein can be modified to improve their functional performances through thermal, chemical, and enzymatic treatments. Heat treatment weakens protease inhibitor activity, disposes

lipoxygenase and volatile compounds, and enhances some specific functional properties (Barac *et al.*, 2004). Thermal denaturation improves emulsification and foaming properties of soy protein through increasing protein surface hydrophobicity and decreasing surface tension (Morr, 1990). Chemical modification is aimed to reduce protease activity and phytic acid content, decrease or enhance protein solubility, enhance nutritional value, and reduce undesirable odor and flavor (Barac *et al.*, 2004). Enzymes such as proteases are used to hydrolyze peptide bond and reduce protein molecular weight, and employing oxidases can eliminate aldehydes through irreversible oxidization. Also, enzymatic modification can remove undesirable flavor, increase nutritional value, and improve functional properties (Barac *et al.*, 2004).

In bakery products, inclusion of soy can decrease amylopectin recrystallization, which improves the shelf life of bakery products; however, it reduces bread volume and cookie spread, darkens the color, leads to dense texture and chewy mouthfeel, and generates undesirable flavor (Vodovotz and Ballard, 2009). Enzyme-active soy flour, which includes lipoxygenase enzyme activity, is mainly used as a whitener in wheat-flour dough. It can also improve rheological properties, increase nutritional value, and lead to better bread (Riaz, 1999). Defatted soy flour increases water holding capacity, improves moisture retention and machinability, and enhances dough strength (Riaz, 1999; Gayas *et al.*, 2012). Degree of thermal processing determines the flavor of soy bread, and emulsifier can be added to improve loaf volume and crumb texture (Council, 1987; Ribotta *et al.*, 2010). Besides, soy protein concentrates and soy protein isolates are added as milk replacement with a lower cost, which further affects color, flavor, and texture of the product (Council, 1987; Riaz, 1999). Lecithinated soy protein product, which possesses various phospholipids, functions as an emulsifier in bakery products to improve volume,

structure, and texture. Usually, lecithinated flour contains 15% lecithin content, which is much higher than that in regular soy flour.

Gluten-free bakery products serve the people who have coeliac disease or gluten-sensitive enteropathy (Ribotta *et al.*, 2004). Due to low level of protein content and lack of gluten in gluten-free flour, gluten-free bakery products always present low quality and less desired mouthfeel. Soy contains high protein, and it is commonly added into gluten-free bakery products to improve protein content, structure, and texture of the products. This chapter reviews the application of different soy products in bakery foods and their effect on wheat and gluten-free bread, cookies, and cakes dough/batter properties and their quality.

1.2 Soy application in wheat bread

1.2.1 Enzyme-active soy flour

Lipoxygenase presents in several legumes, while in North America, soy flour has been a major source. The lipoxygenase in soy flour has stronger enzyme activity than in other sources (Chang and McCurdy, 1985). Approximately 0.5-1.0% of enzyme-active soy flours based on wheat flour amount, is suggested to add in the wheat dough formulation to avoid off-flavors at a higher amount (Gélinas *et al.*, 1998) (Wolf, 1975). Lipoxygenase from soybean can act on polyunsaturated fatty acids in triglycerides, while wheat lipoxygenase is only active on free fatty acids (Ahmad and Ahmed, 2014). There are at least three types of lipoxygenase isoenzymes in enzyme-active soy flour (type 1, 2, and 3) with three optimal pH: 9.0, 6.5, and 6.0, respectively. Among these three types of isoenzymes, type-2 works as a good whitener in an aerobic situation, while type-1 works as an excellent whitener in an anaerobic situation (Luning *et al.*, 1991).

Cumbee *et al.* (1997) used 3% full-fat soy flour with three distinct lipoxygenase isozymes (L1, L2, L3) to determine their effect on wheat flour dough rheological and bread quality.

Enzyme-active soy flour increased dough tenacity and strength but decreased dough extensibility. In addition, it increased bread volume and decreased crumb firmness. Frazier *et al.* (1973) demonstrated that soy lipoxygenase action could provide more rapid oxidation of the gluten during the mixing process, and the oxidation of polyunsaturated fatty acids by lipoxygenase could promote protein oxidation and enhance the rheological properties of dough.

The use of enzyme-active soy flour to whiten wheat bread due to the oxidation of unsaturated fatty acids by lipoxygenase-mediated also reduces the amount of anti-nutritional compounds (e.g., phytate and trypsin inhibitor) in wheat and soy flour mixtures (Weber and Grosch, 1976; Khan *et al.*, 2012). Enzyme lipoxygenase possesses a bleaching effect on flour pigments, which involves several complex reactions with lipids and atmospheric oxygen. Also, it benefits the oxidation of the flour proteins in the same way (Ribotta *et al.*, 2004). Lipoxygenase catalyzes polyunsaturated fatty acid oxidation, produces hydroperoxides with intermediate free radicals that support co-oxidize lipophilic pigments and thiol groups (Lamsal and Faubion, 2009). Recently, several studies used enzyme-active soy flour to control raw soy flour in bakery products to compare with modified soy flour, and the primary function was to improve dough properties (Maforimbo *et al.*, 2006, 2008).

1.2.2 Dough properties

Water absorption of soy flour is much higher than that of wheat flour, and the water absorption level is positively correlated with bread volume. Mashayekh *et al.* (2008) indicated that the amount of protein content in soy flour showed a positive correlation with water absorption and increased water-binding capacity. The degree of protein denaturation affected the water absorption of wheat dough containing soybean products (Katsuharu *et al.*, 1972). Fleming *et al.* (1974) compared water absorption and viscosity of soy flour, soy protein concentrates, and

soy protein isolates (Table 1). Soy isolate has a higher water absorption value and higher apparent viscosity than soy flour and soy protein concentrate. Overall, the value of water absorption and apparent viscosity increased with the protein content increasing due to the higher hydration capacity of the protein, higher solid content, and stronger self-aggregation of soy globulins which cause the dough to become more viscous (Lim *et al.*, 2006).

In wheat bread dough, starch-protein and protein-protein interactions contribute to the rheological properties (Lim *et al.*, 2006). Adding a high amount of soy flour or soy protein negatively affects the dough rheological properties and bread quality. Rocchia *et al.* (2009) investigated the interaction between gluten and soy protein and found that soy protein isolates significantly decreased maximum resistance, extensibility, retarded compliance, and syneresis value, but increased Newtonian viscosity value. Although soy protein can improve the viscosity properties of gluten, soy protein builds a weaker network with gluten and produces a more porous network of wheat dough due to the globular interfere. Adding soy protein in wheat dough increases free sulfhydryl concentration; thus, the globular in soy protein interacts with gluten fibril, dilutes the original gluten concentration, and blocks the continuous gluten network. Texturization improves soy protein functionality in the wheat dough since it improves noncovalent interactions. However, Ryan *et al.* (2002) found that the sulfhydryl groups of soy proteins may not result in the deleterious effects on wheat bread and even can enhance the SS-SH interchange to improve dough development.

Ribotta *et al.* (2007) found that soy protein increased peak viscosity, hot peak viscosity, cold paste viscosity, breakdown, and setback of wheat starch gel. Since soy protein consists of many hydrophilic groups that could interact with starch to form crosslinks in the protein-starch gels, and this interaction affects the adhesion system and amylose aggregation. They also used

scanning electron microscopy to observe the structure of starch gels, which was smoother than the protein-starch gels due to the more condense structure caused by the attachment of globular proteins to the granule surface and continuous phase. Besides, Ribotta *et al.* (2005) indicated that soy flour and soy protein increased wheat dough water absorption. Soy flour extended dough develop time, increased dough stability, decreased softening degree value, increased maximum resistance, and decreased extensibility value. Soy protein and soy flour both interfere with the wheat dough gas retention and provide a negative influence on the dough fermentation and baking process. The wheat-soy dough is weaker compared to wheat dough, since soy flour decreases the free water level and competes with gluten for available water (Ribotta *et al.*, 2010).

Vittadini and Vodovotz (2003) reported that the amylopectin recrystallization was significantly decreased with soy flour, which was caused by the less water available for the starch component in the formulation. Maforimbo *et al.* (2006) found that soy flour modified through moist heat treatment provides more higher molecular-weight proteins and improves dough formation. The wheat flour and soy flour were mixed in 1:1 ratio. Compared with the wheat dough, the inclusion of non-modified soy flour presented significantly lower maximum resistance to extension and stability value, while with addition of modified soy flour, better dough properties were achieved. The modification affects protein secondary and tertiary structure, causes more disulfide bonding formation, and promotes soy protein aggregation into larger polymers. Dough properties of wheat bread with different types of modified soy flour and/or with food additives are summarized in Table 2.

Compared with soy flour and soy protein, okara flour contains lower amount of protein. Campderrós (2017) combined wheat flour with three different levels of okara flour (5, 7.5, and

10%) to produce wheat bread with increased protein and fiber contents and found that the dough could retain more moisture during baking process due to the hygroscopic nature of okara.

1.2.3 Bread quality

Wheat bread volume significantly decreased by adding soy flour, but the loaf volume can be improved using modified soy flour. It has been reported that increasing the amount of soy flour or defatted soy flour decreased bread volume and specific volume, but increased the bread moisture content (Islam *et al.*, 2007; Alam *et al.*, 2013). In a study by Ndife *et al.* (2011), four levels of soy flour (10, 20, 30, and 40%) were tested to evaluate the effect on wheat bread baking properties, nutritional value, quality, and sensory. Bread volume decreased with soy level increased, and the overall acceptability of bread also decreased with the soy level increased due to the soy flavor and dense structure.

Ivanovski *et al.* (2012) evaluated soy-wheat bread prepared from different blends of soy flour and soy protein isolates. The physical attributes and sensory qualities of cookies were compared with wheat flour cookies. Wheat bread and soy flour-wheat bread showed similar firmness, denseness, and stickiness to teeth properties, while wheat bread with soy protein showed significantly larger firmness and denseness than the control wheat bread. In addition, soy-wheat bread presented obvious beany aromas and bitter tastes. Astringent phenolic acids present in soybean also lead to the bitter taste of soy-wheat bread. Compared with soy flour, soy protein contains more daidzein and genistein, affecting the astringent properties of bread. The study also indicated that using soy flour in wheat bread resulted in better performance than using soy protein isolate.

Islam *et al.* (2007) studied the effects of different levels of soy flour on wheat bread. Slightly increasing the amount of soy flour improved the air cell size and uniformity of bread,

which might be due to the high protein content of bread. The higher solid matter in soy flour increased the bread weight. In the sensory test, adding up to 10% soy flour did not affect the acceptable quality attributes.

Shao *et al.* (2009) compared the isoflavones in soy flour-wheat bread and soy protein-wheat bread. Baking of soy flour and soy protein did not destroy the isoflavones but changed the profiles of individual isoflavones under the interconversions of malonyl conjugates. Also, moist heat and dry heat generated different conjugates. Moist heat increased the amount of β -glucoside conjugates, while dry heat generated more acetyl conjugates. Bread properties of different types of modified soy flour with or without food additives are summarized in Table 2.

The specific volume of bread decreased with the amount of okara flour increased, which was caused by the structure weakening and gas retention reducing of the dough (Campderrós, 2017). Okara wheat bread contains about 12.7% of insoluble dietary fiber. Campderrós (2017) also found that okara flour increased bread firmness and cohesion due to the dilution of gluten and disulfide transaction between gluten protein and soy protein. A combination of saccharides and proteins could also improve the bread hardness.

1.3 Soy application in wheat cakes

1.3.1 Batter properties

Soy products are sometimes added in cake formulation to partially replace milk, egg white, or whole egg ingredients. Starch gelatinization, protein coagulation, production of carbon dioxide gas, air incorporation, and ingredients interactions play important roles in cake texture, structure, and volume (Sahin, 2008). Majzoobi *et al.* (2014) investigated the addition of six different levels of soy protein isolates (0, 5, 10, 15, 20, and 30%) in sponge cake formulation. Soy protein isolates decreased Bostwick number and batter density, which is negatively

correlated with batter consistency. Insufficient batter consistency cannot trap the air formed during mixing and the CO₂ produced by sodium bicarbonate during the baking process. Increasing soy protein isolate level increased the batter consistency. Milad *et al.* (2014) found that adding soy flour also resulted in reduced cake volume.

Rahmati and Mazaheri Tehrani (2015) prepared soy milk using full-fat soy flour mixed with hot water (ratio 1:3). The inclusion of soy milk increased batter viscosity and stability since the soy milk can help stabilize the oil-in-water emulsion. Batter density increased with soymilk level increasing, and soymilk improved the retention of air that was incorporated into the batter. Besides, Rahmati and Mazaheri Tehrani (2014) stated that the high water absorption capacity of protein in soy milk reduced the available free water in the batter, increased batter viscosity, and improved the air holding capacity during the high-speed mixing and whipping process. Several modifications and additives were added into the formulation of soy-wheat cake to improve batter properties (Table 3).

1.3.2 Cake properties

Air incorporation in the cake batter system during the mixing process plays an important role in cake volume. Majzoobi *et al.* (2014) found that soy protein isolates increased cake volume, cake height, and gumminess, and decreased cake density, cohesiveness, springiness, and chewiness because the higher amount of protein could retain more air bubble during mixing and the CO₂ produced by sodium bicarbonate. Soy protein can stabilize the films around the air bubble and prevent bubble collapse during baking process. In addition, soy protein can increase starch gelatinization temperature, increase enthalpy of gelatinization, and afford more expansion of the batter during baking. The overall acceptability of cake was improved with moderate soy level but significantly decreased with higher soy level.

Rahmati and Mazaheri Tehrani (2015) reported that using soymilk increased cake density due to a higher water absorption of soy protein. A high level of soymilk even decreased cake volume since high water absorption of the soy reduced the available water in the cake batter, decreased the amount of steam, and reduced the number of air cells formed in cake crumb. Also, soymilk resulted in lighter color for the cake crumbs and had adverse effect on brown intensity of crust due to the enzyme-active properties of soy flour that the lipoxygenase enzyme caused decomposition of xanthophylls in wheat flour and reduce the b^* value (Milad *et al.*, 2014). Rahmati and Mazaheri Tehrani (2014) replaced egg in the cake formulation with soymilk. They found that batters with too low or too high viscosity had a negative effect during the baking process. The less viscous batter cannot trap the air bubble, but the highly viscous batter limits batter expansion during baking and also produces lower volume of cake. Milad *et al.* (2014) used soy flour and guar gum as an egg replacement in cake. Using soy flour or guar gum decreased cake moisture content. Increasing the amount of soy flour level decreased the volume and firmness of the cake. Studies related to soy applications in cake production are summarized in Table 3.

Wang *et al.* (2014) prepared cake with wheat flour, and okara was used to replace part of the wheat flour at 0, 5, 10, 15, or 20%. Okara flour did not change the color significantly due to the higher fiber content in okara compared to protein content, while only protein can participate in Maillard or caramelization reactions. Besides, up to 10% of okara flour was acceptable for consumers based on sensory tests. Adding more than 10% of okara flour significantly decreased overall acceptability, color, flavor, and taste ratings of the cake.

1.4 Soy application in wheat cookies

Cookie is one of the largest categories of snack food in the U.S. due to a wide acceptability, convenience, and long shelf-life. Cookies containing soy protein isolate have better properties in color, crispness, and flavor than the cookies with soy flour or soy concentrate (James *et al.*, 1989). It was also reported that up to 15% of wheat flour could be replaced by soy flour to achieve acceptable cookie quality. Pareyt and Delcour (2008) indicated that the proportion of protein and starch in a cookie formulation influenced the water absorption capacity, dough rheology, and spread ratio of wheat flour cookies. Soft wheat flour provides a higher spread ratio and cookie set time during baking process (Mancebo *et al.*, 2016).

1.4.1 Dough properties

In the study by Singh and Mohamed (2007), three different ratios of gluten to soy protein isolates (70:30, 50:50, and 30:70) were used to replace part of the wheat flour (0, 10, 15, 20, 25, and 30%) to produce sugar-snap cookies. Higher amount of soy protein isolate decreased dough stability, reduced mixing tolerance, and increased the water absorption of cookie dough. The stronger water absorption of soy in cookie dough decreased water availability for wheat flour and resulted in a dryer dough. Lee and Brennand (2005) added soy protein isolates at different ratios (5, 10, and 15%) in fried cookie dough. Soy protein isolates significantly increased dough consistency, while decreased the dehydration and oil absorption of the dough. Rababah *et al.* (2006) prepared wheat cookies with three different levels of isolated soy protein (3, 6, and 9%). Compared with wheat flour, soy protein isolate had lower moisture content and significantly higher protein content. The addition of soy protein isolate increased the protein content and decreased the moisture content of cookie dough.

Ndife *et al.* (2014) investigated the nutrition value and quality of cookies blended with four levels of roasted soy flour (0, 20, 30, and 50%). With the amount of soy flour increasing, the water absorption capacity of the composite flour increased, while the oil absorption capacity decreased, implying that soy flour can absorb more water. The addition of soy flour increases the protein content and improved essential amino acid balance of the cookies (Akubor and Ukwuru, 2003; Banureka and Mahendran, 2011). Besides, for cookie dough pasting properties, inclusion of soy flour significantly decreased pasting viscosity, final viscosity, breakdown, and set back values, as well as increasing pasting temperature. Since the pasting properties are related to the starch content, especially amylose content, replacing part of the wheat flour decreased the starch content, and therefore, resulting in lower viscosity (Devi and Haripriya, 2014).

Okara flour has higher water absorption capacity and protein content than wheat flour. The replacement of wheat flour with okara flour resulted in dryer cookie dough, which restricted dough spreading during baking (AHMED *et al.*, 2018).

1.4.2 Cookies properties

With the addition of soy flour, soy protein concentrate, or soy protein isolate into a cookie formulation, cookie spread decreased dramatically, thickness of the cookie obviously increased, and results from sensory test showed that the color, texture, flavor, and overall acceptability were less accessibility compared to regular wheat cookies, which requires further improvement (Chen *et al.*, 2003).

Singh and Mohamed (2007) found that there was a negative correlation between protein content and diameter of the cookies. Addition of a high amount of soy protein isolate or soy flour significantly decreased cookie spread ratio, while low amount of soy did not significantly affect the spread (Rababah *et al.*, 2006; Zaker Md and TR, 2012; Devi and Haripriya, 2014; Kulthe *et*

al., 2014). For example, a study found that adding 3% of soy protein isolate improves the overall acceptability of wheat cookies, and the product is acceptable based on sensory test (Rababah *et al.*, 2006). Besides, increasing the amount of soy protein isolate led to darker color for the cookies. With a higher amount of soy protein isolate added into the dough, the hardness of cookie significantly increased while the cohesiveness and springiness values decreased. This is because of the higher water-holding and binding capacity of soy that resulted in the low spread ratio and hard texture. Cookies with soy flour have higher content of protein, crude fiber, and minerals due to the high content of these nutrients in soy flour than in wheat flour (Kulthe *et al.*, 2014). Up to 20% of soy flour could be added into cookies with an acceptable sensory score with respect to taste, color, and crispiness. The inclusion of defatted soy flour could also decrease the calorie value of cookies compares with wheat control cookies due to higher fiber content (Singh *et al.*, 2000; Kulthe *et al.*, 2014).

Ndife *et al.* (2014) modified soy flour through a roasting process. Cookie spread factor increased with the amount of roasted soy flour increased due to the differences in soy flour and wheat flour in the particle sizes and other features. Incorporation with roasted soy flour also increased food mineral content (Serrem *et al.*, 2011; Zaker Md and TR, 2012). In addition, blending with soy flour increased the lysine content in the cookies, improved the in vitro protein digestibility, and increased the protein digestibility corrected amino acid score (PDCAAS). Cookies with 30% roasted soy flour had the best overall acceptability among the different treatments (0, 10, 30, and 50% soy flour).

Adding okara flour not only improves the structure and texture but also increase the nutritional value of wheat cookies. Hawa *et al.* (2018) prepared wheat cookies with different ratios of okara, red teff, and wheat flours. Increasing the amount of okara flour significantly

increased the moisture, ash, protein, fat, and fiber content, but decreased the carbohydrate content of cookies. Wheat flour mixed with okaro provided higher amount of minerals such as zinc, and calcium. Grizotto *et al.* (2010) investigated the properties of wheat cookie with 30% of okara flour through physical, physical-chemical, and sensory tests. Adding 30% of okara flour slightly decreased diameter of the cookies due to the high protein content and high water absorption capacity of okara flour, which resulted in the cookie dough lack of expansion capacity and spread during baking. Based on sensory tests, the color, flavor, texture, and overall quality of cookies containing okara flour had significantly lower scores than the control wheat cookies. It still remains a challenge to utilize okara flour into wheat cookies. Related studies are summarized in Table 3.

1.5 Soy applications in gluten-free bread

Different types of gluten-free flour are used in gluten-free bakery products, such as sorghum flour, rice flour, corn starch, potato starch, buckwheat, tapioca starch, and others. Among these flours, rice flour is the most used. The optimal amount of soy flour differs depending on the types of base gluten-free flour. For example, 10% soy flour provided a larger volume than 20% soy in rice flour based gluten-free bread. However, in corn flour based gluten free bread, 20% soy flour performed better (Sciarini *et al.*, 2010). Many gluten-free bread products have low protein content and are deficient in some essential amino acids, with undesirable texture, volume, structure, and flavor. Soy products have high protein content and can be added into gluten-free products to improve the batter/dough functionality and product quality. Related studies are summarized in Table. 4.

1.5.1 Dough properties

Soy products can be added into gluten-free batter to help build a protein network that may help retain gas during fermentation or leavening. Since soy protein has higher water absorption than many gluten-free flours, adding soy protein increases dough development time and stability. Unlike wheat-based bread dough, soy protein decreases the viscosity (peak viscosity and final viscosity) of gluten-free batters, since the presence of soy protein absorbs more water and dilutes the starch. Protein-starch interaction affects starch pasting properties, in which protein works as an inactive filler among starch molecules (Wang *et al.*, 2002; Sciarini *et al.*, 2010). Soy protein significantly increased batter water binding capacity and decreased batter swelling power at 100 °C (Shin *et al.*, 2010).

Marcoa and Rosell (2008) investigated the gluten-free dough properties with different amount of protein isolate and transglutaminase added into rice flour. The presence of soy protein significantly decreased the batter peak and final viscosity, and there was a negative correlation between protein content and peak viscosity. Besides, the inclusion of soy protein significantly decreased elastic (G') and viscous (G'') modulus. The soy-wheat batter presented a more solid-like behavior since the elastic modulus was larger than the viscous modulus. Similar results were also reported by Marco and Rosell (2008a), and this might be due to the high water absorption and higher water-binding capacity of soy protein (Firdevs Dogan *et al.*, 2005; Marcoa and Rosell, 2008). Compared with pea protein, soy protein resulted in better viscoelastic properties of the batter (Ronda *et al.*, 2011; Ziobro *et al.*, 2013).

Sciarini *et al.* (2010) found that adding soy flour decreased starch gelatinization enthalpy, onset temperature, peak temperature, and final temperature, because soy protein has higher water

affinity, which resulted in less water in the dough available for starch gelatinization. Besides, increasing soy flour from 10% to 20% significantly improved batter consistency.

Lian *et al.* (2020) investigated the effect of adding soy and chickpea okara on the properties of gluten-free bread based on rice flour and corn starch blend. Soy okara contains about 3.41g/100g of starch and 50-57g/100g of insoluble fiber. Soy okara has high water absorption and oil absorption capacity than soy flour. The addition of soy okara increased peak viscosity, trough viscosity and final viscosity of gluten-free bread dough due to the greater swelling ability and lower water absorption capacity, resulting in thickening properties of gluten-free bread dough (Krishna Kumar *et al.*, 2019). Besides, protein and lipid content of okara affected the swelling power and pasting properties of starches (Mohammed *et al.*, 2014).

1.5.2 Bread properties

Miñarro *et al.* (2012) prepared gluten-free bread based on legume flour, as well as adding xanthan gum and emulsifier. The presence of soy flour slightly increased bread volume compared to chickpea flour and pea isolate. With the addition of xanthan gum and emulsifier, the bread showed improved crumb structure and staled faster compared to the bread without gum and emulsifier.

However, adding soy protein decreased the specific volume of gluten-free bread, increased hardness, resulted in a small number of large cells (Marco and Rosell, 2008b; Ziobro *et al.*, 2013). The amount of available low molecular weight polysaccharides in the bread formulation is important to the bread volume since it is necessary to act as substrates for yeast (Witczak *et al.*, 2010).

Ziobro *et al.* (2013) prepared gluten-free bread with corn and potato starch. Soy protein significantly reduced bread volume but improved the porosity character of the gluten-free bread.

The inclusion of soy protein significantly decreased the retrogradation enthalpy on the first day. With the storage time increasing, retrogradation enthalpy of amylopectin was still lower than the control bread, indicating that soy protein could provide both nutritional value and anti-staling factor for gluten-free bread. Although albumins are able to bind carbon dioxide and improve the dough structure during fermentation and baking, soybean on contains a small amount, and this would not significantly influence the bread volume. The addition of soy protein significantly reduced the overall acceptability due to the strong soybean flavor.

Soy flour provides better quality for gluten-free bread than soy protein since soy flour contains a variable amount of fat and fiber, improving the bread quality and palatability. Besides, moderate amount of soy flour is better in improving bread quality. Ribotta *et al.* (2004) investigated the effect of heated and unheated full-fat soybean flours on the gluten-free bread based on rice and cassava flour. The enzyme lipoxygenase in soy flour provided a bleaching effect on flour pigments and helped oxidate flour protein. Also, the amount of cold-water-soluble protein in soy flour determined its suitability and performance in gluten-free bread.

Soy okara gluten-free bread generally had high moisture content and low bread volume compared with gluten-free bread without okara. Lian *et al.* (2020) found that the addition of soy okara increased the bread hardness due to the higher insoluble fiber, and the protein content of okara resulted in a strong network of the bread dough. Soy isoflavone provides a yellow color for the bread and better appearance. While the overall acceptability of gluten-free bread and soy okara gluten-free bread was not good and is still a great challenge in the gluten-free area. Besides, the same results were also reported in Guimarães *et al.* (2019) that soy okara had a negative influence on bread volume, although it could improve the nutritional value such as protein content, dietary fiber, and isoflavones.

1.6 Soy applications in gluten-free cookies

Flour, sugar, and fat are the three main ingredients, along with water, to make cookies (Mancebo *et al.*, 2016). Generally, cookies are characterized by low water content with restricted development of gluten network (Pareyt and Delcour, 2008). Several different types of gluten-free flours were used to produce gluten-free cookies such as amaranth (Gambuś *et al.*, 2009; de la Barca *et al.*, 2010), buckwheat (Gambuś *et al.*, 2009; Altindag *et al.*, 2015), rice flour (Chung *et al.*, 2014; Altindag *et al.*, 2015; Sarabhai *et al.*, 2015; Akesowan, 2016), or the mixture of these flours (Dapčević Hadnadev *et al.*, 2013; Maghaydah *et al.*, 2013; Rai *et al.*, 2014; Gerzhova *et al.*, 2016; Mancebo *et al.*, 2016; Šarić *et al.*, 2019). Sugar-snap cookie is a representative cookie with high sugar, high fat, and low water levels. Cookies perform well with low protein content and weak gluten strength, such as soft wheat flour. Also, it has been reported that cookies with higher protein are more acceptable than cookies with higher starch content (Mancebo *et al.*, 2016).

1.6.1 Cookies dough properties

The addition of soy protein dramatically decreases the hardness and gumminess of the cookie dough compared with the control of rice flour cookie dough. Gluten-free cookie dough properties are related to the ingredients, and the cohesiveness and adhesiveness characteristic of dough are important since the dough must remain certain cohesion during handling and baking to ensure the best quality of the final product (Gujral *et al.*, 2003). Sarabhai *et al.* (2015) prepared gluten-free cookie dough based on rice flour and investigated the effects of adding three different levels of soy protein isolates (5, 7.5, and 10%) on cookie dough and cookies quality. Combining soy protein and emulsifier decreased dough hardness, and soy protein seems to balance some of function of gluten proteins and build the structure for gluten-free cookies.

Recently, okara is also added into gluten-free cookies to improve fiber and protein content. Park *et al.* (2015) prepared gluten-free cookies with a standardized method. Compared with okara-starch dough and okara-soy flour dough, okara soy dough had the highest moisture content and lowest water holding capacity. The hardness of dough showed a positive correlation with water holding capacity due to the increase in the cohesiveness of the starch granules in dough and increase in protein content. For the microstructure, the addition of soy and starch decreased the number of holes in the dough structure, while HPMC (hydroxypropyl methylcellulose) could reduce the size of holes in cookie dough, since the lower moisture content resulted in less and smaller holes. With the addition of okara, the moisture content, ash percentage, protein content, fat percentage, fiber content, and total carbohydrates were also increased (Ostermann-Porcel *et al.*, 2017).

1.6.2 Gluten-free cookies properties

Sarabhai *et al.* (2015) found that soy protein reduced the spread ratio and breaking strength of gluten-free cookie but improved cookie texture since soy protein has high water absorption capacity and resulted in less free water that were available to hydrate the rest of the solid ingredients. With 7.5% soy protein isolate added, the texture and structure of rice flour cookie were improved compared with the control cookie. Compared with wheat flour cookie, rice flour cookie without soy protein had much lower quality. Gluten-free cookies with higher soy protein content had a darker color due to the Maillard reaction, which is the reaction between proteins and reducing sugars. Cookies with 7.5% soy protein and 0.5% emulsifiers presented a better overall quality, which was only a little bit lower than the control wheat flour cookie.

Increasing the level of soy protein in cookie, the overall acceptability decreased, since excessive protein in the cookie formulation cause a harder dough texture, lower dough spread

ratio, and dryer mouthfeel (Aly and Seleem, 2015). A relatively lower amount of soy protein could improve the cookie quality (Rababah *et al.*, 2006).

Increasing the amount of soy okara content decreased the spread ratio significantly (Ostermann-Porcel *et al.* 2017). Ostermann-Porcel *et al.* (2017) also found that gluten-free cookies with soy okara were significantly darker due to the Maillard reaction between protein and sugar. Although soy okara improved nutritional value of cookie, the sensory attributes of okara were less desirable. Adding soy okara resulted in negative effects on color, aroma, taste, and overall acceptability of gluten-free cookies.

1.7 Conclusion

Overall, adding soy products in wheat-based or gluten-free bakery products can improve some rheological and texture properties of the dough/batter and end products. In wheat-based bakery products, adding soy products increases water absorption of the composite flour, generates new starch-protein/protein-protein interactions, and improve some rheological properties of the dough/batter. However, adding relatively higher amount of soy products negatively influences wheat dough/batter properties and product quality. Food formulation containing soy products needs to be carefully optimized in order to improve both the nutritional value and quality of the products. In gluten-free bakery products, soy helps to build protein network in bread dough and decrease the cohesiveness and adhesiveness of cookie dough. Soy can be added to gluten-free products to improve the batter/dough functionality and product quality. A moderate amount of soy product is helpful for both wheat and gluten-free bakery products. Pretreatment or modification of soy products can further improve the functional properties, nutritional value, and flavor profile of the bakery products.

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1.9 Tables

Table 1.1 Crude protein content and water absorption (Fleming *et al.*, 1974) .

Protein product		Crude protein*	Centrifuge water absorption (g H ₂ O/g product)	Brookfield apparent viscosity (cP)
		(%)	In water	In water
Soy Flour		50.2	2.77	25
Soy concentrate		63.7	3.65	50
	Supro 610	85.8	6.25	>83,300
Soy isolate:	Promine	86.1	7.75	45,000
	D			

*N x 5.7

Table 1.2 Wheat based dough and bread properties containing soy products.

Soy products	Modification type	Modification	Dough Properties	Bread Properties	Reference
Defatted soy flour	Enzymatic	Transglutaminase (TG); Glucose oxidase (GOX); 1.4-endoxy lanase (XYL)	XYL decreased stability and increased softening degree (SD), GOX increased stability and decreased SD; Both TG and low level of GOX increased G' and G''; Except the higher amount of XYL, all modified flour increased the resistance to extension value and decreased extensible.	XYL and low level of GOX increase the loaf volume; GOX and low level of XYL improve the crumb structure; TG significantly improved crumb cell structure	Roccia <i>et al.</i> , 2012 Basman et al., 2003
Full fatty soy flour	Physical: protein denaturation; Chemical: attributed to SS/SH interchange reactions	Thermal pre-treatment; L-ascorbic acid (L-AA) added	Both thermal pre-treatment and L-AA increased the value of resistance to extension and decreased extensibility; both improved the dough strength, and L-AA showed significant effects. 10% soy flour presented the highest resistance.	N/A	Maforimbo <i>et al.</i> , 2006a

Whole seed soybeans	Physical: upward protein size distribution	1. Soybeans were kept in boiling water for 3 min, blow-dried with hot air (80°C) for 5-6 h, and milled into 0.8mm sieve; 2. Dehulled and flush-steamed soybeans for 3 min, blow-dried for 3hr and milled into 0.8mm sieve.	Boiling the soybeans increased dough resistance to extension, extensibility, and stability.	Both were boiling and steaming soy flour improved the loaf volume, but still below the wheat bread	Maforimbo <i>et al.</i> , 2006b
Soy protein	Hydrolysates	Soy protein hydrolysates (SPH)	SPH decreased stability, increased the viscosity, and a higher degree of starch retrogradation	SPH produces the darker brown color of crust and crumb.	Schmiele <i>et al.</i> , 2017
Soy flour	Physical: heat treatment	Heated in an electric oven with air flux for 3 min at 40, 60, 80, 100, 130, 150, or 200 °C	Heating decreased lipoxygenase activity and supernatant protein content	Increased heating temperature improved bread volume but negatively affected bread quality	(Ribotta <i>et al.</i> , 2004)

Table 1.3 Wheat based cake and cookies properties containing soy products.

	Soy products	Modification type	Dough/batter Properties	Bread Properties	Reference
	Soy Flour	Roasting process	N/A	Improved cookies spread ratio; 30% roasted soy flour provided best sensory test score; increased food energy and mineral content	(Ndife <i>et al.</i> , 2014)
Cookies	Soybean	Genetically modification	Soy reference gene increased with the amount of soy flour added to the dough. The overall DNA fragment length was reduced	There are no significant differences due to the baking process also reduces the overall DNA fragment length	(Gryson <i>et al.</i> , 2007)
	Soy protein	Pomace extracted from cherry and encapsulated in soy protein	Soy protein increased polyphenols and anthocyanins content, but encapsulate powder decreased polyphenols and anthocyanins content	Soy protein and encapsulate powder both improve the antioxidant activity; Dark the color of cookies	(Tumbas Šaponjac <i>et al.</i> , 2016)

	Soy protein	Ethanol-modification and sonication treatment	Increased pH of foam and decreased the foam volume	Increased cake volume provided a more tender texture of the cake, and most of the soy off-flavor compounds were eliminated by ethanol	(Wang <i>et al.</i> , 2012a)
Cake	Soy flour	Particle size modification	At the high level of soy flour (30%) participated, large particle size (>156µm) had the highest moisture content and batter viscosity; increased particle size, coalescence phenomenon appeared	Larger particle size significantly decreased cake hardness, but no significant difference on specific volume; sponge cake had a better texture than a layer cake	(Dhen <i>et al.</i> , 2016)
	Soy milk	Modified with different emulsifiers: DGMS (distilled glycerol monostearate); SMS (sorbitan monostearate); lecithin	The effects of emulsifiers on batter viscosity and density: SMS>DGMS>lecithin	Added emulsifiers decreased cake density; Cake volume: DGMS = lecithin > SMS	(Rahmati and Mazaheri Tehrani, 2014)

Table 1.4 Gluten-free cookie and bread properties containing soy.

	Soy products	Modification	Dough/batter Properties	Properties	Reference
Cookies	Soy protein	Modified with emulsifiers: sodium stearoyl-2-lactylate (SSL); glycerol monostearate (GMS); Lecithin	N/A	Cookies treated with lecithin had the highest spread ratio; Cookies treated with GMS had the highest overall quality and acceptability, GMS improve the textural characteristics; SSL showed fewer effects on cookies	(Sarabhai <i>et al.</i> 2015)
Bread	Soy flour	0, 0.1, 1, and 10 U of Transglutaminase (TGase)/g of protein added into bread dough	The cross-linking networks formed by Tgase and soy protein	No significant different on crumb characteristics, bread volume; 1 U and 10 U Tgase significantly increased hardness and useful to improve the bread quality	(Moore <i>et al.</i> , 2006), (Smerdel <i>et al.</i> , 2012)

Chapter 2 - Effect of formulation and pretreatment of soy flour on the properties of gluten-free cookies

Abstract

Incorporating soy flour into gluten-free (GF) cookie formulation improves nutritional value of the products but negatively influences cookie quality. The objective of this study was to investigate the effect of cookie formulation (e.g., water, shortening, and sugar) and pretreatment of soy flour on the properties of GF dough and cookie containing soy flour. Soy flour was pretreated with glutathione, cysteine, sodium sulfite, and protease Flavourzyme (10 and 30 mins), respectively. GF cookies containing soy were produced by replacing 30% of commercial GF flour with soy flour or pretreated soy flour, respectively. Adding higher amount of sugar, shortening, and water in the formulation significantly improved cookie texture. Among these three ingredients, sugar and shortening are more essential in building the cookie structure, though water is also critical. SDS-PAGE profiles indicated molecular weight changes of soy proteins as a result of the pretreatment. The band distribution was significantly affected by the reducing agents and protease hydrolysis. The pretreatment of soy flours dramatically weakened the dough and improved cookie quality, especially with the soy flour treated with cysteine.

Keywords: Soy; gluten-free; cookie; reducing agent; enzyme protease; ingredients

2.1 Introduction

There has been a steadily increasing demand for gluten-free (GF) products in recent years, partially driven by an increasing number of people diagnosed with celiac disease (CD) or gluten intolerance, as well as more people switching to new diet styles (Man *et al.*, 2014). Celiac patients are permanently intolerant to gluten (Gallagher *et al.*, 2003; Niewinski, 2008; Alvarez-Jubete *et al.*, 2010; Dapčević Hadnadev *et al.*, 2013). Foods containing less than 20 ppm of gluten can be labeled "gluten-free" (Department of Health and Human Services Food and Drug Administration, 2013).

Cookies contain four main ingredients: flour, sugar, shortening, and water. Sugar-snap cookie is one type of cookies with a high amount of sugar and shortening, but a low amount of water. The high fat but low water in sugar-snap cookie restricts gluten network development, increases dough elasticity, and decreases cookie dough extensibility (Schober *et al.*, 2003; Dapčević Hadnadev *et al.*, 2013). With the less desire of gluten development, various gluten-free flours and starches (rice, sorghum, buckwheat, cassava, etc.) can be used to prepare gluten-free cookies, and appropriate formulations and ingredient modifications can further improve the gluten-free cookie quality. In wheat-based cookie, flour plays the most important role in the cookie spreading, while sugar and fat further guarantee the cookie quality. Sugar affects dough viscosity and starch gelatinization of the cookie dough; fat improves the aeration, lubrication of cookie dough, and cookie texture (Pareyt and Delcour, 2008). Different combinations of gluten-free flours (rice flour, maize, sorghum, and pearl millet) could produce different quality of gluten-free cookies (Rai *et al.*, 2014). Sugar-snap cookie has two characteristics: low protein content and

weak gluten strength, which increases cookie set time and spread ratio (Mancebo *et al.*, 2016).

Therefore, starch and protein content and their interactions are critical in cookie making.

Reducing protein disulfide bond decreases the molecular weight and can improve soy protein digestibility (Faris *et al.*, 2008; Wang *et al.*, 2009). Cystine, glutathione (GSH), and sodium sulfite are common reducing agents for the reduction of disulfide bonds to free sulfhydryl groups. Chemical modifications such as using cysteine or sodium sulfite improve soy protein solubility and protein dispersibility index (PDI) (Abtahi and Aminlari, 1997). GSH is a tripeptide consisting of glutamate, cysteine, and glycine residues. Wang, Yang, & Song (2012) and Zhao *et al.* (2019) reported that volatile compounds produced by GSH reacted with sugars. GSH decreased the content of acrylamide in cookies, and the ratios varied depending on the temperature during the baking process (Zhu *et al.*, 2020). Besides, GSH affects the rheological properties of bread dough by changing the protein network through the reduction of the disulfide bonds (Guo *et al.*, 2020; Cui and Li, 2021). A disulfide bond links two cysteine residues to form the cystine. Gluten protein contains a high concentration of cysteine that provides viscoelastic characteristics to the dough due to the sulfhydryl-disulfide exchange reactions (Tozatti, 2020). In wheat cookies, cysteine work as a reducing agent in decreasing flour water absorption capacity (WAC), weakening the dough, and increasing the spread factor of wheat cookies (Kumar *et al.*, 2013). Sodium sulfite is also a reducing agent commonly used in dry food to prevent fruit discoloration.

Hydrolysis of soy protein generates smaller peptides and reduces protein molecular weight that may have better digestibility. Flavourzyme, a commonly used food protease, is an aminopeptidase consisting of peptidase, medium-sized endopeptidase, and exopeptidase (Hoa *et al.*, 2014). Hrčková,

Rusňáková, & Zemanovič (2018) reported that soy flour treated with Flavourzyme had the highest degree of hydrolysis and better gelation and foaming properties, compared to that treated with Novozym and Alcalase.

Pretreatment of soy flour or soy proteins can broaden their food utilization, improve some functional properties, and lead to better food texture, especially for bakery uses. Although there are some previous studies reporting soy flour/soy protein modification, less information is available on the treatment of soy protein for gluten-free cookie applications. In this study, defatted soy flour was pretreated with GSH, cysteine, sodium sulfite, or enzymatic hydrolysis, and then formulated into a GF cookie dough. This study aimed to determine the effect of formulation (e.g., water, shortening, sugar) and soy protein modification on the properties of gluten-free doughs and cookies.

2.2 Materials and methods

2.2.1 Materials

Commercial gluten-free (GF) flour blend (12.12% moisture content) containing garbanzo bean flour, potato starch, tapioca flour, whole grain sorghum flour, and fava bean flour was obtained from Bob's Red Mill (Milwaukie, OR, USA). Defatted soy flour was provided by Cargill (Wayzata, MN, USA). Sugar, shortening, salt, and dextrose were purchased from a local grocery store. Sodium bicarbonate, sodium sulfite, cysteine, GSH, and Flavourzyme were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.2.2 Soy flour pretreatment

The modifier (cysteine, GSH, or sodium sulfite, 1% based on soy flour) was added into 10% soy flour suspension and mixed for 2 hrs at room temperature to allow the reaction. Flavourzyme (1% based on soy flour) was added into 10% soy flour suspension and mixed for 10 and 30 min, respectively, in a water bath (50 °C). After that, the tube was transferred into boiling water to deactivate the enzyme. The pretreated soy flour suspensions were dried using a freeze-dryer (Freezone 4.5L, Labconco Corporation, Kansas City, MO, USA) at -40°C for seven days.

2.2.3 Flour water absorption capacity and mixing properties

Water absorption capacity (WAC) was evaluated according to the method of Shen, Tang, & Li (2021) with some modification. One gram of each flour sample was added into 10 ml distilled water in a 15 ml tube, then vortexed for 30 seconds. After that, the tube was transferred into a water bath at 30 °C for 30 min. Sample tubes were centrifuged at 3,000 xg for 20 min. The sediment was collected and weighed. WAC was calculated according to Eq: (1)

$$\text{WAC (g H}_2\text{O/ g flour)} = (W_2 - W_1)/W_1$$

Where W_2 is the sediment weight, and W_1 is the sample weight.

Flour mixing properties were measured using Mixograph (National Manufacturing Co. Lincoln, NE, USA) following AACC method (50-40.01). Soy flour was pre-mixed with gluten-free flour (30 g soy flour / 100 g total flour) in a KitchenAid mixer before the mixograph test.

2.2.4 Free sulfhydryl concentration and free amino group analysis

Free sulfhydryl concentration was determined according to a previous method (Shen *et al.*, 2021) with some modifications. Approximately 30 mg flour was accurately weighed and added to 6 mL reaction buffer containing 0.05M sodium phosphate, 2% SDS, 3 M urea, and 1 mM Ethylenediaminetetraacetic acid (EDTA) at pH 6.5, then vortexed for 30 s and mixed for 1 hr. The sample was centrifugated at 13,600 xg for 10 min. After that, 300 µL Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid)) (DTNB) (0.1%) was added into 3 mL supernatant, then vortexed for 30 s and allowed to react for 45 min. The absorbance was determined at 412 nm.

Free amino group was determined according to the method of Gujral & Rosell (2004) with some modification. Thirty mg flour was mixed with 6 mL distilled water, then vortexed thoroughly, and the supernatant was collected. One mL NaHCO₃ (4%) and 1 mL TNBS (0.1%) were added into 1 mL sample solution, vortexed for 30 s, and the tube was then transferred to a water bath at 40 °C for 2 hrs. After that, 1 mL SDS (10%) was added into the sample, followed by adding 0.5 mL HCl (1 N) and cooling at room temperature for 15 min. The absorbance was measured at 340 nm. The results were determined against an L-leucine standard curve and tested in duplicate.

2.2.5 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of the protein samples was conducted under non-reducing condition following a literature method (Shen *et al.*, 2021) with small modification. The flour sample was dispersed into distilled water (5 mg/mL) and vigorously mixed overnight, and then centrifuged at 10,000 xg for 5 min at room temperature. A PowerPac 1000 (Bio-Rad, USA) was used to run the electrophoresis.

2.2.6 Cookie dough preparation and stickiness properties

Cookie dough was prepared by mixing gluten-free flour, water (1 time or 2 times), shortening (1 time or 1.5 times), and/or sugar (1 time or 1.5 times). Besides, soy flour or different types of modified soy flour was added to replace 30% of the gluten-free flour. Cookie dough stickiness was tested by using the TA-XTplus Texture Analyzer equipped with an SMS/Chen-Hoseney rig according to a previous method (Chen *et al.*, 2020).

2.2.7 Preparation of cookies

Cookies were prepared following AACC method 10-50.05 (Sugawara and Nikaido, 2014). Formulation of the cookies is shown in Table 1. To investigate soy effect, 30% of the gluten-free flour was replaced by soy flour based on the combination formula (i.e., 2 times water, 1.5 times shortening, and 1.5 times sugar).

2.2.8 Cookie analytical methods

Cookie spread ratio was calculated according to the AACC method (10-50.05, AACC, 1999). Cookie hardness and fracturability were determined by using the TA-XTPlus Texture Analyzer, according to the previous study (Chen *et al.*, 2020). The color analysis was performed using a CIE-LAB color system (XITIAN machine equipment Co., Ltd., Huizhou, China), and the results were presented as L* (lightness), a* (redness-greenness), and b* (yellowness-blueness). Moisture content was measured as described in the AACC method (44-15.02 AACC, 1999b).

2.2.9 Statistical analysis

The data were analyzed using SAS statistical software (SAS Institute, Cary, NC, USA). Tukey's multiple comparisons test was used to determine the significant difference between each individual data. A significant level was defined at $p < 0.05$.

2.3 Results and discussion

2.3.1 Flour water absorption capacity and mixing properties

Table 2 shows the results of water absorption capacity (WAC) of different flours. The WAC of commercial GF flour (0.71 g H₂O/ g flour) was significantly lower than that of soy flour (1.63 g H₂O/ g flour), due to the much higher protein content in soy flour (approximately 50%), which would result in a drier and crumbly cookie dough structure if water addition remains the same (Mcwatters, 1978). Soy protein could trap the water quickly during mixing process (Srikanlaya *et al.*, 2018) and lead to the higher WAC. Similar result was also reported by Akubor and Ukwuru (2003). With the modification of soy flour protein with cysteine, GSH, sodium sulfite, or hydrolysis, the WAC significantly decreased to a similar range as that of the GF flour. The protein structures could affect the water absorption of the protein. The reducing agents broke down the disulfide bonds, which unfolded the protein and denatured the tertiary structure. (Liu *et al.*, 2016; Shen *et al.*, 2021). Hydrolysis of soy protein with Flavourzyme cleaved the peptide bonds and resulted in smaller molecular size of the proteins.

Mixograph shows dough mixing properties and dough strength, and peak time and peak value of the doughs are summarized in Table 2. The mixographs of GF cookie dough with different modified soy flours are presented in Fig. 2. Mixing tolerance of the doughs significantly decreased by adding the

modified soy flours. Unmodified soy flour limited cookie spread due to the higher protein content and strong protein interaction, which negatively affected the quality of cookies (Singh and Mohamed, 2007; Park *et al.*, 2015). The advantage of proteins enrichment is to support dough structure, while the size and shape of molecules decide their polymer or aggregates (Srikanlaya *et al.*, 2018). A previous study showed that including soy protein increased the mixing tolerance index (Singh and Mohamed, 2007). As shown in Table 2, dough with GSH modified soy had the lowest peak time (0.65 min), peak value (11.16%), and peak integral (13.97 %Tq*min). Peak integral shows the average work input for the dough to achieve peak development. This is because the molecular weight of protein in soy flour was decreased by the modification, as a result of the reduction and reoxidation reactions. Sodium sulfite was used to treat soy protein to improve in vitro and in vivo digestibility due to the rearrangement of disulfide bonds during chemical modifications (Wang *et al.*, 2009). Therefore, the dough mixing optimum development time and mixing tolerance were positively correlated with flour WAC.

2.3.2 Free sulfhydryl concentration and free amino group content

The free sulfhydryl group (SH) and free amino group content of soy flours are presented in Table 2. The free SH concentration of soy flour significantly increased after the modification with cysteine, GSH, and sodium sulfite. Adding cysteine increased SH content from 4.18 to 31.42 mmol / g flour. Although cysteine initially contains SH group, Li and Lee (1998) indicated that cysteine increased the SH content in wheat flour after fractionation. Soy flour modified by sodium sulfite generated more SH groups than that with GSH. The oxidation-reduction capability of sodium sulfite is lower than cysteine but is commonly used as a reducing agent due to the protein sulfhydryl, which were broken down by the

sodium sulfite from disulfide bond blocked as a sulfonate group (Petruccelli and Añón, 1995). The SH content was not significantly different between 10 min and 30 min hydrolysis, and the SH concentration was 2.19 and 2.03 mmol/g, respectively. In addition, free SH content was reduced by the hydrolysis process, this might be caused by the thermal deactivation process that promoted disulfide crosslinking. Also, the thermal energy, mechanical mixing, and air available during hydrolysis can favor the oxidation process, resulted in more crosslinking of free SH and transformation into disulfide bond.

Among the different reducing agents, cysteine modification increased the free amino group content from 23.15 mmol/g to 45.88 mmol/g (Table 2). Free amino group is present at one end of each polypeptide, as well as from lysine residue. There was no significant difference on free amino group content between GSH and sodium sulfite modifications. Free amino groups or free carboxyl groups are commonly measured to determine the hydrolysis equivalents (Navarrete del Toro and García-Carreño, 2003). The increasing of free amino group content with reducing agent modification might be due to the preparing process. In soy flour, some of the free amino group from lysine residue are buried inside the protein's hierarchical structure and cannot be accessed directly during analysis. Reducing agent promoted the exposure of free amino group from lysine residue. Increasing hydrolysis time significantly increased free amino group content from 45.59 to 51.93 mmol/g due to the fact that the enzyme (Flavourzyme) cleavage peptide bonds in the primary structure and released free amino groups. These results confirmed that the longer hydrolysis time could improve protein functionality. Anh *et al.* (2020) indicated that the recovery efficiency of soluble protein was positively correlated with hydrolysis time but reached equilibrium after around 120 min.

2.3.3 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE profiles of the proteins in the modified soy flours are shown in Fig 1. In addition to the evidence from the increased content of free SH group and free amino groups demonstrating the effectiveness of the modification, SDS-PAGE was conducted to further analyze the occurrence of disulfide bonding reduction and protein cleavage. The 7S globulin (β -conglycinin) and 11S globulin (glycinin) are the two major components in soy protein (Petrucelli and Añón, 1994). β -conglycinin is formed by three subunits α , α' , and β with molecular weights of 78, 72, and 52 kDa, respectively, and the glycinin is formed by acidic (A) subunits (34 – 43 and 10 – 15 kDa) and basic (B) subunits (18 – 25 kDa) (Islam *et al.*, 2020; Yang *et al.*, 2020). With the reducing agents, larger protein molecules were partially broken down into subunits. Cysteine modified soy flour showed a higher intensity of 11S (A) and 11S (B) bands than the GSH or sodium sulfite modified soy flour. This result was positively correlated with the free SH content and free amino groups in cysteine modified soy flour. However, Abtahi and Aminlari (1997) showed that cysteine's effectiveness was less than sodium sulfite. The reducing agent breaks down the disulfide bonds and results in the loss of larger molecular weight bands and appearance of new bands of smaller molecules (Shen *et al.*, 2021).

Less intensive bands were observed for the hydrolysis of soy flour, while 30 min hydrolysis provided the least intensive bands in SDS-PAGE. This result is expected due to the enzyme deactivation process after hydrolysis that caused the protein denaturation, aggregation, and crosslinking. Flavourzyme-susceptible catalytic sites are acted within globulins instead of surface and provide a limited cleavage effect (Xu *et al.*, 2020c). Protease hydrolysis generates high amount of smaller

peptides, commonly below 15 kDa (Islam *et al.*, 2020). Hydrolysis bands had different distribution with the reducing agent bands.

2.3.4 Cookie dough stickiness properties

Dough stickiness, which indicates the adhesion of dough when it contacts a surface, is an issue commonly seen in the cookie industry (Basri *et al.*, 2020). Cookie dough stickiness properties are shown in Table 3. Increasing to 1.5 times of sugar or shortening showed less effect on dough stickiness, work of adhesion, and dough cohesiveness. Cookie dough with a combination of 2 times of water, 1.5 times of sugar, and 1.5 times of shortening had higher stickiness (29.67 g) than that with 30% soy dough (21.08 g), but still lower than the GF cookie dough (36.94 g). Sugar and fat are the two major components to build the structure of cookies (Ashwath Kumar and Sudha, 2020). However, only increasing to 1.5 times sugar or shortening did not affect the dough stickiness properties. Incorporating 2 times of water in 30% soy flour dough significantly increased the dough stickiness, but the stickiness value of 2 times water cookie dough is still lower than the control GF dough due to the high water absorption of soy flour. This result is consistent with a previous study (Hamed *et al.*, 2015) that excessive water decreased dough stickiness. Sugar provides lower glass transition temperature and higher hygroscopic character for food (Jaya and Das, 2009). In addition, increasing the amount of water, sugar, and shortening, respectively, did not change the work of adhesion and cohesiveness value of the cookie dough.

Partial replacement of GF flour with modified soy flour significantly increased the stickiness, work of adhesion, and dough cohesiveness compared to the control (i.e., combination). The modified soy flour cookie doughs had similar stickiness properties as the GF cookie dough, and among these, the

dough with soy flour of 30 min hydrolyses had the highest stickiness value (36.41 g). These results are negatively correlated with flour water absorption. There was no significant difference in dough stickiness after using cysteine, GSH, sodium sulfite, and protease enzyme to modified the soy flours. Arepally *et al.* (2020) indicated that water activity affects moisture content, fat melting zone, solubilization, and crystallization of cookies during baking; besides, flour hydration and protein content affect cookie spread ratio. With gluten protein breaking down by adding GSH and cysteine, protein depolymerized, the resistance to dough decreased, and the extensibility increased (Maforimbo *et al.*, 2007). Disulfide bonding improved dough's stability; thus, breaking down of disulfide bonds would result in a weaker dough (Maforimbo *et al.*, 2008). Incorporating the modified soy flours led to a similar dough consistency with the original GF cookie dough.

2.3.5 Cookie characterization

2.3.5.1 Baking loss and moisture content

Baking loss of the cookies is shown in Table 4, which indicates the water loss/retention of cookies during the baking process. The highest baking loss was observed in the control GF cookie (13.5%). With 30% of soy flour incorporated, the baking loss decreased significantly. This is because soy flour with much higher protein content could better retain the water during baking compared to the commercial GF flour. This result is consistent with a previous study by Traynham *et al.* (2007), which showed that combining soy flour with wheat flour increased water holding capacity. Increasing the amount of water slightly increased the baking loss of cookie because more water was participated to dissolve sugar during baking. There were no significant changes of baking loss between the cookies

added with higher amount of sugar or shortening. Increasing the sugar amount slightly decreased baking loss because sugar improves starch gelatinization (Kweon *et al.*, 2009).

Compared to the cookie with the high amount of water, sugar, and shortening, replacing 30% of GF flour with modified soy flour slightly increased baking loss, especially for the soy flour treated with sodium sulfite (11.41%) or 10 min hydrolysis (11.64%). This might be related to the water absorption properties of the modified soy flours. The chemical modification resulted in the cookie dough absorbing a lower amount of water during mixing and more water evaporation during the baking process. In addition, breaking down of the disulfide bonds or peptide bonds promoted starch and protein interactions, and improved the water-holding capacity of the starch-protein gel (Yu *et al.*, 2020).

As shown in Table 4, incorporation with 30% soy flour significantly increased cookie moisture due to the higher water absorption of soy flour. The same results were also presented in Shrestha and Noomhorm (2002). With higher amount of water addition, the moisture content increased (8.17%). Incorporated with higher amount of sugar or shortening decreased the moisture content of cookie, compared with control soy cookie. Adding modified soy flour significantly decreased the cookie moisture content. Cookies with soy flour from 30 min hydrolysis had the lowest moisture content (2.8%). The tendency of cookie moisture content was slightly negatively related with the baking loss.

2.3.5.2 Spread ratio and cookie firmness

Cookie spread ratio and hardness are summarized in Table 4. Incorporation of 30% soy flour into GF cookie dramatically decreased the spread ratio from 9.22 to 5.47. This is not only because of the high water absorption of soy flour but also due to the wheat starch granules material leached out of the

granules interact with protein and/or the surface granules interact protein (Ribotta *et al.*, 2007). The higher amount of sugar and shortening significantly increased the spread ratio. Besides, sugar increased the hardness of cookies from 5887.75 g to 10143.12 g, while shortening reduced the hardness from 5887.75 g to 4902.88 g. Adding 2 times of water, 1.5 times of sugar, and 1.5 times of shortening significantly decreased cookies' hardness and increased spread quality. Increasing the amount of shortening led to a softer texture of cookies. Increasing the amount of sugar causes more un-dissolved sugar melt gradually during baking, improves the extensive spread of the dough, and produces more crispy and crunchy cookies (Arepally *et al.*, 2020). Besides, Xu *et al.* (2020a) noticed that adding fat prevented the diffusion of carbon dioxide during leavening action, produced amorphous glass around flour particles, and improved cookies spread quality. Doubling water amount had less obvious effect on the cookie spread and texture quality.

Adding modified soy flour significantly improved the spread ratio of GF cookies. The smaller molecular size of proteins in the treated soy flour weakened the GF cookie dough. Cookie with cysteine treated flour had the highest spread ratio, about 22.83. The spread ratio of cookies with reduced soy flours was overall positively related with free amino group content and the SDS-PAGE results. Spread ratio increased in the order of sodium sulfite < GSH < cysteine. Cysteine presents the most profound effect on cookie texture, and this might be due to the intensive reducing reaction that broke more disulfide bonds than that with the other two reducing agents. Besides, a higher amount of proteins with smaller molecules might also help to increase the cookie spread ratio. Elkhaila and El-Tinay (2002)

indicated that directly adding cysteine into wheat dough did not affect cookies' spread ratio, which might be because the dough mixing process would not allow sufficient reduction reaction.

The spread ratio of cookies also increased with increasing soy protein hydrolysis time. This result is also consistent with the free amino group trend, which indicates that the free amino group plays an important role in the spread quality of GF cookies. For the cookie hardness, all the cookies with modified soy flours had a higher value than the combination control (1249.97 g), especially for sodium sulfite (2878.25 g), hydrolysis at 10 min (2729.38 g), and hydrolysis at 30 min (2683.54 g).

Fracturability determines the tendency of the fracture, shatter, and crispiness of cookies using a small amount of force. Incorporation of modified soy flour significantly reduced the fracturability values. This consequence might be due to the degradation of disulfide and/or peptide bonds, as peptide degradation and crosslinking are significantly related to protein's molecular weight (Liu *et al.*, 2012).

2.3.5.3 Color analysis

The color properties of GF cookies are summarized in Table 5, and pictures of the cookies are shown in Fig. 3. Cookies with a higher amount of water, sugar, and/or shortening did not obviously differ in terms of surface lightness, Hunter a*, and Hunter b*. Cookies containing modified soy flours had significantly reduced Hunter L* and slightly decreased Hunter b*, indicating darker and blue color tones of the cookie surface, respectively, caused by the higher protein content and more intensive browning reaction. Cookies with soy flours from 30 min hydrolysis presented the darkest color among all the treatments, which was related to the higher amount of free amino groups that were available for Maillard reaction. A higher amount of free amino group promoted the Maillard reaction, which resulted

in darker color for the GF cookies. The higher amount of available amino acid residues reacted with sugars during the Maillard reaction in generating color compounds (Shrestha and Noomhorm, 2002). Overall, higher amount of free amino group led to weaker dough, higher spread ratio, and flatter texture of the cookies.

2.4 Conclusions

Cookies can be produced from gluten-free flours as the needs for gluten development in cookie dough are less desired compared to bread dough; however, GF flours are of lower nutritional value and contain low amount of protein. Incorporating soy flour into GF cookie formulation increased protein content and nutritional value, but decreased cookie spread. Increasing the amount of water, sugar, and shortening in the formulation improved the cookie quality. Pretreatment of soy flours with reducing agent or protease greatly decreased protein molecular size, cookie dough strength, mixing peak time, and peak value. Reducing agent significantly increased free SH content due to the breakdown of the disulfide bond. Cysteine presented more profound performance compared to GSH and sodium sulfite in reducing soy protein. Free amino group was positively correlated with the degree of hydrolysis. Incorporation of pretreated soy flours dramatically improved cookie texture. Among these pretreated soy flours, cysteine pretreatment improved the cookie texture more obviously because of its stronger potent as a reducing agent in breaking more disulfide bonds. Overall, our study demonstrated that pretreatment of soy flour with 1% cysteine, GSH, or sodium sulfite, or Flavourzyme improves baking performances of soy flour for gluten-free cookie application. In the future, the pretreatment conditions (modifier

amount, reaction time, temperature) of soy flour with reducing agent or protease need to be further optimized to achieve the best flour functionality for cookie applications.

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2.6 Tables

Table 2.1 Formulations of different types of cookie (based on g per 100g flour).

Formulation	GF flour, g	(Treated) soy flour (SF), g	Shortening, g	Sugar, g	Water, g
Control	100	0	28.44	57.78	7.11
30% soy flour	70	30	28.44	57.78	7.11
2x Water	70	30	28.44	57.78	14.22
1.5x Shortening	70	30	42.66	57.78	7.11
1.5x sugar	70	30	28.44	86.67	7.11
Combination	70	30	42.66	86.67	14.22
Cysteine	70	30	42.66	86.67	14.22
GSH	70	30	42.66	86.67	14.22
Sodium sulfite	70	30	42.66	86.67	14.22
Hydro 10 min	70	30	42.66	86.67	14.22
Hydro 30 min	70	30	42.66	86.67	14.22

Note: Each formulation also included 0.93 g salt, 1.11 g sodium bicarbonate, and 14.67 g dextrose solution per 100 g flour.

Table 2.2 Physical properties of gluten-free (GF) flour and soy flours with different pretreatments.

	Water absorption	Free S-H	Free amino group	Mixograph peak		Mixograph 1 min right of the peak	
	g H ₂ O / g flour	mmol/g	mmol/g	Time, min	Value, %	Width, %	Integral, %Tq*Min
GF flour	0.71 ± 0.03 ^b	-	-	-	-	-	-
Soy flour	1.63 ± 0.01 ^a	4.18 ± 0.12 ^d	23.15 ± 0.37 ^e	7.26 ± 0.23 ^a	25.42 ± 0.82 ^a	37.29 ± 0.90 ^a	187.02 ± 4.98 ^a
Cysteine	0.78 ± 0.09 ^b	31.42 ± 0.14 ^a	45.88 ± 3.18 ^{ab}	1.63 ± 0.51 ^{bc}	16.54 ± 3.31 ^{bc}	8.32 ± 6.42 ^b	28.95 ± 5.06 ^c
GSH	0.92 ± 0.03 ^b	17.61 ± 0.50 ^c	37.33 ± 1.84 ^{cd}	0.65 ± 0.13 ^c	11.16 ± 2.14 ^c	11.94 ± 1.98 ^b	13.97 ± 4.11 ^c
Sodium sulfite	0.80 ± 0.10 ^b	24.20 ± 0.10 ^b	33.10 ± 0.65 ^d	0.95 ± 0.16 ^{bc}	15.60 ± 1.35 ^{bc}	12.12 ± 2.84 ^b	22.96 ± 2.99 ^c
Hydrolysis 10min	1.00 ± 0.10 ^b	2.19 ± 0.01 ^e	43.59 ± 0.38 ^{bc}	1.85 ± 0.20 ^b	21.88 ± 1.08 ^{ab}	20.16 ± 7.41 ^{ab}	50.03 ± 4.43 ^b
Hydrolysis 30min	1.00 ± 0.05 ^b	2.03 ± 0.04 ^f	51.93 ± 0.87 ^a	0.92 ± 0.01 ^{bc}	15.66 ± 0.11 ^{bc}	15.04 ± 1.79 ^b	22.56 ± 0.21 ^c

*Means with different letters within each property denote significant differences (P < 0.05)

Table 2.3 Cookie dough stickiness properties with soy flour incorporation from different formulations and pretreatments.

Formulation	Stickiness, g	Work of adhesion, g.sec	Dough cohesiveness
100% GF	36.94 ± 1.38 ^a	1.62 ± 0.08 ^a	0.68 ± 0.06 ^a
30% Soy flour	21.08 ± 2.26 ^d	0.55 ± 0.07 ^d	0.35 ± 0.02 ^b
2 times of water	27.27 ± 0.61 ^c	0.73 ± 0.06 ^d	0.44 ± 0.05 ^b
1.5 times of sugar	18.27 ± 1.92 ^d	0.46 ± 0.04 ^d	0.31 ± 0.02 ^b
1.5 times of shortening	18.80 ± 2.03 ^d	0.49 ± 0.06 ^d	0.34 ± 0.04 ^b
Combination	29.67 ± 0.73 ^{bc}	1.05 ± 0.07 ^c	0.62 ± 0.05 ^a
Cysteine	34.92 ± 1.94 ^{ab}	1.47 ± 0.14 ^{ab}	0.75 ± 0.13 ^a
GSH	35.53 ± 1.24 ^a	1.53 ± 0.09 ^{ab}	0.74 ± 0.07 ^a
Sodium sulfite	33.27 ± 0.52 ^{ab}	1.32 ± 0.06 ^{bc}	0.63 ± 0.01 ^a
Hydrolysis 10min	35.60 ± 2.79 ^a	1.46 ± 0.17 ^{ab}	0.73 ± 0.08 ^a
Hydrolysis 30min	36.41 ± 2.54 ^a	1.52 ± 0.13 ^{ab}	0.68 ± 0.03 ^a

*Means with different letters within each property denote significant differences ($P < 0.05$).

Table 2.4 GF cookie baking loss, spread ratio, hardness, and fracturability.

	Baking loss, %	Cookie moisture content, %	Spread ratio	Hardness, g	Fracturability, mm
100% GF	13.05 ± 0.40 ^a	4.60 ± 0.00 ^d	9.22 ± 0.07 ^f	3931.05 ± 217.98 ^{cd}	46.59 ± 0.26 ^{ab}
30% Soy flour	8.80 ± 0.26 ^{cde}	7.28 ± 0.02 ^b	5.47 ± 0.07 ^h	5887.75 ± 596.26 ^b	46.62 ± 0.36 ^{ab}
2 times of water	11.03 ± 0.25 ^{abcd}	8.17 ± 0.00 ^a	5.66 ± 0.10 ^h	4902.88 ± 357.88 ^{bc}	45.97 ± 0.52 ^b
1.5 times of sugar	8.18 ± 0.26 ^e	5.73 ± 0.05 ^c	6.49 ± 0.11 ^g	10143.12 ± 616.06 ^a	46.66 ± 0.16 ^{ab}
1.5 times of shortening	9.04 ± 0.03 ^{bcd}	5.85 ± 0.14 ^c	6.67 ± 0.08 ^g	4147.12 ± 468.94 ^c	47.34 ± 0.29 ^a
Combination	8.64 ± 0.66 ^{de}	6.85 ± 0.05 ^b	10.04 ± 0.04 ^e	1249.97 ± 210.90 ^f	42.27 ± 0.48 ^c
Cysteine	9.89 ± 0.90 ^{bcd}	3.76 ± 0.15 ^e	22.83 ± 0.27 ^a	2325.79 ± 250.85 ^{ef}	40.79 ± 1.03 ^d
GSH	11.21 ± 0.85 ^{abcd}	3.48 ± 0.10 ^{ef}	21.68 ± 0.32 ^b	1914.96 ± 147.32 ^{ef}	41.61 ± 0.16 ^{cd}
Sodium sulfite	11.41 ± 0.69 ^{abc}	3.24 ± 0.14 ^{fg}	19.07 ± 0.04 ^{cd}	2878.25 ± 226.12 ^{de}	41.93 ± 0.15 ^{cd}
Hydrolysis 10min	10.03 ± 1.71 ^{bcd}	3.40 ± 0.14 ^{ef}	18.65 ± 0.09 ^d	2729.38 ± 568.80 ^e	42.13 ± 0.05 ^c
Hydrolysis 30min	11.64 ± 1.89 ^{ab}	2.80 ± 0.24 ^g	19.35 ± 0.08 ^c	2683.54 ± 338.20 ^e	42.34 ± 0.14 ^c

*Means with different letters within each property denote significant differences ($P < 0.05$).

Table 2.5 Color analysis of GF cookies with different formulations or soy flour pretreatments.

	L*	a*	b*
100% GF	53.78 ± 1.55 ^{bc}	8.91 ± 0.23 ^{abc}	33.23 ± 0.40 ^{bcd}
30% Soy flour	57.02 ± 0.06 ^{ab}	10.65 ± 0.01 ^{ab}	36.07 ± 0.18 ^a
2 times of water	62.08 ± 0.37 ^a	7.36 ± 0.06 ^{bc}	35.19 ± 0.18 ^{ab}
1.5 times of sugar	54.92 ± 0.76 ^b	10.50 ± 0.33 ^{ab}	35.39 ± 0.52 ^{ab}
1.5 times of shortening	57.76 ± 0.81 ^{ab}	8.63 ± 0.98 ^{bc}	36.08 ± 0.60 ^a
Combination	54.91 ± 0.75 ^b	6.77 ± 1.25 ^c	34.82 ± 1.06 ^{ab}
Cysteine	48.76 ± 2.43 ^{cd}	6.27 ± 1.42 ^c	31.26 ± 0.02 ^{cd}
GSH	46.99 ± 1.81 ^d	8.69 ± 1.33 ^{bc}	33.30 ± 0.24 ^{bc}
Sodium sulfite	47.19 ± 0.07 ^d	6.82 ± 1.24 ^c	33.07 ± 0.53 ^{bcd}
Hydrolysis 10min	44.38 ± 0.08 ^d	8.98 ± 0.31 ^{abc}	32.07 ± 0.27 ^{cd}
Hydrolysis 30min	36.85 ± 2.69 ^e	12.24 ± 0.39 ^a	30.65 ± 1.53 ^d

*Means with different letters within each property denote significant differences ($P < 0.05$).

2.7 Figures

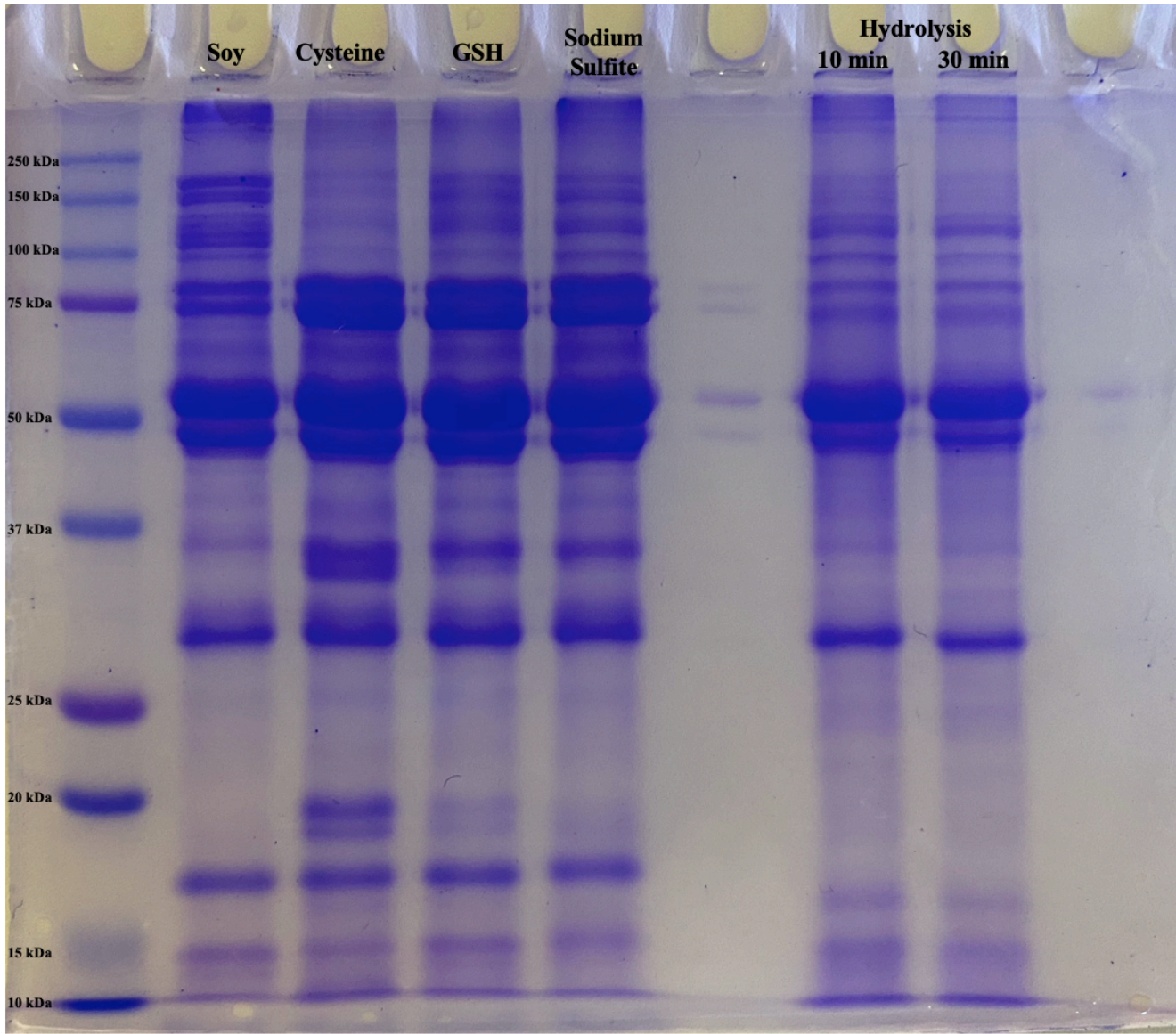


Figure 2.1 SDS-PAGE of soy proteins in soy flours with different pretreatments.

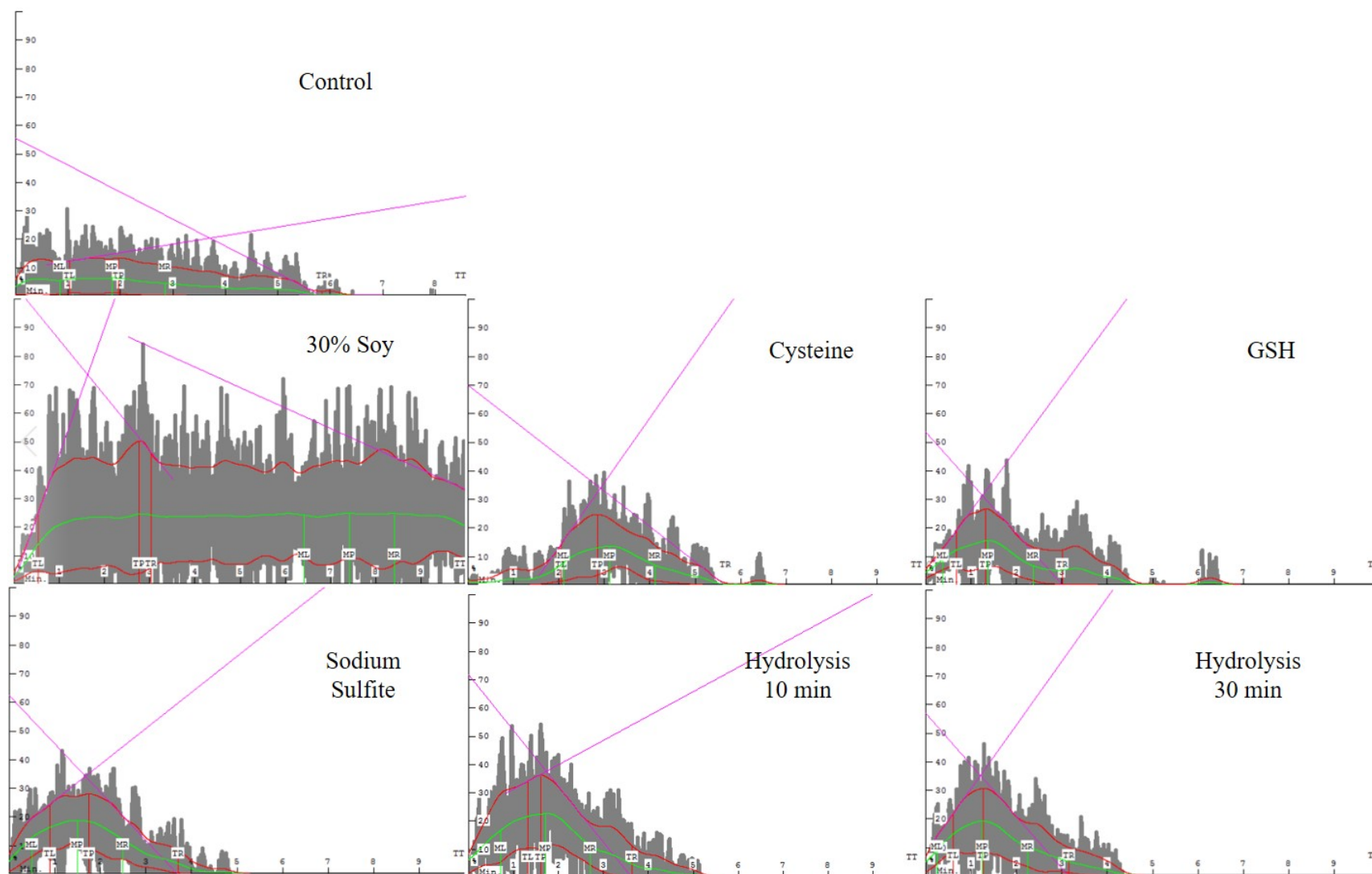


Figure 2.2 Mixograms of gluten-free flour and its composite with soy flour from different pretreatments.

A.



B.

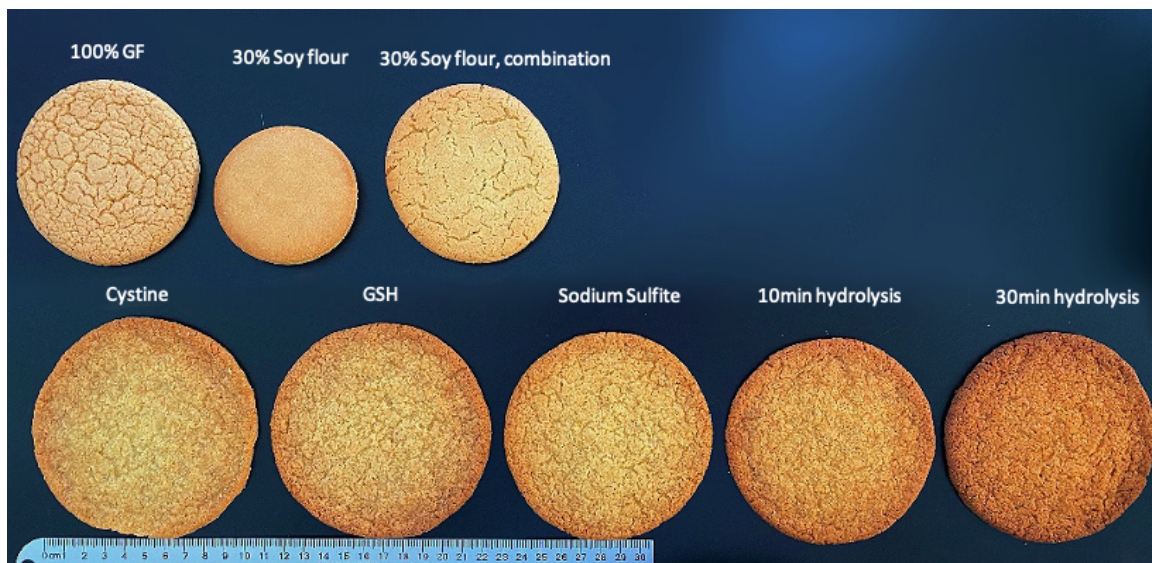


Figure 2.3 Images of GF cookies from different treatments.

Chapter 3 - Dough rheology and baking quality of whole wheat/pulse flour blends

Abstract

Pulse flours are commonly added to food products to improve the functional properties, nutritional profiles, and product quality and health benefits. This study aimed to understand the effect of different types (yellow pea, green pea, red lentil, and chickpea) and amounts (0, 5, 15, and 25%) of whole pulse flours on whole wheat dough properties and bread quality. Whole pulses were grounded through a Wiley mill to obtain whole pulse flour. Mixolab was used to determine the mixing and pasting properties of whole wheat/pulse composite flour, and bread quality was analyzed through volume test, texture analyzer, C-cell, and moisture analysis. Pulse flours had higher contents of protein and ash, but lower moisture content and smaller average particle size compared to whole wheat flour. Commercial yellow pea flour had a smaller particle size than lab-grounded yellow pea flour and negatively affected both dough and bread properties. Increasing the substitution level of pulse flours decreased dough viscosity, stability, and development time and bread volume, and accelerated bread retrogradation. Incorporation of 5% yellow pea flour led to similar bread quality as that with only whole wheat flour. Among all the tested pulse flours, the composite flour containing yellow pea flour had overall better baking performances.

Keywords: Whole wheat bread, pulse flours, Mixolab, mixing and pasting properties

3.1 Introduction

Pulses, such as peas, lentils, chickpeas, and dry beans, are widely consumed as a staple food in many countries due to their high nutritional values (Zucco *et al.*, 2011). Pulses contain high amount of dietary fibers, proteins, vitamins, minerals, and phytochemical antioxidants (e.g., phenolic acids, flavonoids, isoflavones, and tannins), which are beneficial to human health (Hall *et al.*, 2017; Millar *et al.*, 2019b; Rebello *et al.*, 2014). Fully cooked pulses can function as low-glycemic foods that inhibit appetite and glycemia in a short term (Smith *et al.*, 2012). Consuming pulses may reduce the risk of cardiovascular disease, gastrointestinal disorders, obesity, type-2 diabetes, cancer and lower cholesterol levels (Roy *et al.*, 2010; Bazzano *et al.*, 2011; Curran, 2012; Rebello *et al.*, 2014; Padhi and Ramdath, 2017).

Nowadays, consumers are becoming more advocates for health and wellness (Tyler *et al.*, 2017). The tendency of including pulses to improve the nutritional values of food has become more popular. Whole grain foods are considered as healthier food as they contain all the original nutrients present in bran, germ, and endosperm. Whole wheat flour contains better nutritional profiles and more health benefits than refined wheat flour, especially that it is rich in vitamins, minerals, fibers, antioxidants, and phytochemicals (Tebben *et al.*, 2018). Consumption of whole wheat foods can reduce the risk of cardiovascular disease, obesity, cancer, diabetes, and maintain body weight (Jiang and Peterson, 2013). However, baking with whole wheat flour always leads to bread with smaller size, bitter taste, and coarser and harder texture compared to white bread, which is less appealing to consumers. Moreover, wheat contains relatively low concentration of protein (8-15%), and it is an incomplete protein source due to a lower amount of lysine. On the other side, protein content in

pulses is much higher, around 18-30%, and pulses are rich in lysine. Incorporating pulse flours into wheat breads can improve the lysine content and amino acid profile (Wani and Kumar, 2014).

Marchini et al. (2021) found that adding 10% red lentil flour with larger particle size resulted in better dough properties compared to that with higher amount of red lentil or smaller particle size flour. Pulse flour blends showed higher water absorption due to the high amount of polysaccharides and protein content (Man *et al.*, 2015). A study on white flour/pea flour blends showed that the bread properties are positively related to dough rheological properties, and bread specific volume decreased with higher amount of pea flour (Millar *et al.*, 2019a). Besides, several studies demonstrated that incorporating pulse flour significantly improved nutritive values of wheat-based bakery products (Shrestha and Noomhorm, 2002; Zucco *et al.*, 2011). Compared with the continuous network and unique viscoelasticity of wheat dough' protein matrix, the protein matrix of pulse flour was less desirable for bread making. Pulse may also have some negative effect on food products, such as strong beany flavor, intense aroma, and introducing anti-nutritional compounds. A study showed that that higher fiber content in wheat flour and chickpea flour resulted lower wheat bread volume due to the interaction between its hydroxyl groups and water through hydrogen bonding and influence on dough stability (Man *et al.*, 2015). Including pulse flours into bread dough would dilute gluten protein and affect gluten development and starch-protein complex that are important to dough rheology and quality of bread (Mohammed *et al.*, 2012; Millar *et al.*, 2019a). Pea flour also interrupted starch-gluten matrix, resulting in weaker and less elastic dough (Millar *et al.*, 2019a). Different modification of pulses, such as germination, extrusion, fermentation, were used to improve the quality of food containing pulses (Setia *et al.*, 2019).

So far, few literatures are available on incorporating various pulse flours into whole wheat bread products. This study aimed to determine the effect of different types and amount of whole pulse flour (e.g., yellow pea, green pea, red lentil, and chickpea) on the whole wheat dough properties and bread quality.

3.2 Materials and methods

3.2.1 Materials

Whole wheat flour (protein content 15.1%, moisture content 12.9%) was supplied by Mennel Milling Company (Fostoria, OH, USA). Commercial yellow pea flour was obtained from Harvest Innovations (Indianola, IA, USA). Dried whole yellow pea and whole green pea were purchased from Rani (Houston, TX, USA). Whole red lentil was provided by Food to Live (Brooklyn, NY, USA). Whole chickpea was from Palouse Brand (Palouse, WA, USA). Active dry instant yeast, sucrose, salt, and shortening were purchased from a local grocery store.

3.2.2 Flour characterization

Pulse grains were ground with a Wiley Mill (Arthur H. Thomas Company, PA, USA) equipped with a 0.1 mm sieve, where the grains went through the hammer mill and the particle size was reduced through the impact. The yields of yellow pea, chickpea, green pea and red lentil flour recovered after the milling were 82.6%, 76.9%, 71.4%, and 69.7%, respectively, based on 1000 g of each grains. Besides, pulse mill ability was decided by the seed moisture content (Thakur *et al.*, 2019). Protein, moisture, and ash of the flours were measured following AACC Approved methods (46-30.01, 44-19.01, 08-01.01). Lipid content was measured according to AACC Approved method 30-10.01 with some modification. Briefly, flour and ethyl ether were mixed at a ratio of 1:20 (w:v) in

a conical flask for 30 min, then centrifuged at 3000 xg for 10 min, and the extract was collected. The extraction process was repeated twice. The extract was then pooled and left in a fume hood overnight to evaporate the solvent, and then the weight of the lipids was measured. Total carbohydrate was determined based on the percentage of ash, total fat, moisture, and protein content using Eq: (1)

$$\text{Carbohydrates (\%)} = 100 - \text{Ash} - \text{Total Fat} - \text{Moisture} - \text{Protein}.$$

Particle size of the flours was measured using a Ro-Tap sieve shaker (Model B, Combustion engineering, Inc., OH, USA) with sieve sizes of 53 to 3360 μm . Approximately 100 g sample was weighed, transferred to the top of the shaker, and then shaken for 10 min. The weight of flour on each sieve and the pan was measured. The average particle size was calculated by Eq: (2)

$$\text{Avg particle size } (\mu\text{m}) = \Sigma \left[\frac{W_i}{W_t} \times d_i \right]$$

Wi: weight of flour remains in each sieve; Wt: weight of total flour; di: diameter of ith sieve in the stack.

3.2.3 Mixolab analysis of whole wheat/pulse flour blends

Dough mixing and pasting properties of blends of whole wheat flour with different types (commercial yellow pea flour, yellow pea flour, green pea flour, and chickpea flour) and amounts (5, 15, and 25%) of pulse flours were analyzed using Mixolab (Chopin Technologies, France) according to AACC method 54-60.01. Whole wheat flour was also measured for comparison. The flours were pre-mixed in an external mixer before the testing.

3.2.4 Bread baking of whole wheat/pulse blends

Breads from different whole wheat/pulse flour blends were prepared following AACC method 10-10.03 (AACC Internationals, 1999). The formulation included 100 g flour, 2 g instant dry

yeast, 3 g shortening, 6 g sucrose, 1.5 g salt, 0.2 g malt flour, and optimum amount of water. The amount of water and dough mixing time were determined based on mixograph analysis. The bread-making was conducted in duplicate for each formulation.

3.2.5 Bread quality analysis

Bread volume and specific volume were determined following AACC Method (10-05.01). The breads were then cut into slices of approximately 15 mm thick and stored in foil bags with oxygen absorbers for further analysis. Bread crust color was measured using a CIE-LAB color system (XITIAN machine equipment Co., Ltd., Huizhou, China) to obtain the Hunter L*, a*, and b* values. Bread crumb structure was determined using a C-Cell Bread Imaging System (Calibre Control International Ltd., Warrington, UK). The central slices from each loaf were used for testing, and number of cells, cell wall thickness, and cell volume were collected.

Texture profiles of bread crumb were measured using a TA-XT plus (Stable Micro Systems, Surrey, UK) following a previous method (Tebben and Li, 2019). Three slices from each treatment were measured on day 1, 4, and 7, respectively. The first texture profile analysis (TPA) was conducted after 24 hours of storage (i.e., day 1).

Moisture content of the bread was determined following AACC Method (44-15.02). Approximately 2 g crumb was cut from the loaf, accurately weighed, transferred to an aluminum pan, and dried in the air-oven at 135 °C for 3 hr. In order to investigate the effect of different types and amount of pulse flour on the water retention of the bread, moisture content was also measured on day 1, 4, and 7, respectively.

3.2.6 Statistical analysis

All the experiments and tests were conducted in at least duplicate. Data were analyzed using SAS statistical software, Version 9.4 (SAS Institute, Cary, NC, USA) following Tukey's multiple comparisons test and analysis of variance (ANOVA). The significant level was considered at $p < 0.05$.

3.3 Results and discussion

3.3.1 Flour properties

Moisture content of the whole wheat flour and pulse flours is summarized in Table 1. The whole wheat flour contained 12.92% moisture, which was much higher than the pulse flours. Among all the pulse flours, green pea flour (8.04%) and chickpea flour (7.95%) had significant lower moisture content. This result is consistent with a previous study (Borsuk *et al.*, 2012). Overall, flour moisture content is related to the post-harvest and environmental condition of grains and milling process.

Protein, ash, lipid, and total carbohydrate contents are also shown in Table 1. The whole wheat flour had significantly lower protein content (15.13%) compared to the pulse flours. The protein content of pulse flours ranged from 19.82 to 24.22%. The protein content of pulse flours are highly variable and related with the genetic and environmental factors (Man *et al.*, 2015). There was no significant difference in the protein content among different types of the pulse flour. Rebello *et al.* (2014) indicated that pulse flours have higher protein content and a higher amount of lysine, which are much higher than that in cereals such as wheat. Whole wheat flour possessed the lowest ash content (1.8%), and chickpea flour contains the highest amount of ash content (2.66%) among these

six types of flours. The ash content of whole wheat flour and pulse flours ranged from 1.80 to 2.66%. The higher ash content in pulse flours might be due to the higher mineral content, fiber content, and thicker seed coat outside the pulse grains (Summo *et al.*, 2019). These results were consistent with the previous literature (Rebello *et al.*, 2014), which also showed that pulse flours had higher protein and fiber content than whole wheat bread flour. Chickpea presented the highest amount of lipid (5.10%). The same results were also presented by Dalgetty and Baik (2003). Chickpea contains high protein digestibility and high lysine content (Mohammed *et al.*, 2012; Wani and Kumar, 2014). The carbohydrate content of whole wheat flour was 68.36%, and the pulse flours ranged from 61.96 to 67.56%. Starch and dietary fiber are two primary components that build up the carbohydrates. Most of the polysaccharide is in the form of starch granules in whole wheat flour, and humans have better starch digestibility of cereal products than pulse products (Rebello *et al.*, 2014). Lentil contains 20.6 – 31.4% protein, a high amount of fiber, and a low amount of fat, with a high proportion of globulins (Jarpa-Parra, 2018).

The average particle sizes of whole wheat flour and pulse flours was shown in Table 1. Chickpea had a significantly larger particle size (427.01 μm). The average size of whole wheat and commercial yellow pea flour was 160.77 and 131.62 μm , respectively, and were much smaller than the chickpea flour. There were no significant differences between the average particle size of the yellow pea (242.55 μm), green pea (331.59 μm), and red lentil flour (236.84 μm). The particle size distribution curves are shown in Fig. 1. The hull and hardness of the seeds affect the grinding properties of whole pulses. Most of the flours fell in the range of 53 to 105 μm screen for whole wheat flour, and this is attributed to endosperm flour fraction (Doblado-Maldonado *et al.*, 2012).

Doblado-Maldonado et al. (2012) also indicated that although a smaller particle size of whole wheat flour could release more nutritional value, moderate particle size performed better in the bread structure and quality. Most of the particles of yellow pea (81.95%), green pea (84.23%), and red lentil (76.70%) flour were fell between 149 and 420 μm screen. However, 43.93% of the chickpea particle was on the 595 μm screen. The largest particle size showed in chickpea is because the highest lipid content, which resulted in the high amount of flour agglomeration on the 595 μm screen and cannot be sifted. Since there was a small peak of chickpea flour around 250 μm in the particle size distribution curve, it could be concluded that the actual particle size of chickpea should be similar with the other milled pulse flours. The overall particle size of lab-grinded flour is larger than the whole wheat flour and commercial yellow pea flour, due to the different milling process and grain properties. The same trend also presented in Bourré et al. (2019), and they also concluded that there was more starch damage in the finely ground flours.

3.3.2 Dough properties

3.3.2.1 Mixing characteristics

The mixing characteristics of the whole wheat dough incorporated with pulse flours are summarized in Table 2. Fig. 2 shows an example of mixolab graphs of whole wheat with 0, 5, 15, and 25% yellow pea flour. The mixing properties of dough were determined at 30 °C, and the optimal water absorption was determined by the Mixolab when the mixing torque value reached 1.1 Nm. The addition of pulse flours considerably affected the water absorption of the dough. Increasing the amount of pulse flours presents a higher water absorption value compared to the whole wheat flour (70.9%), except for 25% commercial yellow pea flour (70.0%). Incorporation of 25% red lentil

flour significantly increased the water absorption (74%). This might be due to the larger particle size and non-dehulled coat. There was no significant difference between yellow pea and chickpea flour on water absorption when the amount of pulse flours increased. The water absorption was significantly increased by adding green pea and red lentil flour, especially with 25% red lentil flour (74%). This might be due to the better hydrophilic factor of red lentil protein. Adding 25% commercial yellow pea flour presented a lower water absorption value than that with 25% yellow pea flour, which might be due to the smaller particle size. Besides, the proportion of bran in whole wheat flour also influenced the water absorption. Bourré et al. (2019) found that water absorption is affected by pulse flours' particle size and finer flours presented lower water absorption value. However, this study also indicated that the water absorption is related to the particle size and damaged starch, surface area, protein content, fiber content, and starch content.

Dough development time was affected by pulse flour addition, especially by adding the yellow pea flour. Adding 5% chickpea flour slightly increased the development time of the dough (8.88 min); this result was consistent with the previous study (Mohammed *et al.*, 2012). There was no significant difference between the whole wheat flour (7.41 min) incorporated with 5% of different types of pulse flour, and also no difference was found between 15% of different types of pulse flour. Increasing the amount of pulse flour decreased development time when the dough reached the maximum consistency, especially for the addition of 25% pulse flour; among these, adding 25% of yellow pea flour presented the shortest development time (4.50 min).

The stability of dough was positively correlated with development time and also significantly affected by pulse flour addition. Adding 25% of yellow pea flour and green pea flour significantly

decreased the dough's stability, and the value was 4.85 and 5.15 min, respectively. These results are also presented in the dough index properties (Fig. 3). The mixing index of adding 25% of yellow pea flour and green pea flour decreased drastically compared to other pulse flours. Yellow pea flour possessed less protein content than other pulse flours. The addition of 5% chickpea flour presented the highest value of stability (10.05 min), which was similar to the control whole wheat flour (9.80 min). Besides, adding 15% of chickpea flour did not significantly affect stability, and adding chickpea flour showed a less negative influence on dough stability. The stability time of dough indicated the flour strength. Incorporated with pulse flour decreased the dough's strength; this might be because the dilution of gluten concentration blocks the gluten network development in the whole wheat dough (Roccia *et al.*, 2009). The same results were also reported by Sadowska *et al.* (2003). No significant difference was shown in mechanical weakening value, ranging from 0.069 to 0.110 Nm, which indicated the protein weakening is similar between different types and amount of pulse flours in the whole wheat dough. Overall, the addition of 5% pulse flour did not obviously negatively affect the dough mixing properties and demonstrated better potential for the preparation of pulse-fortified bakery products.

3.3.2.2 Pasting characteristics

The pasting characteristics of the whole wheat dough incorporated with pulse flours are shown in Table 3. Increasing the amount of pulse flours decreased the dough's gluten strength index, since the lower proportion of whole wheat flour resulted in the gluten's lower resistance during heating (Fig. 3). Besides, the addition of pulse flours decreased maximum viscosity and reduced amylase activity of whole wheat dough properties. Adding 15% yellow pea flour, 15% chickpea

flour, and 25% chickpea flour slightly increased retrogradation index resulted in a shorter shelf-life of whole wheat bread. The minimum torque in the protein weakening phase, which was ranged between 58.2 and 61.8 °C, decreased as the amount of pulse flours increased. There was no significant difference for the temperature at minimum torque. The highest value of minimum torque is presented in the whole wheat flour (0.486 Nm). Incorporated with 25% of commercial yellow pea (0.330 Nm), yellow pea (0.351 Nm), green pea (0.376 Nm), red lentil (0.411 Nm), and chickpea flour (0.332 Nm) showed lower minimum torque among different types and amount of pulse flours. The commercial yellow pea flour presented a smaller value in minimum torque than yellow pea flour. The minimum torque values of lab-grinded pulse flours (except for chickpea flour) were positively correlated with particle size. These results may relate to the smaller particle size. Particle size and substitution level affected the pasting and thermal properties, where increasing the amount of pulse flour decreased the consistency of the dough (Marchini *et al.*, 2021). Besides, pulse flour diluted the gluten proteins which can also result in lower minimum torque. The thermal weakening parameter was calculated by the torque (C1) in the mixing process minus minimum torque. There was no significant difference in the value of thermal weakening among adding 5% different pulse flours. The value of thermal weakening was positively correlated with the amount of pulse flour. Intolerance to thermal weakening was important to determine bread-making quality (Simsek *et al.*, 2016).

During the thermal process, the protein starts unfolding and aggregating. The starch starts swelling in the limited available water, ruptures and separates until the amylose, amylopectin, and granule fragments are formed (Rosell *et al.*, 2007). The peak viscosity of whole wheat dough was

1.510 Nm. Peak torque was significantly affected by the substitution level of pulse flours, especially the 25% of yellow pea presented the lowest peak viscosity (1.152 Nm). Increasing the substitution level of pulse flour decreases peak viscosity (peak torque) due to increased protein content in pulse flour. The gluten blocks the contact area between separated starch granules and influences the dough gelatinization; gluten absorbs excess water and affects the starch swelling and gelatinization time (Xu *et al.*, 2020a). The peak viscosity of pulse flours is in the order of yellow pea flour < green pea flour < chickpea flour < red lentil flour < commercial yellow pea flour. The value of peak torque on each substitution level had a positive relationship with the protein content of pulse flour. The interaction between starch and protein affects food matrix through the inter-and intramolecular forces in macroscopic structure (Jekle *et al.*, 2016).

Overall, the cooking stability was not significantly affected by the different types and substitution levels, except for 25% yellow pea flour (0.895 Nm), 5% chickpea flour (1.174 Nm), and 15% chickpea flour (1.138 Nm). Cooking stability, also called hot gel stability, indicated the stability of previously broken starch granules during the heating process (Rosell *et al.*, 2007). 25% yellow pea flour significantly decreased the hot gel stability, while 5 and 15% chickpea flour significantly increased the hot gel stability. These results might be due to the starch content in the pulse flours. The setback was calculated through the torque in starch retrogradation in the cooling phase minus the torque in the hot gel stability phase ($C_5 - C_4$). There was no significant difference in the value of setback between different treatments. The setback indicated the straight-chain amylose molecules' realign and form the stable gel structure (Gadallah, 2017). The dough incorporated with 25% yellow pea flour presented the lowest value (0.128 Nm), while the dough with 15% chickpea flour presented

the highest value (0.260 Nm). This might be due to the higher protein content providing better stability for starch granules at high temperatures (Shevkani *et al.*, 2015). The overall tendency of the setback value was slightly increased at the 15% level and then decreased at the 25% level.

The alpha, beta, and gamma values of the fortified whole wheat flour dough are shown in Table 4. The alpha, beta, and gamma slopes represent the protein weakening speed, starch gelatinization speed, and enzyme degradation speed. The protein weakening speed was not significantly changed by the types and the amount of pulse flour. The starch gelatinization and enzyme degradation speed are mostly related to the starch component. Adding 5% red lentil flour (0.424), 5% chickpea flour (0.428), and 15% chickpea flour (0.442) slightly increased the starch gelatinization speed of the dough. Again, this might be due to the protein content and starch components.

3.3.3 Bread properties

3.3.3.1 Bread volume and structure

The specific volume of whole wheat breads with pulse flours is shown in Table 5, and the photos of bread products are presented in Fig. 4. Increasing the amount of pulse flours slightly decreased the specific volume of the bread, except for 5% yellow pea flour. Overall, there showed no significant difference after adding 5, 15 and 25% of various pulse flour. The bread with the addition of 5% yellow pea flour (3.81 cm³/g) had a similar specific volume with the control whole wheat bread (3.82 cm³/g). Among the different pulse flour treatments, the dough containing 5% yellow pea flour presented better pasting properties and good mixing properties and remained the values similar to the whole wheat control dough, which may explain its better bread volume. The 5% yellow pea

bread also had smaller cell diameter (1.832 mm) and large number of cells (2969) among all the breads fortified with pulse flours. For the number of cells, there was no significant difference between the 5% yellow pea bread and the whole wheat control (2941). Yellow pea flour had the lowest protein content and larger particle size than commercial yellow pea flour. Adding 25% of yellow pea flour significantly decreased the number of cells and enlarged the cell volume, also decreased the bread volume. Adding 15% of yellow pea flour slightly decreased the specific volume and the number of bread cells. The same trend was also found in Kamaljit et al. (2010). Increasing the amount of pulse flours worsened the cell structure, except for red lentil flour. Adding 5 and 15% of pulse flours did not significantly influence the bread crumb structure. The number of cells and cell diameter are related to the gluten network production. Incorporated with higher amount of pulse flours not only had influence on the gluten development but also affected the dough stability during baking process.

Adding 25% green pea flour ($2.90 \text{ cm}^3/\text{g}$) and 25% red lentil flour ($2.84 \text{ cm}^3/\text{g}$) dramatically decreased the bread volume, compared with the control, which might be due to that the highest starch content hindered the gluten development. Lower water absorption of pulse flour might also resulted in the lower specific volume of bread. Jekle et al. (2016) proposed two interactions between protein and starch: the competitive hydration between protein and starch, and the gluten proteins produced a diffusion fraise on the starch granule surface and changed the starch granules diffuse water. The higher amount of pulse starch resulted in a larger cell wall and a higher amount of open crumb grain (Sadowska *et al.*, 2003). Incorporation of 5 or 15% of green pea flour and red lentil flour into whole wheat flour was acceptable due to the volume and structure of bread were not

reduced significantly. Unlike the other pulse flours, chickpea flour provided better specific volume and crumb structure when the substitution level arrived at 25%, and the specific volume was even higher than the 15% chickpea level. This might be related to the pasting characteristics of 25% chickpea flour, where the dough had a similar setback value with the whole wheat dough, and the flour had the highest lipid content. Mohammed et al. (2012) found that adding 20% of chickpea flour significantly decreased the white bread specific volume. Whole wheat bread dough had a more diluted gluten network than the white bread dough, thus the effect might be less obvious in the former system.

3.3.3.2 Color analysis

Bread crumb color properties are summarized in Table 6. The whole wheat breads fortified with different types and amount of pulse flours did not differ in terms of crust redness-greenness (a^*) and yellowness-blueness (b^*). The addition of pulse flours decreased Hunter L^* , which indicates that the bread became darker, especially with green pea, red lentil, and chickpea flours. Commercial yellow pea contained the lowest carbohydrate content, shown lighter color. This might be due to the Maillard reaction that reducing carbohydrates react with amino acids and results in an amino acid-sugar reaction (Mohammed *et al.*, 2014). Both carbohydrates and protein content are related to crust color. Besides, red lentil flour provided darker red color for the bread crumb, and green pea flour provided green color for the bread crumb.

3.3.3.3 Moisture loss and texture properties

The moisture content and moisture loss of bread are shown in Table 7. The overall tendency of bread moisture content decreased as the storage day increased (Fig. 5). On day 1, day 4 and day 7,

increasing the substitution level of pulse flours decreased the moisture content. Moisture content of the bread with commercial yellow pea flour significantly decreased with the storage day increased and was the lowest among all the breads and at all the three testing days. The bread supplemented with 5 and 15% yellow pea, green pea, red lentil, and chickpea flour did not differ in moisture content, ranged from 46.02 to 47.06%. Adding 5% chickpea flour provides the lowest moisture loss, which is about only 1.47%. From day 4 to day 7, adding 15% green pea presented the highest moisture decrease rate, and adding 5% chickpea presented lowest moisture decrease rate. Overall, largest moisture decrease rate was found for the bread with 25% commercial yellow pea flour, and the smallest moisture decrease rate for 5% chickpea bread. Incorporation with 5% of pulse flour slightly decreased the moisture loss during the one week storage. This might be due to the slightly higher water absorption of pulse flour to help the bread hold the moisture. Wang et al. (2004) found that protein is more attractive for water molecules than starch, results in the water in the protein is less mobile than in the starch granular. Whole wheat bread had large moisture loss which might be due to the higher gluten content. The bread moisture loss was related to several factors, e.g., gluten content, pulse flours protein content, and pulse flours particle size. From day 1 to day 4, 15% yellow pea bread showed the highest moisture decrease rate, and 15% green pea bread showed the lowest moisture content decreasing.

The whole wheat bread texture is significantly affected by pulse flours and storage time (Table 8). The tendency of whole wheat bread's hardness significantly increased as the storage day increased (Fig 6). On the first day, incorporating 5% pulse flours and 15% commercial yellow pea flour, yellow pea flour, green pea flour, and chickpea flour did not significantly change the hardness

of the bread. Especially, adding 5% red lentil flour slightly decreased the hardness of the bread, though not significantly ($p > 0.05$). With storage time increasing, breads with 5% yellow pea, green pea, red lentil, chickpea flour, and 15% yellow pea did not reveal a significant difference in bread hardness. These results might be related to the dough's starch retrogradation value from Mixolab analysis, that these doughs had similar starch retrogradation value in the cooling phase. Compared with the bread hardening rate of whole wheat bread, incorporating 5% lab-milled pulse flour delayed the hardening change of bread. Increasing the substitution level of pulse flours significantly increased the starch retrogradation value and resulted in a harder texture. The polysaccharides in the pulse flour protein contribute to the bread hardness (Aider *et al.*, 2012), and resulted the firmer texture of the bread with pulse flour.

3.4 Conclusions

Pulse flour particle size and content of protein and carbohydrate greatly affected the dough mixing and pasting properties. The addition of pulse flours led to gluten dilution, increased maximum consistency, decreased dough development time and stability. Adding pulse flours reduced shelf-life of whole wheat bread due to a higher rate of retrogradation. The smaller particle size of commercial yellow pea flour provided a worse effect on dough and bread properties compared to the lab-milled yellow pea flour with larger particle size. Dough viscosity decreased as the substitution level of pulse flours increased. Adding 5% of yellow pea flour did not obviously affect the bread volume, structure, and texture. Incorporating 25% of green pea flour or red lentil flour significantly decreased bread specific volume. Increasing the substitution level of pulse flours decreased the moisture content and increased the hardness of the bread. Overall, adding up to 5% of

pulse flours was acceptable with minimal effect on dough properties and bread quality and improved the nutritional value of whole grain bread. Compared with commercial yellow pea flour, lab-milled yellow pea flour is considered more suitable for bread preparation, and up to 15% of yellow pea flour is acceptable to substitute whole wheat flour.

Future research is necessary to understand the composition and molecular features of various pulse flours and their effect on dough properties, bread quality, and sensory attributes. Furthermore, based on the previous whole-grain formulation, in order to increase the loaf volume, decrease the crumb hardness, and improve shelf-life of whole wheat bread, vital wheat gluten, enzymes, hydrocolloids, or emulsifiers could be incorporated into the formula. Moreover, definition of Mixolab parameters need to be further understood and better predicted in the future since there presented some ambiguity.

3.5 Reference

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3.6 Tables

Table 3.1 Proximate composition (as-is) and average particle size of whole wheat and pulse flours.

	Moisture, %	Protein, %	Ash, %	Lipid, %	Total carbohydrate, %	Average particle size, µm
Whole wheat flour	12.92 ± 0.03a	15.13 ± 0.62b	1.80 ± 0.02e	1.80 ± 0.02b	68.36 ± 0.60a	160.77 ± 1.15c
Commercial yellow pea	10.45 ± 0.10b	24.22 ± 0.31a	2.18 ± 0.01cd	1.21 ± 0.06c	61.96 ± 0.49c	131.62 ± 12.05c
Yellow pea	9.47 ± 0.04c	19.82 ± 0.45ab	2.32 ± 0.04bc	0.84 ± 0.11d	67.56 ± 0.33ab	242.55 ± 6.26bc
Green pea	8.04 ± 0.03d	21.78 ± 0.04a	2.38 ± 0.01b	1.18 ± 0.04c	66.62 ± 0.10abc	331.59 ± 15.05ab
Red lentil	9.80 ± 0.15c	23.72 ± 1.19a	2.10 ± 0.08d	0.96 ± 0.06cd	63.43 ± 1.20bc	236.84 ± 0.41bc
Chickpea	7.95 ± 0.30d	22.19 ± 2.74a	2.66 ± 0.01a	5.10 ± 0.05a	62.10 ± 2.50c	427.01 ± 80.97a

*Means with different letters within each column denote significant differences ($P < 0.05$)

Table 3.2 Mixing characteristics of whole wheat/pulse flour blend.

	Water absorption, %	Development time, min	Stability, min	Mechanical weakening, Nm
Whole wheat	70.90	7.41 ± 0.46abc	9.80 ± 0.42a	0.096 ± 0.00a
5% Comm YP	72.00	7.65 ± 0.57abc	9.10 ± 0.14abc	0.075 ± 0.01a
15% Comm YP	71.80	6.35 ± 0.18bcde	7.35 ± 0.35defg	0.082 ± 0.02a
25% Comm YP	70.00	5.10 ± 0.03de	6.10 ± 0.14gh	0.076 ± 0.02a
5% YP	71.50	8.09 ± 0.52abc	8.70 ± 0.14abcde	0.081 ± 0.01a
15% YP	71.80	6.77 ± 0.78bcd	7.40 ± 0.14defg	0.082 ± 0.01a
25% YP	71.80	4.50 ± 0.04e	4.85 ± 0.21h	0.069 ± 0.00a
5% GP	71.80	7.77 ± 0.45abc	8.90 ± 0.28abcd	0.072 ± 0.02a
15% GP	73.00	7.52 ± 1.06abc	8.15 ± 1.34bcdef	0.089 ± 0.01a
25% GP	72.50	4.95 ± 0.32de	5.15 ± 0.07h	0.086 ± 0.00a
5% RL	71.80	8.29 ± 0.69ab	9.65 ± 0.21ab	0.089 ± 0.01a
15% RL	72.80	6.95 ± 0.21abcd	7.86 ± 0.07def	0.106 ± 0.02a
25% RL	74.00	6.18 ± 0.21cde	7.10 ± 0.14fg	0.110 ± 0.00a
5% CP	71.80	8.88 ± 0.18a	10.05 ± 0.07a	0.091 ± 0.00a
15% CP	72.00	7.21 ± 0.62abc	9.00 ± 0.14abc	0.101 ± 0.01a
25% CP	71.50	6.09 ± 0.54cde	7.20 ± 0.14efg	0.087 ± 0.01a

* Values are expressed as the mean and SD of three measurements. Means with different letters within each property denote significant differences ($P < 0.05$); CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea

Table 3.3 Pasting characteristics of whole wheat/pulse flour blend.

	Minimum torque (Nm)	Thermal weakening (Nm)	Temperature at minimum (°C)	Peak torque (Nm)	Cooking stability (Nm)	Setback (Nm)
Whole wheat	0.486 ± 0.02a	0.631 ± 0.002fg	58.6 ± 1.6a	1.510 ± 0.03a	1.041 ± 0.04bcde	0.213 ± 0.02abc
5% Comm YP	0.434 ± 0.01bcd	0.641 ± 0.004efg	58.9 ± 1.3a	1.401 ± 0.01abcde	1.063 ± 0.01abcd	0.171 ± 0.04abc
15% Comm YP	0.379 ± 0.01ef	0.675 ± 0.006cdef	58.2 ± 1.2a	1.332 ± 0.00cdefg	0.980 ± 0.02cdef	0.154 ± 0.00abc
25% Comm YP	0.330 ± 0.00g	0.741 ± 0.004ab	60.5 ± 2.5a	1.322 ± 0.04defg	1.018 ± 0.00bcdef	0.169 ± 0.02abc
5% YP	0.475 ± 0.00a	0.654 ± 0.018ef	58.6 ± 2.5a	1.425 ± 0.05abcd	1.073 ± 0.02abcd	0.186 ± 0.03abc
15% YP	0.423 ± 0.01cd	0.711 ± 0.002bc	61.2 ± 0.7a	1.353 ± 0.03bcdef	0.993 ± 0.02cdef	0.199 ± 0.02abc
25% YP	0.351 ± 0.01fg	0.775 ± 0.013a	58.8 ± 0.4a	1.152 ± 0.02h	0.895 ± 0.00f	0.128 ± 0.00c
5% GP	0.469 ± 0.02ab	0.650 ± 0.012ef	59.5 ± 0.1a	1.438 ± 0.06abcd	1.092 ± 0.00abc	0.195 ± 0.04abc
15% GP	0.430 ± 0.01bcd	0.678 ± 0.022cde	59.6 ± 2.3a	1.406 ± 0.05abcde	1.077 ± 0.10abcd	0.224 ± 0.06abc
25% GP	0.376 ± 0.01ef	0.750 ± 0.004ab	60.4 ± 0.6a	1.212 ± 0.00gh	0.954 ± 0.03def	0.147 ± 0.05bc
5% RL	0.477 ± 0.00a	0.651 ± 0.015ef	61.1 ± 1.6a	1.461 ± 0.03ab	1.054 ± 0.04abcd	0.144 ± 0.02bc
15% RL	0.452 ± 0.01abc	0.671 ± 0.018cdef	58.7 ± 1.4a	1.399 ± 0.05abcde	1.039 ± 0.01bcde	0.183 ± 0.00abc
25% RL	0.411 ± 0.01de	0.706 ± 0.020bcd	60.7 ± 0.7a	1.282 ± 0.03efg	0.922 ± 0.04ef	0.135 ± 0.02bc
5% CP	0.467 ± 0.01ab	0.600 ± 0.005g	59.8 ± 0.4a	1.470 ± 0.01ab	1.174 ± 0.00a	0.227 ± 0.01abc
15% CP	0.423 ± 0.00cd	0.664 ± 0.002ef	58.9 ± 1.3a	1.451 ± 0.01abc	1.138 ± 0.01ab	0.260 ± 0.01a
25% CP	0.332 ± 0.00g	0.727 ± 0.006b	61.8 ± 0.4a	1.272 ± 0.01fgh	0.963 ± 0.02def	0.242 ± 0.00ab

* Values are expressed as the mean and SD of three measurements. Means with different letters within each property denote significant differences ($P < 0.05$);

CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea

Table 3.4 Comparison of protein weakening (alpha), starch gelatinization (beta) and enzyme degradation (gamma) parameters.

	Alpha	Beta	Gamma
Whole wheat	-0.079 ± 0.007bcd	0.413 ± 0.010ab	-0.023 ± 0.016a
5% Comm YP	-0.077± 0.010abcd	0.388 ± 0.028ab	-0.030 ± 0.011a
15% Comm YP	-0.066 ± 0.003abc	0.389 ± 0.021ab	-0.054 ± 0.014a
25% Comm YP	-0.065 ± 0.001abc	0.382 ± 0.045ab	-0.033 ± 0.021a
5% YP	-0.074 ± 0.000abcd	0.362 ± 0.037ab	-0.047 ± 0.007a
15% YP	-0.069 ± 0.001abc	0.376 ± 0.045ab	-0.045 ± 0.007a
25% YP	-0.062 ± 0.006ab	0.302 ± 0.034ab	-0.032 ± 0.003a
5% GP	-0.079 ± 0.004bcd	0.410 ± 0.037ab	-0.029 ± 0.004a
15% GP	-0.087 ± 0.013cd	0.405 ± 0.041ab	-0.041 ± 0.001a
25% GP	-0.055 ± 0.001a	0.276 ± 0.074b	-0.023 ± 0.010a
5% RL	-0.076 ± 0.003abcd	0.424 ± 0.042a	-0.044 ± 0.003a
15% RL	-0.067 ± 0.004abc	0.401 ± 0.027ab	-0.034 ± 0.014a
25% RL	-0.073 ± 0.004abcd	0.327 ± 0.018ab	-0.046 ± 0.025a
5% CP	-0.081 ± 0.013bcd	0.428 ± 0.011a	-0.035 ± 0.007a
15% CP	-0.093 ± 0.004d	0.442 ± 0.011a	-0.040 ± 0.003a
25% CP	-0.074 ± 0.003abcd	0.392 ± 0.023ab	-0.053 ± 0.004a

* Values are expressed as the mean and SD of three measurements. Means with different letters within each property denote significant differences ($P < 0.05$); CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea

Table 3.5 Specific volume and C-cell properties of whole wheat/pulse bread.

	Specific volume, cm ³ /g	Number of cells	Cell Diameter /mm	Wall thickness, mm
Whole wheat	3.82 ± 0.07a	2941 ± 54a	1.879 ± 0.04bcd	0.420 ± 0.001bc
5% Comm YP	3.15 ± 0.12ab	2867 ± 31ab	1.825 ± 0.01cd	0.420 ± 0.003bc
15% Comm YP	3.43 ± 0.03ab	2818 ± 8ab	1.822 ± 0.01cd	0.425 ± 0.002abc
25% Comm YP	3.10 ± 0.05ab	2425 ± 60cde	2.066 ± 0.01ab	0.443 ± 0.001ab
5% YP	3.81 ± 0.22a	2969 ± 109a	1.832 ± 0.06cd	0.423 ± 0.009bc
15% YP	3.59 ± 0.11ab	2592 ± 34bcd	1.988 ± 0.11abc	0.435 ± 0.009abc
25% YP	3.16 ± 0.05ab	2220 ± 94e	2.157 ± 0.04a	0.450 ± 0.016a
5% GP	3.54 ± 0.06ab	2825 ± 60ab	1.753 ± 0.13d	0.415 ± 0.010c
15% GP	3.18 ± 0.13ab	2682 ± 76abc	1.815 ± 0.03cd	0.421 ± 0.000bc
25% GP	2.90 ± 0.11b	2285 ± 43de	2.008 ± 0.02abc	0.439 ± 0.004abc
5% RL	3.44 ± 0.24ab	2780 ± 17ab	1.855 ± 0.07bcd	0.428 ± 0.008abc
15% RL	3.00 ± 0.11ab	2732 ± 161abc	1.808 ± 0.07cd	0.424 ± 0.005bc
25% RL	2.84 ± 0.05b	2411 ± 132cde	1.892 ± 0.04bcd	0.423 ± 0.001bc
5% CP	3.50 ± 0.14ab	2919 ± 154ab	1.852 ± 0.04bcd	0.423 ± 0.003bc
15% CP	3.13 ± 0.11ab	2738 ± 37abc	1.932 ± 0.03abcd	0.425 ± 0.000abc
25% CP	3.31 ± 0.14ab	2663 ± 42abc	1.922 ± 0.02bcd	0.430 ± 0.000abc

* Values are expressed as the mean and SD of three measurements. Means with different letters within each property denote significant differences ($P < 0.05$); CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea

Table 3.6 Color analysis of whole wheat/pulse breads crumb.

	L*	a*	b*
Whole wheat	36.25 ± 0.64a	11.46 ± 0.89a	25.90 ± 1.40a
5% Comm YP	36.53 ± 0.69a	12.20 ± 0.12a	26.40 ± 0.54a
15% Comm YP	34.27 ± 2.04abcd	11.54 ± 0.23a	24.55 ± 0.09a
25% Comm YP	32.21 ± 0.48bcd	10.49 ± 1.86a	21.72 ± 1.36a
5% YP	34.81 ± 0.42abc	17.52 ± 8.93a	18.98 ± 10.02a
15% YP	33.96 ± 0.78abcd	11.45 ± 0.20a	24.05 ± 0.41a
25% YP	31.56 ± 0.00cd	11.40 ± 0.39a	22.72 ± 0.85a
5% GP	36.21 ± 2.23a	11.65 ± 0.01a	25.20 ± 0.13a
15% GP	33.13 ± 0.06abcd	11.45 ± 0.39a	23.86 ± 0.88a
25% GP	30.51 ± 0.57d	11.04 ± 0.01a	23.03 ± 0.69a
5% RL	35.57 ± 0.04ab	11.54 ± 0.37a	24.72 ± 0.04a
15% RL	33.09 ± 0.31abcd	11.21 ± 0.05a	22.92 ± 0.69a
25% RL	30.59 ± 0.83d	11.29 ± 0.43a	22.45 ± 1.78a
5% CP	36.41 ± 0.09a	12.28 ± 0.76a	25.97 ± 0.23a
15% CP	33.51 ± 1.85abcd	12.18 ± 0.54a	25.36 ± 1.43a
25% CP	30.74 ± 0.88d	10.94 ± 0.11a	20.68 ± 1.03a

* Values are expressed as the mean and SD of three measurements. Means with different letters within each property denote significant differences ($P < 0.05$); CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea. L* Lightness, a* redness-greenness, b* yellowness-blueness.

Table 3.7 Moisture content of whole wheat/pulse bread during storage.

	Day 1, %	Day 4, %	Decrease rate day 1- day 4, %	Day 7, %	Decrease rate Day4- day 7, %	Overall decrease rate, %
Whole wheat	46.82 ± 0.05ab	46.37 ± 0.28a	0.96	42.98 ± 0.49abcd	7.31	8.20
5% Comm YP	46.45 ± 0.47abc	45.92 ± 0.28ab	1.14	43.16 ± 0.78abcd	6.01	7.08
15% Comm YP	45.73 ± 0.27cde	44.38 ± 1.03ab	2.95	41.12 ± 1.01de	7.35	10.08
25% Comm YP	44.79 ± 0.14f	43.02 ± 0.94b	3.95	39.67 ± 1.38e	7.79	11.43
5% YP	47.06 ± 0.16a	45.81 ± 1.30ab	2.66	44.75 ± 1.11a	2.31	4.91
15% YP	46.17 ± 0.03abcd	44.24 ± 1.28ab	4.18	41.73 ± 0.67bcde	5.67	9.62
25% YP	45.58 ± 0.30cdef	44.64 ± 0.02ab	2.06	41.53 ± 0.35cde	6.97	8.89
5% GP	46.77 ± 0.08ab	46.40 ± 0.32a	0.79	44.29 ± 0.77abc	4.55	5.30
15% GP	46.02 ± 0.46bcd	45.61 ± 0.20ab	0.87	41.01 ± 0.90de	10.09	10.87
25% GP	45.27 ± 0.07def	43.50 ± 0.64ab	3.91	42.06 ± 0.29abcde	3.31	7.09
5% RL	47.00 ± 0.18a	46.12 ± 0.69ab	1.87	44.54 ± 0.24ab	3.43	5.23
15% RL	46.71 ± 0.30ab	45.77 ± 0.80ab	2.01	44.73 ± 0.03a	2.27	4.24
25% RL	45.94 ± 0.25bcd	45.42 ± 0.18ab	1.13	42.24 ± 1.11abcde	7.00	8.05
5% CP	46.48 ± 0.07abc	45.94 ± 0.54ab	1.16	45.01 ± 0.50a	2.02	3.16
15% CP	46.17 ± 0.00abcd	45.18 ± 0.27ab	2.14	42.47 ± 0.56abcd	3.78	5.85
25% CP	44.88 ± 0.06ef	43.92 ± 1.65ab	2.14	41.46 ± 0.25cde	5.60	7.62

* Values are expressed as the mean and SD of three measurements. Means with different letters within each property denote significant differences ($P < 0.05$);

CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpe

Table 3.8 Hardness of whole wheat/pulse bread during one-week storage.

	Day 1, g	Day 4, g	Increase rate day 1 – day 4, %	Day 7, g	Increase rate day 4 – day 10, %	Overall increase rate, %
Whole wheat	567.06 ± 36.32fg	853.74 ± 43.06e	50.56	1199.38 ± 260.09e	40.49	111.51
5% Comm YP	712.1 ± 61.83efg	973.47 ± 12.43de	36.70	1663.07 ± 90.51bcde	70.84	133.54
15% Comm YP	725.12 ± 59.52defg	1122.67 ± 123.00cde	54.83	1713.21 ± 96.98bcde	52.60	136.27
25% Comm YP	1056.81 ± 123.45abc	1392.67 ± 144.21abcd	31.77	2041.58 ± 258.22bcd	46.61	93.18
5% YP	580.03 ± 112.71fg	843.91 ± 28.76e	45.49	1231.22 ± 173.69e	45.89	112.27
15% YP	667.15 ± 112.71fg	897.78 ± 5.82e	34.57	1138.07 ± 165.77e	26.76	70.59
25% YP	993.23 ± 139.79bcd	1410.41 ± 68.88abc	42.00	2191.13 ± 135.79bc	55.35	120.61
5% GP	722.84 ± 110.58efg	1073.61 ± 112.58cde	48.53	1163.77 ± 109.99e	8.40	61.00
15% GP	822.15 ± 5.88cdef	1219.51 ± 141.75bcde	48.33	1907.13 ± 76.45bcd	56.38	131.97
25% GP	1219.74 ± 132.70ab	1552.77 ± 283.37ab	27.30	2276.93 ± 220.38ab	46.64	86.67
5% RL	550.99 ± 2.28g	1051.97 ± 40.25cde	90.92	1460.46 ± 169.68de	38.83	165.06
15% RL	992.63 ± 94.40bcd	1362.45 ± 184.44bcd	37.26	1588.22 ± 66.76cde	16.57	60.00
25% RL	1296.06 ± 105.56a	1793.29 ± 118.36a	38.36	2367.41 ± 81.26bcd	32.01	82.66
5% CP	734.98 ± 62.23defg	1131.76 ± 175.65bcde	53.99	1409.70 ± 38.27de	24.56	91.80
15% CP	963.08 ± 56.84bcde	1460.00 ± 71.59abc	51.60	2873.91 ± 249.33a	96.84	198.41
25% CP	1029.22 ± 102.67abc	1224.73 ± 272.05bcde	19.00	1971.11 ± 72.52bcd	60.94	91.51

* Values are expressed as the mean and SD of three measurements. Means with different letters within each property denote significant differences ($P < 0.05$);

CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea.

3.7 Figures

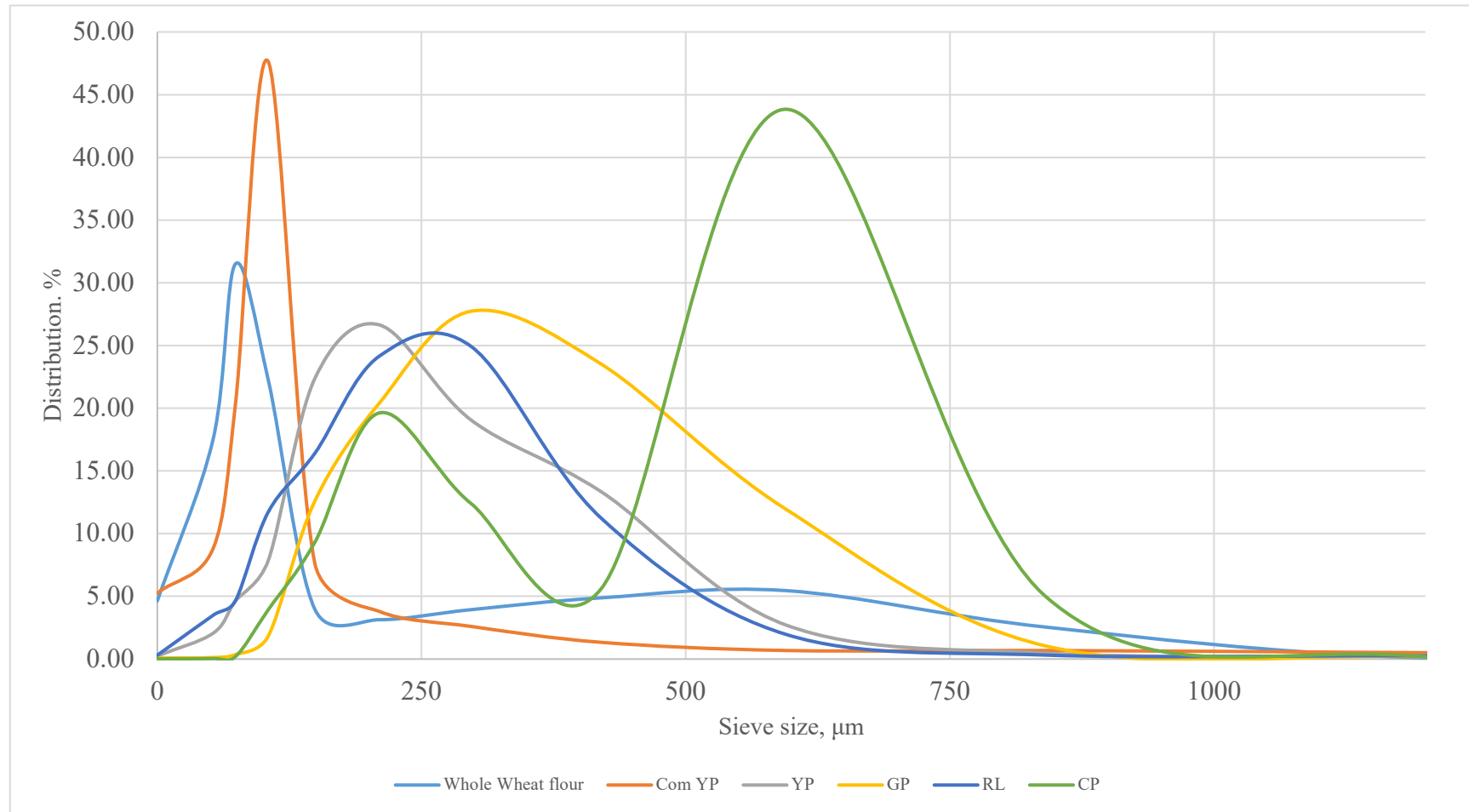


Figure 3.1 Particle size distribution of whole wheat flour and pulse flour. (CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea)

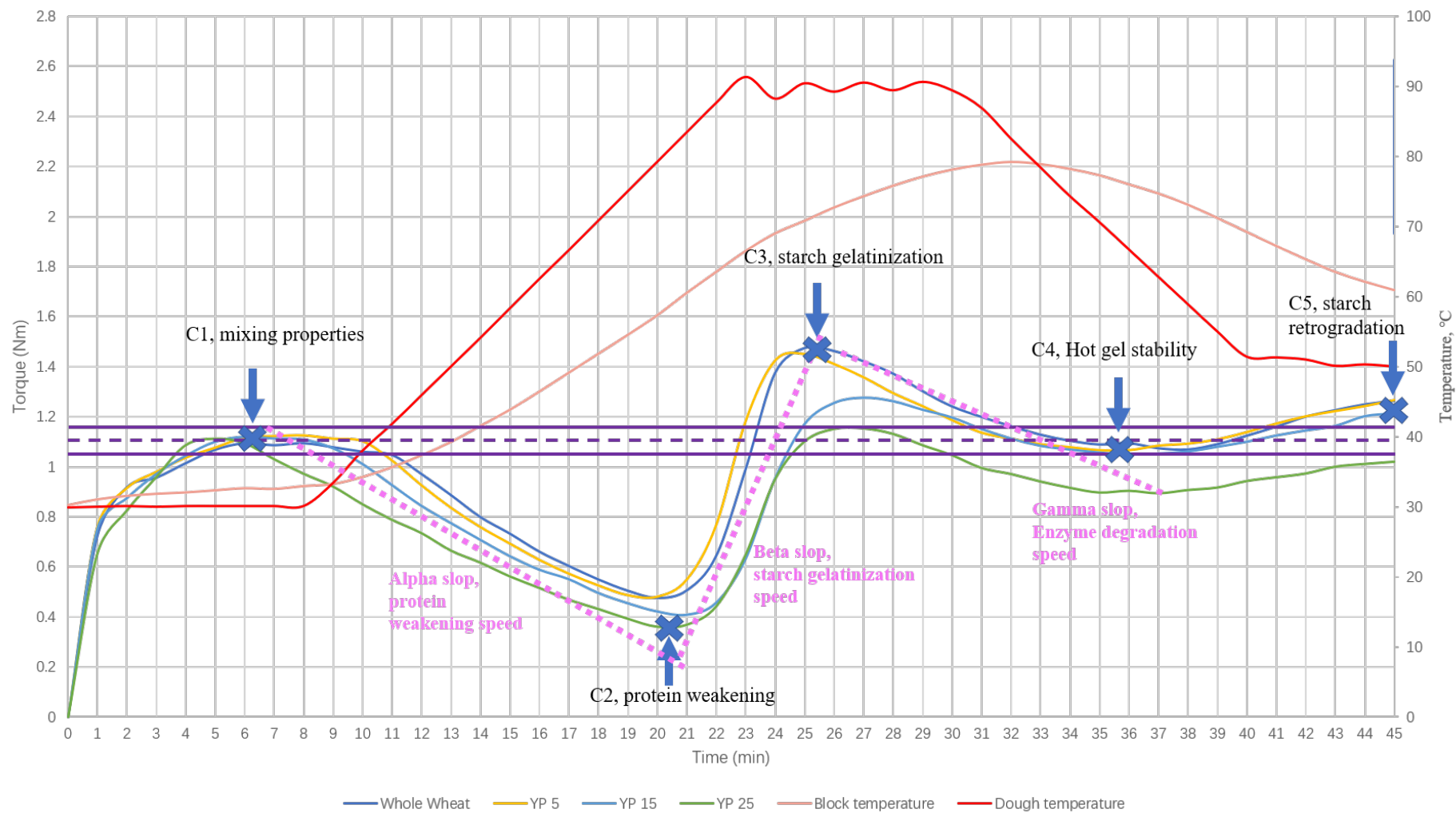


Figure 3.2 Example Mixolab graph (whole wheat, 5, 15, and 25% yellow pea flour). *YP yellow pea flour

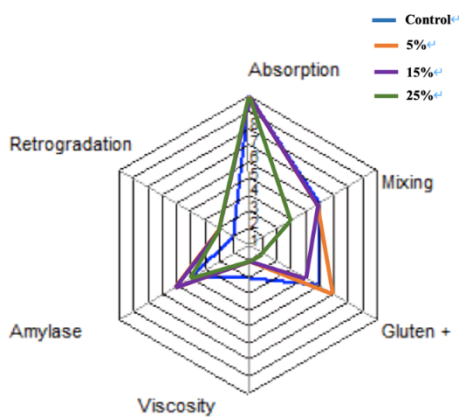
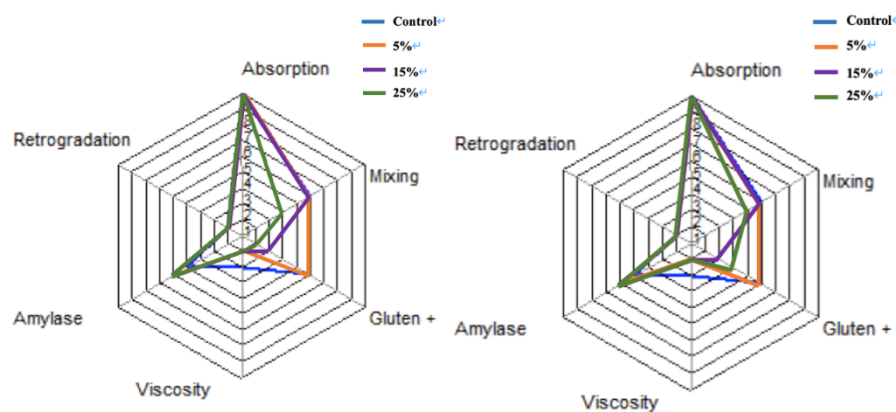
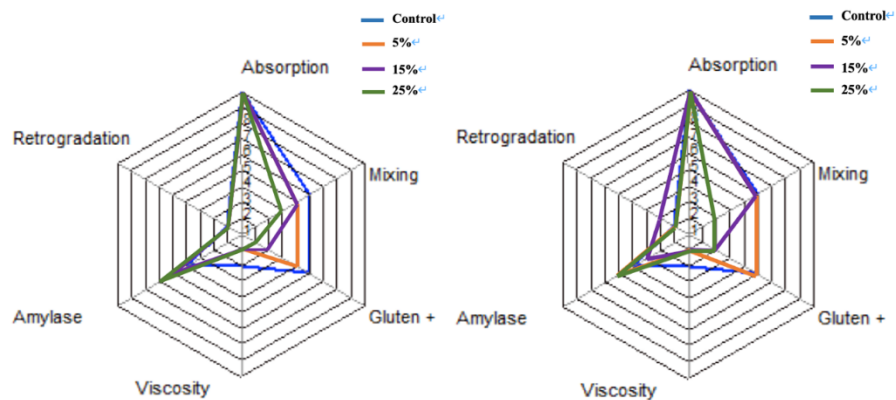


Figure 3.3 Mixing index of the whole wheat/pulse flours from Mixolab.

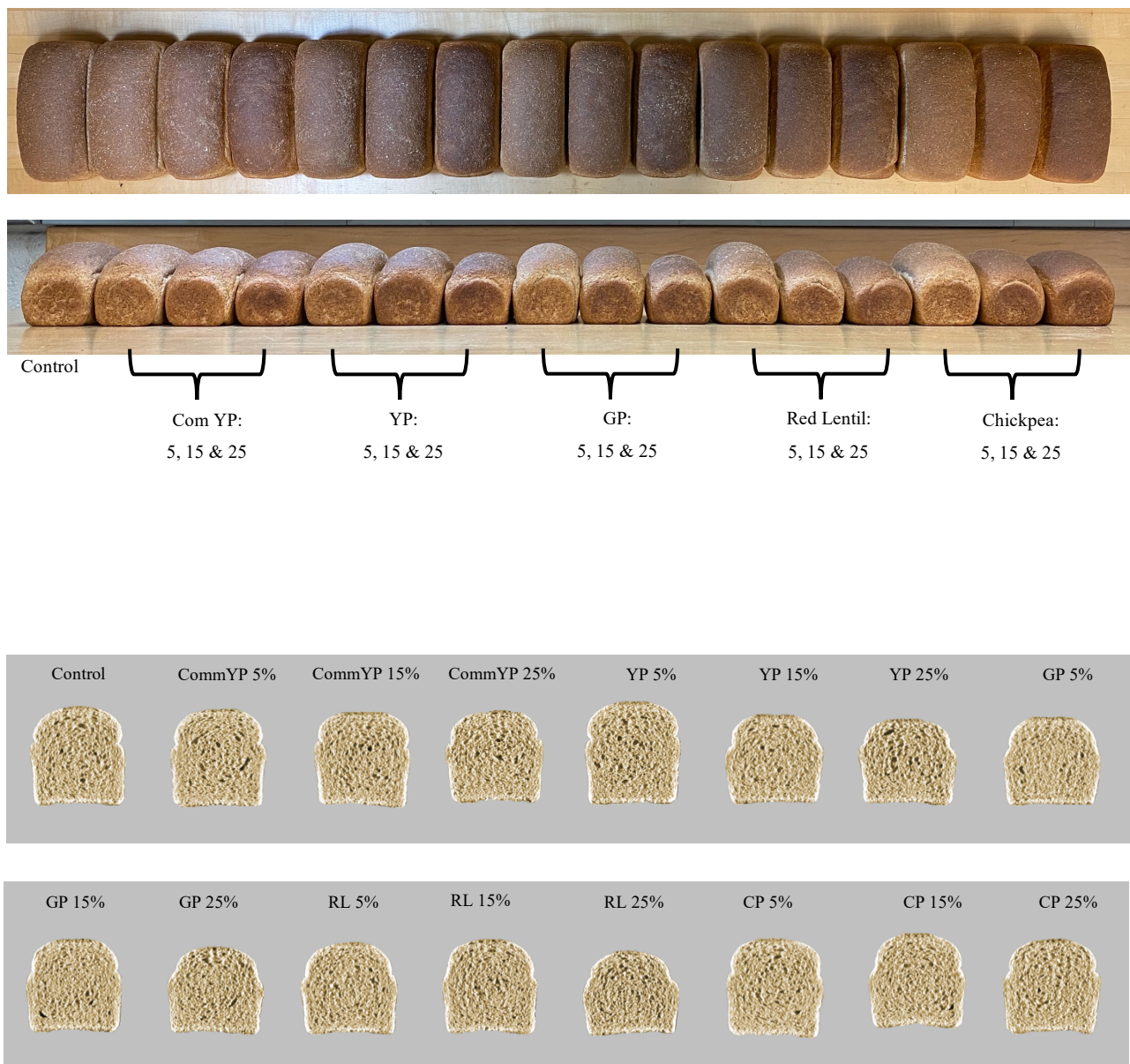


Figure 3.4 Bread photos and C-cell images.

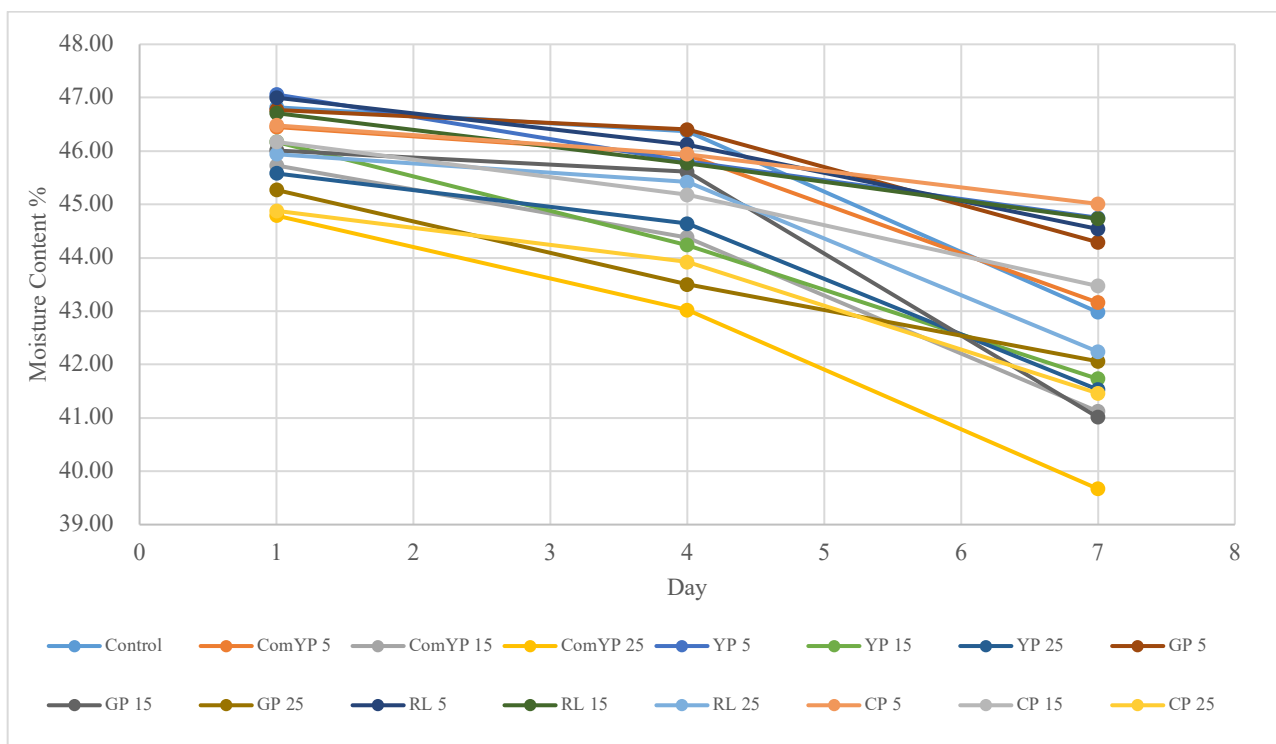


Figure 3.5 Changes of moisture content of whole wheat/pulse bread during one-week storage. (CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea)

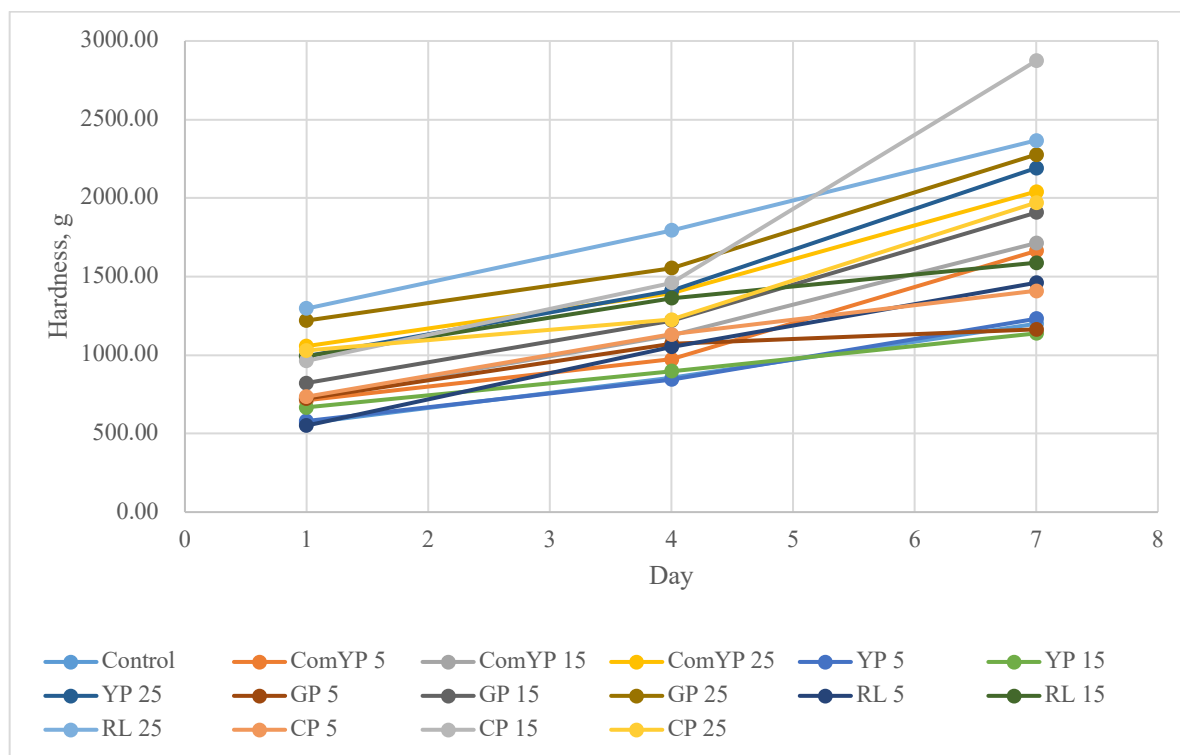


Figure 3.6 Hardness changes of whole wheat/pulse bread during one-week storage. (CommYP commercial yellow pea, YP yellow pea, GP green pea, RL red lentil, CP chickpea)

Appendix A - Statistical analysis code

A.1 SAS: Tukey's test for pairwise comparisons

```
proc glm data =stickiness=none;  
class treatment;  
model stickness = Fraction;  
means treatment;  
means treatment/ tukey;  
run
```