APPLICATION OF RESPONSE SURFACE METHODOLOGY IN THE DEVELOPMENT OF GLUTEN-FREE BREAD WITH YELLOW PEA FLOUR ADDITION.

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Yellow pea (*Pisum sativum* L.) flour fortified gluten-free (GF) bread formulation was optimized by response surface methodology (RSM) and the final product was evaluated for shelf life and sensory acceptability. A second-order model was fitted to the precooking temperature of pea flour (PTPF), water level, and proof time as the factors. Higher PTPF and lower proof time significantly ($P<0.05$) reduced the brightness of bread crumb. Crumb firmness was influenced by the PTPF, water level and proof time. The optimized parameters for PTPF, water level, and proof time were 156.9 °C, 523.8 g, and 18.0 min, respectively. The optimized bread had a brightness (L* value), specific volume, crumb firmness, and cell diameter of 68.2, 2.6 ml/g, 174.2 g_f, and 3.81 mm, respectively. The optimized GF bread had longer shelf-life, but had significantly ($P<0.05$) lower acceptance scores, than the commercial premix bread product.

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LIST OF ABBREVIATIONS

ANOVA .....Analysis of variance

CCD .....Central composite design

CD .....Celiac disease

CL .....Chain length

CMC..... Carboxymethylcellulose

COM .....Commercial premix gluten-free bread

cP..... Centipoise

FDS .....Fraction of design space

GF .....Gluten-free

HLA .....Human leukocyte antigens

HPMC .....Hydroxypropylmethylcellulose

IgA .....Immunoglobulin A

IgG .....Immunoglobulin G

LOX .....Lipoxygenase

MM .....Molecular Mass

OPT .....Optimized yellow pea gluten-free bread

P .....Probability level

PTPF .....Precooking temperature of pea flour

R^2.....Multiple correlation coefficients

RSM .....Response surface methodology

RVA .....Rapid Visco Analyzer

RVU .....Rapid Visco Unit

SMSS ..... Sequential model sum of square

SPV .....Scaled prediction variances

TIA ..... Trypsin inhibitor activity

TPA ..... Texture Profile Analysis

1. GENERAL INTRODUCTION

Dietary avoidance of gluten-containing cereals or gluten-free (GF) diet is a therapy for several conditions, such as celiac disease (CD), the skin rash dermatitis herpetiformis, and neurologic conditions such as gluten-sensitive ataxia (Hischenhuber and others 2006; Pietzak 2012). Following a GF diet is sometimes suggested to patients with Crohn's disease, ulcerative colitis, and irritable bowel syndrome (Engleson and Atwell 2008). Additionally, preliminary studies showed some degree of improvement in autistic children when GF and/or casein free diet is prescribed (Cromley 2008).

Population-based screening studies suggest that prevalence of CD may be as high as 1 in 100 persons (Mustalahti and others 2002). Family members of those with CD may choose to adopt the GF diet to reduce cross-contamination at home and aid in the ease of preparation of GF foods. There is also an increasing consumer sector that choose GF diet because they believe that a GF diet can help improve their overall health (Engleson and Atwell 2008). There are substantial GF foods market opportunities, due to the diverse population requiring or having an interest in consuming GF diets.

According to the World Health Organization (WHO) and the Food and Agricultural Organization (FAO) in 2000, GF foods are food stuffs "a) consisting of or made only from ingredients which do not contain any prolamins from wheat (*Triticum aestivum* L.) or all species such as spelt (*Triticum spelta* L.), kamut (*Triticum polonicum* L.) or durum wheat, rye, barley, [oats] or their crossbred varieties with a gluten level not exceeding [20 ppm]; or b) consisting of ingredients from wheat, rye, barley, oats, spelt or their crossbred varieties, which have been rendered "gluten-free"; with a gluten level not exceeding [200 ppm]; or c) any mixture of the two ingredients as in a) and b) with a gluten level not exceeding [200 ppm]."

Despite many on-going research studies, GF baking still represents significant technological challenges to food manufacturers (Gallagher and others 2004). Gluten is responsible for the viscoelastic properties of wheat dough. Removal of gluten protein significantly decreases bread flavor, loaf volume, color, and yields a crumbling crumb (Arendt and others 2002; Gallagher and others 2004; Sabanis and Tzia 2011b; Ylimaki and others 1991). Baking GF requires different technology than the traditional baking (Cauvain and Young 2007). Blends of GF flours, starches, hydrocolloids, enzymes, soybean proteins, and egg white have been suggested to mimic the viscoelastic properties of gluten in wheat dough (Defloor and others 1993; Gujral and others 2003; Gujral and Rosell 2004; Kim and Deruiter 1968; Kobylanski and others 2004; Sanchez and others 2002; Sanchez and others 2004; Torres and others 1999; Toufeili and others 1994).

Dry pea or edible field pea (*Pisum sativum* L.) is a leguminous crop grown abundantly in the United States. Dry pea is a good source of protein, total dietary fiber, minerals and vitamins. They can be milled into flour and classified as a GF ingredient. The state of North Dakota is the major producer of dry peas. United States Department of Agriculture National Agricultural Statistics Service (USDA-NASS 2011) reported that North Dakota ranked first in dry peas production, producing 57 percent of the Nation's dry peas. Currently, there is limited research available on how to incorporated yellow pea flour into GF breads

Pulse production and GF breads demand highlight a great opportunity to develop GF bread with yellow pea flour addition. The challenge of utilizing yellow pea flour is the undesirable pea flavors; therefore, pre-treatment or precooking of the flour is necessary. During the precooking stage, different temperature levels produce pea flour with different colors and pasting properties. This can affect the baking and end product characteristics of GF breads, as

well as the required amount of water and proof time. In this study, we determined the optimal precooking temperature of pea flour (PTPF), water, and proof time on GF breads. Because there is no standard GF baking method available, RSM was applied to identify optimal conditions for producing GF bread with yellow pea flour addition. This study was intended to demonstrate the technology of RSM and the value-added food application of yellow pea flour.

2. HYPOTHESIS

Yellow pea flour can be used to fortify GF bread with acceptable sensory and shelf life characteristics, by optimizing the PTPF, water addition and proof time during the baking process.

Ho: Yellow pea flour can be used to fortify GF bread.

H_A: Yellow pea flour cannot be used to fortify GF bread.

3. OBJECTIVES

1. To identify the optimal PTPF, level of water addition, and proof time to produce GF bread fortified with yellow pea flour.
2. Evaluate sensory and shelf life characteristics of the optimized GF bread fortified with yellow pea flour.

4. LITERATURE REVIEW

4.1. Celiac disease

4.1.1. Introduction

Gluten-sensitive enteropathy or CD is an autoimmune disorder that occurred in genetically predisposed individuals (Chirdo and others 2002; Thompson 2001). Those affected have reactions when exposed to wheat and other *Triticum* species. Prolamin fractions of *Triticum* species contain certain amino acid sequences that provoke autoimmune response and deteriorate small-intestinal villous (Chirdo and others 2002; Thompson 2001), resulting in a range of symptoms (Green and Jabri 2003). Population-based screening studies suggest that prevalence of CD may be as high as 1 in 100 persons (Mustalahti and others 2002).

4.1.2. Mode of action

The exact cause of CD is unknown. CD patients have strong genetic association with human leukocyte antigens (HLA) DQ2 and DQ8, as well as other currently unknown non-HLA genes located on chromosome 6 (Hunt and others 2008). Proteins with amino acid sequence domains rich in proline were found to be toxic for celiac patients (Wieser and Koehler 2008). Proteins with high proline are inadequately digested; consequently, large proline and glycine-rich peptides accumulate in the small intestine. These peptides can induce a variety of autoimmune responses depending on the amino acid sequences. The immune responses result in mucosal destruction and intestinal epithelial apoptosis (i.e., cell suicide) (Wieser and Koehler 2008). Serum Immunoglobulin A (IgA) and Immunoglobulin G (IgG) antibodies were produced as the result of stimulated T-cells activated B-cells. These antibodies can be used for noninvasive screening tests to diagnose CD (Pietzak 2012).

4.1.3. Symptoms and treatments

Celiac patients exhibit a wide range of symptoms including severe malnutrition, abdominal pain, chronic diarrhea, steatorrhea (excess fat in feces), vomiting, fatigue, and weight loss (Alaedini and Green 2005). In contrast, many celiac patients do not exhibit gastrointestinal symptoms (Bizzaro and others 2012). Instead, CD is commonly found associated with dermatitis herpetiformis, a disease causing blistering and severe itchiness of the skin. This condition is seen in about 10-20% of patients with CD (Leeds and others 2008). CD can also accompany neurological disorders that manifest as ataxia or neuropathy (Zone 2005), including multifocal leukoencephalopathy (inflammation of the white matter of the brain at multiple location), dementia, myoclonus (involuntary twitching of a muscle), myopathy (muscle weakness), myelopathy (inflammation of the spinal cord), stiff man syndrome, and multiple sclerosis (Volta and Giorgio 2010). Untreated celiac patients may develop serious complications such as osteoporosis, refractory sprue and malignant lymphoma (lymphoma of bone) that can be prevented if diagnose early (Rashtak and Murray 2012). The only treatment for diseases is the lifelong avoidance of gluten through a gluten free diet. However, gluten-sensitive individuals may tolerate minimum amount of gluten.

4.2. Yellow peas (*Pisum sativum* L.)

4.2.1. Introduction

Dry peas (*Pisum sativum* L.), which is also known as field pea, green pea, yellow pea, etc., is a cool season crop. Two phenotypes of field peas exist, namely smooth and wrinkled peas (Ratnayake and others 2002). This review only concentrates on field pea of smooth peas.

Pea is a predominant U.S. export crop in world trade (FAOSTAT 2012). The productions of dry peas in the world in 2010 reach approximately 7.5 MT (Figure 1).

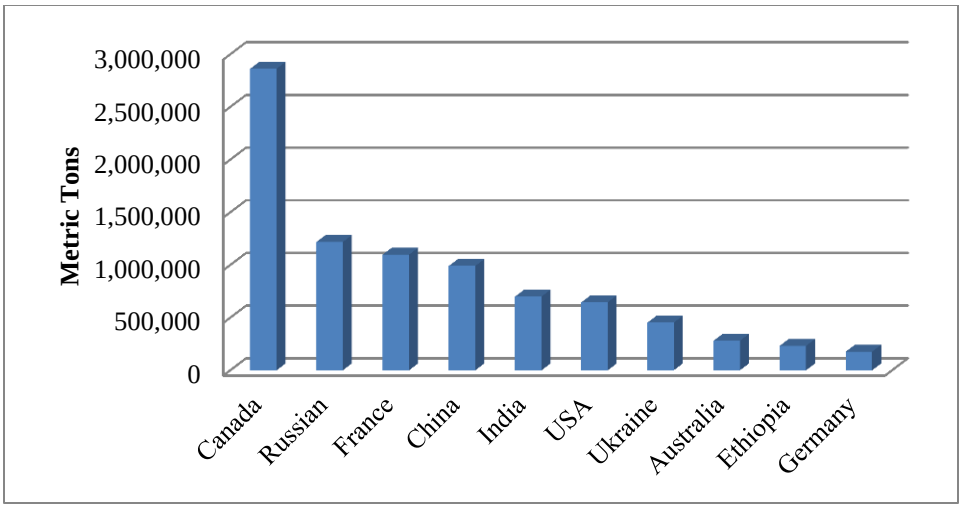

Figure 1. Countries with the highest dry pea production in 2010. Data from FAO Statistics Division, World Dry Peas Productions (FAOSTAT 2012).

In 2012, Canada had the highest production of dry peas at 2,862,400 MT, followed by Russia and France at 1,217,840 MT and 1,098,120 MT, respectively. United States of America ranked 6th in dry pea production, producing 645,050 MT.

4.2.2. Nutrition

Dry peas are economical source of calories, protein, dietary fiber, mineral and vitamins. Dry peas have high concentrations of potassium, magnesium and phosphorus and may provide sufficient amount of minerals to meet the human mineral requirement according to the recommended dietary allowance (National Research Council 1980; Iqbal and others 2006). Although dry peas are low in methionine, cysteine and cysteine, they are considered one of the cheapest high protein foods to offset the lysine deficiencies of cereal (Bahnassey and others 1986). Approximate levels of nutrient of mature split pea seeds are presented in Table 1 (USDA 2012).

Table 1. Nutritional content of mature split pea seeds (100 g).

Nutrient	Unit	Split peas
Water	g	11.27
Energy	kcal	341.00
Protein	g	24.55
Total lipid	g	1.16
Ash	g	2.65
Carbohydrate, by difference	g	60.37
Total dietary fiber	g	25.50
Total sugars	g	8.00
Minerals		
Calcium, Ca	mg	55.00
Iron, Fe	mg	4.43
Magnesium, Mg	mg	115.00
Phosphorus, P	mg	366.00
Potassium, K	mg	981.00
Zinc, Zn	mg	3.01
Vitamins		
Thiamin	mg	0.73
Riboflavin	mg	0.22
Niacin	mg	2.89
Pantothenic acid	mg	1.76
Vitamin B-6	mg	0.17
Folate, total	µg	274.00
Amino Acids		
Tryptophan	g	0.28
Threonine	g	0.87
Isoleucine	g	1.01
Leucine	g	1.76
Lysine	g	1.77
Methionine	g	0.25
Cystine	g	0.37
Phenylalanine	g	1.13
Tyrosine	g	0.71
Valine	g	1.16
Histidine	g	0.60

Data from the USDA National Nutrient Database for Standard Reference, Release 24 (USDA 2012).

4.2.3. Composition

4.2.3.1. Protein

Protein contents ranged from 23.1% to 30.9% (Gueguen and Barbot 1988; Boye and others 2010) with albumin and globulin representing 15–25% and 50–60% of the total protein, respectively (Gueguen and Barbot 1988). Two major globulins in peas are vicilin and legumins. The ratio of vicilin: legumin in dry pea ranged from 0.6 to 3.7 (Gueguen and Barbot 1988). The major albumin protein contains two polypeptides with molecular mass (MM) of ~25,000 Da; whereas, the minor albumin protein is a low MM protein containing polypeptides with MM of approximately 6,000 Da (Rao and others 1989).

4.2.3.2. Lipid

Peas have free and bound lipid contents of approximately 1.8-2.0%, and 0.8%, respectively (Chung and others 2008). Neutral, polar, and total lipids are present in smaller amounts and negatively correlated with protein. Table 2 shows the neutral and polar lipid composition of dehulled peas varying in protein content.

Table 2. Lipid percentage of dehulled peas varying in protein content.*

	Lipid, %		
Protein,%	**Neutral**	**Polar**	**Total**
14.5	2.5	1.6	4.1
18.3	2.1	1.6	3.7
24.2	1.8	1.5	3.3
28.5	1.5	1.5	3.0
Mean	**2.0**	**1.6**	**3.5**

*****Source: Data from Reichert and MacKenzie (1982)

Linoleic acid (18:2) is the major fatty acid in legumes (Table 3). Dry pea lipids contain 31.2% linoleic acid (Grela and Gunter 1995). High concentration of linoleic makes pea susceptible to lipoxygenase (LOX) activity. Dry pea has a complex LOX gene family made up of

at least five LOX enzymes (Domoney and others 1990). LOX promotes the oxidation of polyunsaturated fatty acids into hydroperoxide products, which negatively affect color, off-flavor and antioxidant status of pea (Casey and others 1996). LOX reduce shelf life in dry peas by contributing to the beany off-flavor to the end products (Wilson 1996). Crude LOX extract of dry peas are active under a broad range of temperatures between 10 and 30 °C. Trace LOX activities in the crude extract were observed at 50 °C or higher. Dry peas crude LOX was almost completely inactivated after heating for 3.0 min at 70 °C and 1.5 min at 75 °C (Gokmen and others 2002).

Table 3. Fatty acid composition of legume seeds (g per 100 g total fatty acids).

Legume	**14:0**	**16:0**	**18:0**	**18:1**	**18:2**	**18:3**	**Other***
Common bean	0.2	16.8	3.5	13.9	43.1	12.4	10.1
Field pea	0.4	17.1	4.8	19.1	31.2	7.1	20.3
Garden pea	0.3	13.7	8.1	12.7	35.3	5.4	24.5
Lentil	0.4	17.9	2.0	20.1	37.6	6.9	15.1
Soybean	0.9	5.9	3.4	26.9	53.3	6.4	3.2

*Includes C10:0, C12:0, C20:0, C20:1, C20:2, C22:1 and unknown acids, adapted from Grela and Gunter (1995).

4.2.3.3. Starch

Scanning electron microscopy revealed that most dry pea starch granules are oval, some spherical, round and elliptical and irregularly shaped granules are also present (Ratnayake and others 2002). The granules varied in size around 2-40 µm (Ratnayake and others 2002). The total amylose content of dry pea starches was between 33.1 and 57.0% (Czuchajowska and others 1998; Ratnayake and others 2001). X-ray diffractometry revealed that dry pea starch exhibit a ‘C’ type diffraction pattern (Davydova and others 1995). Gernat and others (1990) have reported that the ‘C’ crystalline polymorph is a mixture of ‘A’ type (cereal) and ‘B’ type (tuber) (Figure 2). The crystalline polymorph is determined mainly by the chain length (CL) of amylopectin; ‘A’

type CL <19.7, B type CL ≥ 21.6, and starches exhibiting CL between 20.3 and 21.3 are A, B, or C type patterns (Hizukuri and others 1983).

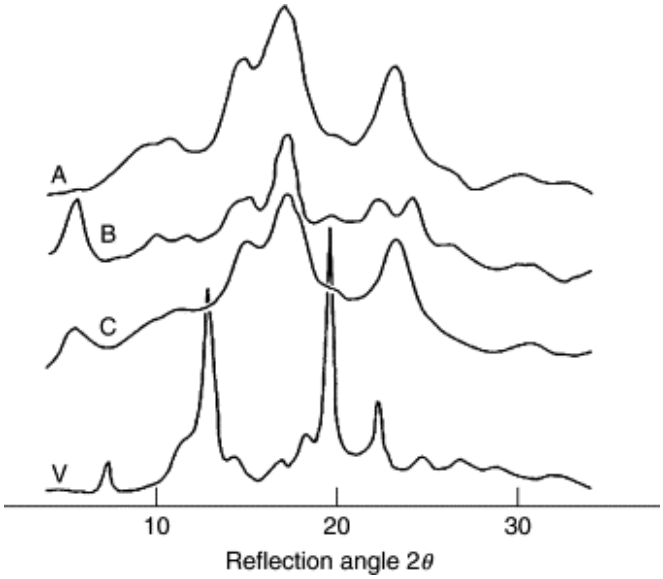

Figure 2. X-ray diffraction patterns exhibited by cereal starch (A pattern), tuber starch (B pattern), root and legume starches (C pattern), and amylose-lipid complexes starch (V pattern) as presented by Zobel (1988).

When starch is heated in excess water, the crystalline structure is disrupted, water molecules bonds to amylose and amylopectin, starch granules swell and solubilize, and disorder phase transition (gelatinization) occurs. Swelling power and solubility is influenced by the amylose/amylopectin ratio and distribution, degree of branching, length of branches, and molecule conformation (Ratnayake and others 2002). Gelatinization is associated with the hydration and swelling of starch granules, loss of optical birefringence, uptake of heat, loss of crystalline order, dissociation of double helices in the crystalline regions, and amylose leaching (Donovan 1979; Hoover and Hadziyev 1981; Hoover and Manuel 1996b).

Dry pea has the swelling factor of 4 to 27 and temperature ranged between 30.7 and 95 °C (Hoover and Manuel 1996b; Ratnayake and others 2001), significantly lower than values reported for mung bean (*Vigna radiate*), lentil (*Lens culinaris*), and beach pea (*Vigna marina*)

(Chavan and others 1999; Hoover and Manuel 1995; Ratnayake and others 2001), but higher than pinto *(Phaseolus vulgaris* L.) (Hoover and Manuel 1996a). Dry peas have the onset, midpoint, and end gelatinization temperatures of 61, 67, and 76°C, respectively (Ratnayake and others 2001).

4.2.3.4. Starch pasting properties

The starch pasting properties reflects the starch water retention and swelling capacity. The pasting properties can be determined using the Rapid Visco Analyzer (RVA). RVA is conducted by suspending samples in a solvent (water), apply appropriate shearing and temperature conditions, and pasting curve is then obtained when the test is completed. Figure 3 illustrates the typical RVA pasting curve profile.

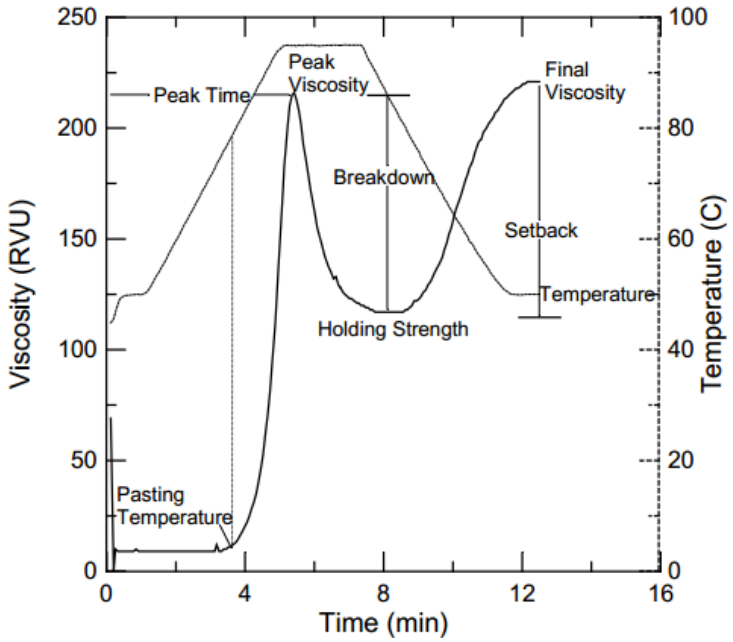

Figure 3. Typical starch pasting curve showing the measured values obtained from Rapid Visco Analyzer. Source: Newport Scientific operation manual (1998).

During heating, the starch granule imbibes water and swells, the crystalline structure melts (gelatinization), granules break down, and gel forms (Batey 2007). The peak viscosity is

obtained when the number of swollen intact starch granules is at the maximum. Sciarini and others (2010) suggested the peak viscosity relates to the amount of water that the starches can uptake or the water binding capacity. Once the maximum viscosity is reached, the viscosity decrease due to the disruption of starch granule (breakdown). The decrease in viscosity ceases after the cooling stage begins. During the cooling stage, glucan chains from starch begin to reassociate and form gel (retrogradation), which is indicated by the setback value. The viscosity continues to increase even after the final temperature is reached. The viscosity at the end of the test is called the final viscosity. The peak viscosity, breakdown, setback, and final viscosity of peas are similar to lentil and chickpea (*Cicer arietinum* L), but lower than wheat, and brown rice (*Oryza sativa* L.) (Table 4).

Table 4. Comparison of pasting properties between pulses, wheat, and brown rice.

Sample*	Pasting temperature (°C)	Peak Viscosity (cP)	Breakdown (cP)	Setback (cP)	Final Viscosity (cP)	References**
Pea[1]	69.6	1214.3	143.3	650.7	1722.0	1
Lentil[2]	70.6	1272.0	189.5	633.5	1716.0	1
Chickpea[3]	70.3	1120.3	ND	480.0	1591.7	1
Wheat [4]	68.5	5310.0	2212	2364.0	5458.0	2
Brown rice[5]	69.4	2567.4	189.5	1159.2	2921.9	3

*[1]*Pisum sativum* L.; [2]*Lens culinaris;* [3]*Cicer arietinum* L.;[4]*Triticum spp;*[5]*Oryza sativa* L..
**Data from 1 Chung and others (2008); 2 Chung and others (2012); 3 Hossen and others (2011); Songtip and others (2012).

4.2.3.5. Fiber

Hull fraction of the pulse seeds contribute the majority of dietary fiber in pulse seeds, ranging from dry weight contents of 78% (chickpeas) to 89% (peas) (Dalgetty and Baik 2003; Tosh and Yada 2010). Dietary fiber of pulse consists of insoluble dietary fiber (IDF), i.e. cellulose, lignin, and some hemicelluloses, and soluble dietary fiber (SDF), i.e. pectin, gums, and

some hemicelluloses (Tharanathan and Mahadevamma 2003). The distribution between IDF and SDF of dry pea, common bean, chickpea, and lentil are similar (Table 5).

Table 5. The range of total dietary fiber, insoluble dietary fiber, and soluble dietary fiber in raw pulses (g/100 g).

Pulses*	Total dietary fiber	Insoluble dietary fiber	Soluble dietary fiber	References**
Beans, dry[1]	23–32	20–28	2–6	1, 2, 3, 4
Chickpeas[2]	18–22	10–18	4–8	5, 4, 6
Lentils[3]	18–20	11–17	2–7	5, 4
Peas, dry[4]	14–26	10–20	2–9	1, 7, 5, 8, 9

*[1] *Phaseolus vulgaris* L.; [2]*Cicer arietinum* L.; [3]*Lens culinaris*; [4]*Pisum sativum* L.
**Data from 1 Almeida Costa and others (2006); 2 Granito and others (2002); 3 Kutos and others (2003); 4 Perez-Hidalgo and others (1997); 5 Dalgetty and Baik (2003); 6 Rincon and others (1998); 7 Borowska and others (1998); 8 Martín-Cabrejas and others (2003); 9 Wang and Zhao (2008).

4.2.3.6. Precooking of pea flour

Legumes contain antinutrients such as trypsin inhibitors and lectins (hemagglutinins), which must be inactivated by processing. Lectin are very toxic to the intestinal cells but can be eliminated by heat treatment (Armour and others 1998; Van Der Poel and others 1990). Trypsin inhibitors could reduce nutritional quality of proteins and may cause pancreatic hypertrophy (Liener 1979). Soni and others (1978) found that trypsin inhibitor activity (TIA) of peas were reduced to 12.5, 4.3, and 0% of the original activity after boiling for 12.5 min, autoclaving at 15 lb/sq. inch for 15 min, and roasting with dry heat for 2 min at 200 °C, respectively. In addition to antinutrients, LOX responsible for the beany off-flavor and color changes of pea flour, is also inactivated by heat. The inactivation of LOX by heat treatment was present in the lipids section (p 9) in this review. Currently, there is no standard method for precooking of pea flour.

4.2.3.7. Food application of pulse ingredients

Pulse as whole or split seeds are common ingredients in the traditional cuisine of many countries. Pulses are an inexpensive protein source in the diets where animal proteins are either unaffordable or culturally unacceptable (i.e. Hiduism, Muslim) (Anonymous 1991). Extensive research has been conducted on the development of novel pulse-based products. Pulses can be milled into flour, and utilized directly into a variety of foods, or fractionated into the protein, starch, and fiber fractions. Pulse fractions can be used as functional ingredients for textural improvement, or nutrient enrichment (Boye and others 2010; Tosh and Yada 2010).

Addition of pulse flours to cereal based food products has been studied. Anton and others (2008) investigated that the effects of bean flours on tortillas at 15, 25, and 35% wheat flour substitution. Bean flour negatively affects dough rheology, firmness, cohesiveness, and rollability. Nutritionally, bean tortillas had significantly higher levels of protein, total phenols, *in vitro* antioxidant activity, and antinutritional compounds such as trypsin inhibitors than wheat bread. Tortillas with acceptable texture and improved nutrition profile were produced at 25% bean flours substitution.

Eneche (1999) fortified biscuits with millet (*Pennisetum glaucum*) and pigeon pea (*Cajanus cajan* Millsp.) flour blends at various ratio, and found that biscuits contained high proportions of protein (7.5-15.2%), fat (17.1-18.1%), and carbohydrates (60.2-66.5%). Sensory evaluation results indicated that the modified recipe bicuits had high sensory rating. Biscuits with the highest sensory score were made with 65% millet flour and 35% pigeon pea flour blends.

Pea fiber can be used to increase the dietary fiber level and enhance shelf-life of bread products. Edwards and others (1995) enriched pasta with pea fiber at 5% and 10%. However, only up to 10% addition was recommended because pea fiber decreased pasta firmness. In

breads, 2% pea fiber can be added to flour without deteriorating bread palatability. Pea fiber increased bread shelf life according to the texture study. The crumb and crust color of bread with pea fiber was comparable to the bread with cellulose fiber. Pea and cellulose fiber did not significantly impact bread specific volume, but increased the crumb firmness when compare to the control (Edwards and others 1995).

The use of pulse flours and fractions had been investigated in pasta products. Bahnassey and others (1986) fortified spaghetti with lentil, navy bean and pinto bean flours and protein concentrates at 10% and 15% levels. Pulse fortified spaghetti had higher nutrient levels when compared to the control. However, TIA in the enriched samples was higher than the control. The highest and lowest TIA was reported in the navy bean and lentil products, respectively. Pulse fortification at 10% yielded acceptable products according to the taste panels (Bahnassey and others 1986). Pasta-like products can be made from starch-rich pea flour from twin screw extruder. The extrusion cooking is a high temperature, short time processed, where the extrudates are texturally and histologically restructured (Smith 1971). Compared to commercial spaghetti, the pasta-like product had shorter cooking times, firmer and less sticky texture, and higher cooking loss (Wang and others 1999). High temperature extrusion pasta-like pea flour products had superior texture and flavor quality than those prepared using a low- temperature pasta extruder (Wang and others 1999).

Cai et al (2001) conducted a study on bean curd formation using pulse protein extracts from chickpea, faba bean (*Vicia faba* L.), lentil, mung bean, smooth pea and winged bean (*Psophocarpus tetragonolobus L.*) using calcium sulfate ($CaSO_4$) coagulation. The best result for curd formation occurred from a protein concentration of 2.3-3% and 1.5% $CaSO_4$. Soybean had

the best textural quality, followed by chickpea and faba beans. Low springiness and cohesiveness in curds were observed in lentil, smooth pea and mung bean.

4.3. Gluten-free baking

4.3.1. Introduction

There are two major types of storage protein found in seeds, prolamins and globulins (Shewry and Casey 1999). Prolamins are alcohol-soluble and found predominantly in cereal grains. Globulins are salt-soluble and found largely in dicots, but also are present in cereal grains (Stoger and others 2001). Prolamin could be divided further into alcohol-soluble gliadin and alcohol-insoluble glutenin in wheat, or glutelins in other species (Shewry and Tatham 1990). Wheat is composed of 15% albumin, 5% globulin, 33% gliadin, and 14% soluble glutenin, and 33% insoluble glutenin (Bushuk 1986). Dry peas are mainly composed of 15–25% albumin and 50–60% globulin of the total protein (Gueguen and Barbot 1988). However, peas lack gliadin and glutenin.

When wheat flour is mixed with water, glutenin and gliadin form gluten. Hydrated gliadin provides viscosity and extensibility of the gluten, while hydrated glutenin is both cohesive, elastic and contributes to the gluten elasticity and strength (Shewry and Casey 1999). Gluten provides structure to bread flour, and contributes to the appearance and crumb structure of many baked products. Removal of gluten present challenges for bakers (Krupa-Kozak and others 2011); therefore, production of quality baked products without wheat is difficult.

Current GF products in the market are low quality and exhibits poor texture and flavor (Arendt and others 2008; Moroni and others 2009) due to the absence of wheat. A literature search has indicated a limited number of papers on GF bakery products. In addition, GF baking

methods have not been standardized. Furthermore, there is no literature on GF baking with pulse flour available, which indicates the lack of knowledge of pulse flours.

4.3.2. Fiber in gluten-free baking

GF breads are usually low in dietary fiber because they are made commonly from refined flours and starches. A study involving forty-nine Swedish celiac patients on a GF diet showed that their intake of dietary fiber was lower when compared to a control group of people consuming normal diets (Grehn and others 2001). However, there are limited literatures on the effect of different fibers on GF breads.

Fiber is known to restrict expansion of GF dough gas cells, resulting in compact texture and structure in finished products (Collar and others 2007). Several research investigated methods to increase fiber contents in GF products. Chestnut flour contains high fiber and essential amino acids. Combination of chestnut (*Castanea sativa* Mill.)and rice flour were tested at different ratios (0:100, 10:90, 20:80, 30:70, 40:60, 50:50 and 100:0 w/w) (Demirkesen and others 2010). Elevated levels of chestnut flour at 40% level or higher, led to low loaf volume, hard texture and dark color. The optimal bread was obtained at 30:70 chestnut flour to rice flour ratio.

Bread fortified with Inulin, oligosaccharide syrup and bitter-free chicory (*Cichorium intybus*) flour at 3, 5, and 8% reduced crumb hardening rate, but reduced the crumb springiness in comparison to the control during the 3-day storage period (Korus and others 2006). The supplemented prebiotic were reported to delay water migration from crumb to crust in GF formula. The highest sensory scores were observed in breads with 5% inulin supplement.

Pseudocereal flours were used to replace corn (*Zea mays* L.) starch in GF breads to enhanced protein and fiber contents (Alvarez-Jubete and others 2010). Compared to the rice

flour-potato (*Solanum tuberosum* L.) starch based control, buckwheat (*Fagopyrum esculentum* Moench) and quinoa (*Chenopodium quinoa* Willd.) breads had higher volume, and softer crumb texture. No significant differences were observed in the acceptability of pseudocereal containing GF breads compared to the rice flour/potato starch (50:50 w/w) based control.

Wheat, maize, oat (*Avena sativa* L.), and barley (*Hordeum vulgare* L.) fiber were added at 3, 6, and 9 g/100 g levels in GF bread formulation based on corn starch and rice flour to enhance the fiber content. Maize and oat fiber addition had a positive impact on bread nutritional and sensory properties (Sabanis and others 2009). The sensory evaluation indicated that when fiber content increased, the powdery taste of GF bread also increased. Barley fiber bread had high loaf specific volume and intense color. Sabanis and others (2009) concluded that the presence of fibers, due to their high water binding capacity, keeps the crumb softer than the GF control.

4.3.3. Hydrocolloids in gluten-free baking

The development of GF bread is difficult because gluten is the main structure of the bread (Ylimaki and others 1991). Addition of hydrocolloids in GF bread is necessary to mimic viscoelastic properties of gluten and to increase gas retention capability. Sabanis and Tzia (2011a) reported that 1% and 1.5% gum addition (except from xanthan gum), contributed to bread with higher loaf volume, better color, and increased shelf life compared to control GF bread. Sensory evaluation by trained panel preferred 1.5% hydroxypropylmethylcellulose (HPMC) addition because of the loaf volume, appearance, and crumb firmness characteristics. However, Lazaridou and others (2007) reported highest overall acceptability score was for GF formulation supplemented with 2% carboxymethylcellulose (CMC).

Different hydrocolloids (pectin, CMC, agarose, xanthan, and oat β-glucan) as gluten substitutes were investigated (Lazaridou and others 2007). Generally, the volume of breads increased with addition of hydrocolloids except for xanthan. However, loaf volume decreased when the level of hydrocolloids increased from 1% to 2%, except for pectin. In most cases, addition of hydrocolloids did not significantly affect the water activity of crumb. Crumb firmness values were not significantly different between the control bread and breads with pectin, CMC and agarose (1-2%), and β-glucan (1%) (Lazaridou and others 2007). However, addition of xanthan (1-2%) and β-glucan (2%) increased crumb firmness.

4.3.4. Proof time of gluten-free bread

Proof time in GF bread studies varied from 20 to 75 min (Ahlborn and others 2005; Lazaridou and others 2007; Ribotta and others 2004). Bauer (1980) reported high quality GF breads, made with potato, corn and rice starches, when the proof time was increased. Pruska-K-Ödzior and others (2008) reported that a 40 min proof time, compared to 20 min, improved the taste, aroma, and mouth feel of GF breads based on maize flour, rice starch, and buckwheat flour.

Proof time of GF breads are generally lower than wheat breads, despite the relatively high yeast (*Saccharomyces cerevisiae*) concentration in the formula (McCarthy and others 2005). The low proof time necessary could be due to the lack of viscoelastic gluten network. It is possible that gluten network in wheat loaves requires high pressure from the carbon dioxide produced by yeast to promote expansion. GF breads can be expanded easier and faster, but the negative effect is the lack of loaf stability (McCarthy and others 2005). Rather than proofing the GF breads to time, Schober and others (2005) used proofing to height method in a GF

sorghum (*Sorghum bicolor* (L.) Moench) bread study. Drifts in room temperature or yeast activity were possible causes for varying results when GF breads were proofed to time.

4.3.5. Gluten-free bread shelf life

Bread shelf life is influenced by moisture loss, staling, and microbial growth (Willhoft 1971). Various mechanism of bread staling have been proposed, however nothing definite can be concluded at present time. The most acceptable hypothesis is that retrogradation of amylopectin occurs, and because water molecules are incorporated into the crystallites, the distribution of water is shifted from gluten to starch/amylopectin, thereby changing the nature of the gluten network (Gray and Bemiller 2003; Sciarini and others 2010). Water migrates from crumb to crust leads to a glass to rubber transition of the two components, also play significant role in bread firming (Baik and Chinachoti 2000).

GF breads stale faster than wheat breads because: 1) GF dough/batter lacks a gluten network, and 2) GF breads are high in moisture. Without any gluten present in the GF bread systems, moisture migration from crumb to crust can occur more rapidly because gluten aids in retarding water movement (Sciarini and others 2010). The rate of starch retrogradation in wheat bread increases as moisture content is increased (Rogers and others 1988). High water level is necessary for making GF bread with higher specific volume, less dense crumb structure, and softer crust and crumb texture (Gallagher and others 2003a; McCarthy and others 2005). As GF breads typically have higher moisture level than wheat breads, the starch retrogradation and the staling rate may progress more rapidly (McCarthy and others 2005). Many studies also have reported that GF bread stale faster than wheat related products (Nishita and others 1976; Toufeili and others 1994; Kadan and others 2001). Studies on shelf life of GF breads indicated that the greatest changes in crumb hardness occurred in the first week (Gallagher and others 2003b;

McCarthy and others 2005; Ronda and Roos 2011). After that, crumb hardness stabilizes and did not increase significantly.

Figure 4 illustrates the comparison between crumb hardness between different GF bread studies. GF breads based on maize starch/rice flour (75:25 w/w), had the highest staling rate when compared to GF formulations based on wheat starch, rice flour/potato starch (50:50 w/w), and rice flour. The difference between the staling of the breads could be due to the differences in formulations and processing conditions. Currently, there is no literature identifying how different GF flours affect the staling properties of bread. However, the water absorption capacity of each GF flour could also be responsible for the different in staling rates. The importance of water on the shelf life of GF breads has been described above.

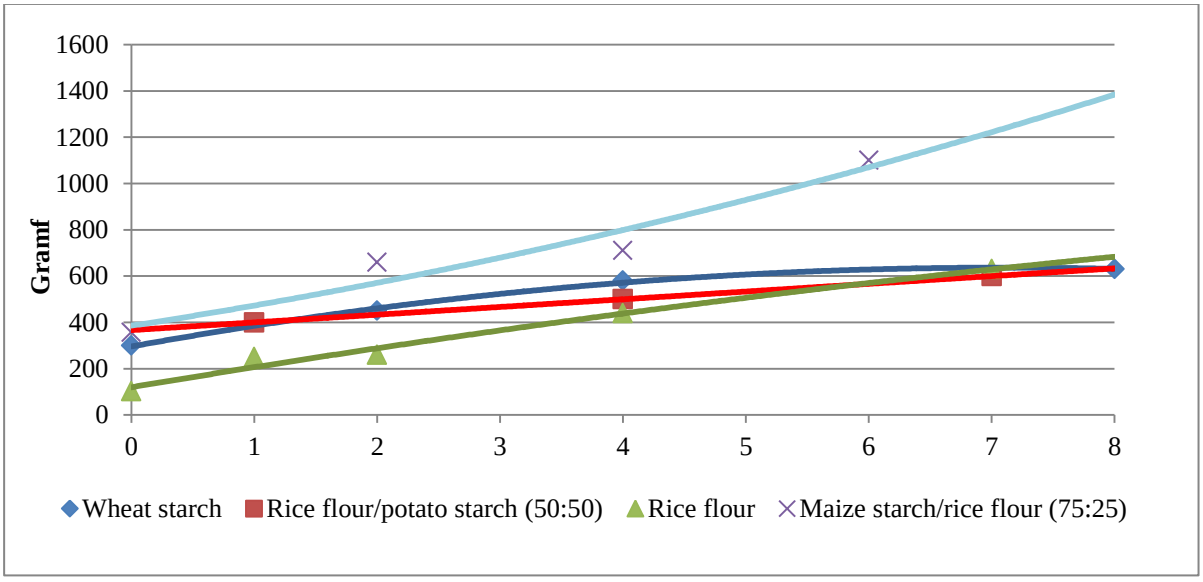

Figure 4. Comparison of crumb hardness between different gluten-free bread studies. Data for breads made with wheat starch (Gallagher and others 2003a); rice flour/potato starch (McCarthy and others 2005); rice flour (Ronda and Roos 2011); and maize starch/rice flour (Sabanis and Tzia 2011b).

4.3.6. Gluten-free breads made with various flour bases

Various GF flours have been tested in bread applications. Table 6 provides comparisons for crumb brightness (L*), crumb firmness (g_f), specific volume (ml/g), and cell diameter (mm) of bread made with different types of GF flour. Although the type of flours greatly affect the GF bread quality, it is important to note that the overall formulations, and processing methods also play significant roles in the final GF bread products. L* value range from 0 (white) to 100 (black). GF breads made from rice and potato starch had the brightest crumb color (L* =86). GF breads made from quinoa and buckwheat exhibited dark crumb color (L*= 51-52) when comparing the L* values. Crumb firmness, specific volume, and cell diameter results varied, and could be due to the overall formulations and processing conditions, as opposed to the flour sources.

Table 6. Comparison of bread characteristic made with various gluten-free flours.

Main flour**	Crumb brightness (L*)	Crumb firmness (g_f)	Specific volume (ml/g)	Cell Diameter (mm)	References***
Rice and potato starch	86	313	3.03	1.7- 13.0	1
Wheat starch	-	350	2.57	-	2
Sorghum flour	55- 63	479-826	1.71	2.07-2.68	3, 4
Rice flour	68 - 79	858-1500	1.92	-	5
Cassava starch	-	-	2.04	-	6
Corn/soy (90:10)	57	682-1342	-	-	7
Amaranth	56	-	1.31	-	8
Quinoa	52	-	1.40	-	8
Buckwheat	51	-	1.63	-	8

** Amaranth (*Amaranthus* L.); Buckwheat (*Fagopyrum esculentum* Moench); Cassava (*Manihot esculenta* Crantz); Corn (*Zea mays* L.); Potato (*Solanum tuberosum* L.); Quinoa (*Chenopodium quinoa* Willd.); Rice (*Oryza sativa* L.); Sorghum (*Sorghum bicolor* (L.) Moench); Wheat (*Triticum aestivum* L.).

*** Data from 1 McCarthy and others (2005); 2 Gallagher and others (2003c); 3 Schober and others (2005); 4 Frederick (2009); 5 Lazaridou and others (2007); 6 Lopez and others (2004); 7 Sciarini and others (2010); 8 Alvarez-Jubete and others (2010).

4.4. Response surface methodology

4.4.1 Introduction

Response Surface Methodology (RSM) is a statistical technique that has been successfully applied to developed cereal products (Gallagher and others 2003c; McCarthy and others 2005; Sanchez and others 2004; Toufeili and others 1994). RSM is useful to assess the effects and interactions of independent variables (factors), and enable us to estimate dependent variables (responses) and predict the optimum conditions for the process. RSM uses an experimental design such as the CCD (Box and Wilson 1951) to fit a model by least squares technique (Mason and others 2003; Myers and Montgomery 1995). The CCD contains fractional factorial matrix with the center point surrounded by factorial or cube points and axial points (Figure 5). Regular central composite designs have 5 levels for each factor. One unit was designated as the distance from the center of the design space to the cube point, while alpha designated the distance from the center of the design space to a star point. The axial point represents the new extreme values for each factor in the design (Plasun, 1999).

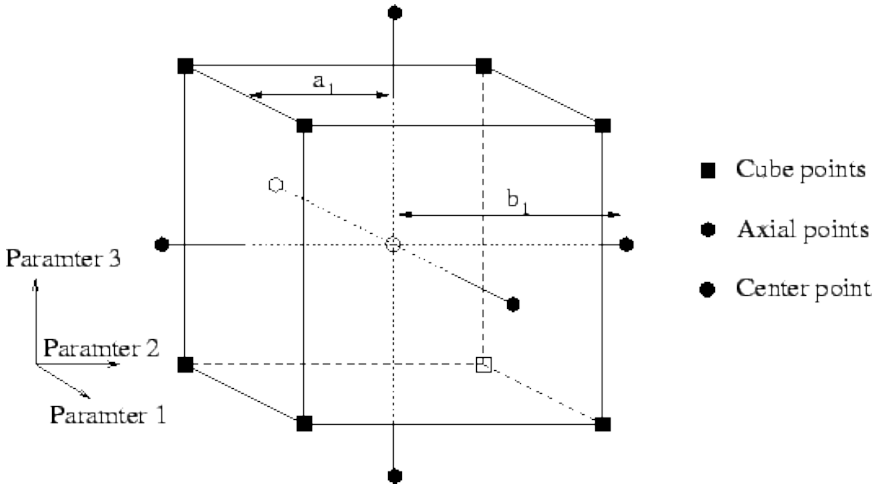

Figure 5. Central composite design as presented by Plasun (1999).

The response surface plots can be employed to study the surfaces and locate the optimum. With the CCD, circumscribed design is widely utilized because of the rotatability characteristic, allowing the experimenter to search for the optimum and estimate response value with equal precision in any direction (Myers and Montgomery 1995). The proposed model undergoes diagnostic checking tests using the analysis of variance (ANOVA).

Fraction of Design Space (FDS) is a tool used to asses prediction capability of response surface designs, allow an experimenter to see patterns of scaled prediction variances (SPV) throughout a design space, and can be used to validate if the numbers of CCD runs were sufficient (Zahran and others 2003). FDS curve is a line graph showing the relationship between the volume of the design space and amount of prediction error. The FDS curve is the percentage of the design space volume containing a given standard error of prediction or less (Anderson and Whitcomb 2005). Standard error of prediction relates to the prediction interval around a predicted response at a given combination of factor levels and/or components. The larger the standard error of prediction, the less likely the results can be repeated, and the less likely a significant effect will be detected. Therefore, FDS is an effective tool use to validate if the number of runs in CCD is adequate for predicting the true average in RSM optimization (Anderson and Whitcomb 2005).

4.4.2 Use of response surface methodology to develop gluten-free products

Currently, there are limited studies on the use of RSM to optimize GF breads. The available studies used RSM to optimize the formulation of GF breads. The optimized variables were HPMC and water (Sabanis and Tzia 2011; McCarthy and others 2005)), xanthan gum, skim milk, and water (Schober and others 2005), and levels of soy flour and dry milk (Sanchez and others 2004). Preliminary studies were conducted to obtained: 1) a manageable formula for the

RSM optimization (Schober and others 2005), and 2) the upper and lower limits of each variables (McCarthy and others 2005). All the baking was performed in random order according to the CCD using five levels of each variable. Replicates at the center of the design were used to allow for estimation of the pure error of sequential model sum of square (SMSS) (Sanchez and others 2004). To establish predictive models for the GF breads from the varying variables, the experimental data for each response variable were used to fit second-order models and generate response surface plots.

Model selection for each response was made based on the SMSS, lack-of-fit tests and the multiple correlation coefficients (R^2). In SMSS, the highest degree model should be selected, for which the F-tests show significant (Sabanis and Tzia 2011; Schober and others 2005; McCarthy and others 2005; Sanchez and others 2004). Only one study reported the model quality (McCarthy and others 2005). The resulted R^2 ranged from 0.51 to 0.82 with the insignificant ($P > 0.05$) SMSS for crumb firmness, and significant ($P < 0.05$) lack-of-fit test for loaf height, and number of small cells.

The GF breads were successfully optimized for maximize loaf specific volume, minimize crumb firmness, maximize number of small cells, minimize number of large cells, minimize batter viscosity , maximize crumb grain score, maximize bread score, maximize bread protein content, and maximize over all acceptability (Sabanis and Tzia 2011; Schober and others 2005; McCarthy and others 2005; Sanchez and others 2004). McCarthy and others (2005) reported that poor quality breads with large gas cells were obtained when maximizing loaf specific volume, loaf height, and the number of small cells, and minimizing crumb firmness and number of large cells. High quality breads were obtained when optimizing GF breads based on maximum number of small cells and minimum number of large cells.

5. PRELIMINARY EXPERIMENT

The preliminary study was conducted at the Northern Crops Institute to identify GF formula. Precooking of pea flour, gums, extreme maximum and minimum limits of level of water, and proof time were also identified in this experiment. Five GF formulas (Frederick 2009; Gallagher and others 2003b; Lopez and others 2004; McCarthy and others 2005; Sabanis and Tzia 2009) were modified and tested with yellow pea flour. None of the formulas produced GF bread with acceptable quality. However, the base formula and bread baking procedures were identified after several ingredient and processing condition adjustments (Table 7).

Table 7. Yellow pea gluten-free bread base formula.

Ingredients	Percent	Grams
Canola oil	5.5	80
Water	36.2	524
Egg yolk	1.0	15
Egg white	8.6	125
Precooked Whole pea flour	8.3	120
Potato starch	13.8	200
Brown Rice flour	16.6	240
Xanthan gum	1.0	14
Salt	0.4	6
Instant yeast (SAF gold)	0.9	13
White granulated sugar	3.5	50
Baking powder	1.4	20
Apple cider vinegar	2.6	37
Calcium proprionate	0.2	3.6
Total Amount	100.0	1447.4

5.1. General preparation

For each treatment, water, eggs, and oil were mixed together at speed 5 for 45 s on a small cake mixer (Kitchen Aid, Kitchen Aid professional 600, St. Joseph, MI). Gums were added and mixed at speed 5 (126 rpm) for 45 s. The rest of dry ingredients were sifted and

mixed at speed 3 (104 rpm) for 30 s. Bowl was scraped and mixed at speed 6 (149 rpm) for 2 min. Apple cider vinegar was added and the mixture was mixed for 30 s at speed 5. The batter was proofed at 38 °C for 0 min (low limit), 45 min, or 70 min (high limit) at 85% relative humidity. Breads were baked for 1 hour and 30 min at 171 °C. Loaf volume, texture, and firmness determinations were completed on breads one day after baking (Table 8).

Table 8. Average bread weight, loaf volume, and crumb firmness values of the gluten-free breads from preliminary experiments.

Descriptions	Bread Weight (g)	Loaf Volume (CC)	Firmness (g_f)
Precooked pea flour	648	1425	1296
Raw pea flour	644	1650	1322
Replace xanthan gum with guar gum	670	1288	1646
Replace xanthan with CMC gum	655	1288	1621
Replace xanthan with HPMC gum	649	1013	2943
218 g total water (low limit)	675	775	12851
760 g total water (High limit)	664	1063	1468
Change proof time to 0 min (low)	636	1238	2002
Change proof time to 70 min (high limit)	644	1325	1329

5.2. Results and discussions

5.2.1. Effects of hydrocolloids

Xanthan, CMC, HPMC, guar, and carrageenan caused different effects on bread final quality (Table 8; Appendix Figure A-1). Bread with soft crumb and solid structure were achieved for bread with guar gum and CMC. Breads made with HPMC had a compact and dense structure compared to others. Unless used in combination with other gums, carrageenan did not provide structures to the GF breads. The final product crumbled upon cutting. Breads with xanthan gum had the best appearance, color, texture, and structure. Therefore, xanthan was chosen for final bread formulas in the RSM.

5.2.2. Water addition

To test for the limit of water addition, 218 g and 760 g of water were used for low and high limit, respectively (Table 8; Appendix Figure A-2). The water level was too low at 218 g. The bread was very dense and compact. The GF breads were too wet after baking formula with 760 g of water, indicated by wet and collapsed loaves. Therefore, the low limit and high limit for water was estimated at 270 g and 660 g, respectively.

5.2.3. Proof time

The lower and upper values for batter proof time were 0 and 60 min, respectively (Table 8; Appendix Figure A-3). At 0 minute, the GF loaf was dense and small. However, the cell diameter was small and evenly distributed. Sixty minutes proof time provided bread with irregular cell structure. Due to prolonged proofing, the loaf structure was noticeably weaker than the control.

5.2.4. Treatments of yellow pea flour

To reduce the after taste of yellow pea flour, yellow pea flours were subjected to many treatments. Pea flours were ozonated, ethanol treated, extruded, and heat treated (precooked flour). Ozonated and ethanol treated flours presented desirable appearance, but unacceptable flavor. Extruded pea flour exhibits gummy texture and dark appearance. Heat treatment was found to be the most effective in reducing the aftertaste of yellow pea flour and providing acceptable appearance and texture.

5.3. Conclusions

During the preliminary experiment, GF base formula and baking procedures, type of gums, and extreme maximum and minimum limits were identified for the RSM study, as well as

the method of treatment of pea flour. Extreme maximum and minimum limits for PTPF, amount of water in the batter, and proof time are presented in Table 9.

Table 9. Lower and upper limits for precook temperature, water absorption level and proof time.

Variables	**Low**	**High**
Precooking temperature of pea flour (°C)	150	180
Water (g)	270	660
Proof time (min)	0	60

6. MATERIALS AND METHODS

6.1. Materials

Commercial whole yellow peas were obtained from two North Dakota suppliers, Dakota Dry Bean (Grand Forks, ND) and United Pulse Trading (Bismarck, ND). All yellow peas were blended to make one homogenized sample. Brown rice, potato starch, and xanthan gum were purchased from Bob's Red Mill (Milwaukie, OR). Canola oil (Crisco), whole eggs, non-iodized salt (Morton), white granulated sugar (American Crystal Sugar Company), double acting baking powder (sodium bicarbonate, Tones), apple cider vinegar (Favorite Foods) were purchased from Hornbachers (Fargo, ND).

6.2. Methods

6.2.1. Preparation of precooked yellow pea flour

To precook the yellow peas, yellow peas were rinsed and soaked in 50 °C tap water at 3:1 water/pea ratio for 30 min. Yellow peas were then drained on a mesh bottom tray and passed through an impingement oven (Lincoln Impinger 1600, Fort Wayne, IN) at 150, 157, 167, 177 and 184 °C for 12.5 min. The processed peas were cooled in a clean container for 2 hours before being packaged and stored in the freezer.

Table 10. Particle size distribution of precooked pea flour.

Particle size (mesh)	%
Over 20	0.0
Over 30	0.2
Over 40	1.0
Over 60	14.7
Over 80	14.3
Over 100	43.0
Thru 100	26.9
Total	100.0

Precooked peas were milled in a Hammer mill (Fitzmill Model DAS-06, Fitzpatrick Company, Elmhurst IL) at the feed rate of 15 rpm, and the knife speed of 7400 rpm. Collected sample passed through 0033 inch screen during milling. The particle size of milled peas (50 g) was determined by Ro-tap sieve shaker with 20, 40, 60, 80, and 100 mesh (Table 10).

6.2.2. Pasting properties of various flours

The pasting properties of the yellow pea flour, brown rice flours, and potato starch were determined according to Chung and others (2008). RVA (Super 4, Newport Scientific Pty. Ltd., Warriewood, NSW, Australia) was used in the study. Flours at 3.5 g (dry basis) and 25.5 g of distilled water were combined. The STD 2 profile AACC International method 76-22.01 (2002a) was used in which the sample was equilibrated at 50 °C for 1 min, heated at 6 °C/min to 95 °C, held at 95 °C for 5 min, cooled at 6 °C/min to 50 °C, and held at 50 °C for 2 min. Peak viscosity, final viscosity, breakdown, setback, and pasting temperature were determined from the viscogram. The results were obtained as centipoise (cP). Twelve cP is equivalent to 1 rapid visco unit (RVU). The reported values are means of duplicate measurements.

6.2.3. Gluten-free yellow pea bread baking

The formula used in this study was from a preliminary study (Table 11). The amount of water in the formula varied between treatments (Table 12 [X_2]) in the experimental design section. The temperature used to precook pea flour, the amount of water, and proof time were followed according to CCD (Table 12). Because different mixing time and speed can affect the results of the breads, particular attention was spent on the mixing. For each treatment, water, eggs, and oil were mixed together at speed 5 (126 rpm) for 45 s in a mixer (Kitchen Aid Professional 600, St. Joseph, MI, USA). Xanthan gum powder was added and mixed at speed 5 for 1 min. The remaining dry ingredients were added and mixed at speed 3 (104 rpm) for 30 s.

Table 11. Gluten-free bread formula containing yellow pea flour.

Ingredients	Percent	Grams
Canola oil	5.5	80
Water	Varied	Varied
Egg yolk	1.0	15
Egg white	8.6	125
Precooked Whole pea flour	8.3	120
Potato starch	13.8	200
Brown Rice flour	16.6	240
Xanthan gum	1.0	14
Salt	0.4	6
Instant yeast (SAF gold)	0.9	13
White granulated sugar	3.5	50
Baking powder	1.4	20
Apple cider vinegar	2.6	37
Calcium proprionate	0.2	3.6
Total Amount	100.0	1447.6

The bowl was scraped and mixed at speed 6 (149 rpm) for 3 min. Apple cider vinegar was added and mixed for 30 s at speed 5. Five hundred and seventy grams of batter was placed into 12.5 x 21.5 x 7.5 cm pans. Each batch of batter made two pans of bread. The breads were proofed to the specified time (Table 12 [X_3]) under 85% relative humidity at 38 °C. Breads were baked for 1 hour and 30 min at 171 °C.

6.2.4. Bread analyses

Two loaves of breads were produced from each batch of treatment. Therefore, analyses for each treatment were conducted on two loaves of bread. For color, texture, and c-cell analysis, breads were sliced to 12.5 mm thickness.

6.2.4.1. Crust and crumb color

Crust color was determined at three locations per loaf with a color analyzer (CR-310, Minolta, Osaka, Japan). Crumb color was determined from two different slices of breads per loaf.

Table 12. Central composite design with experimental value of color, specific volume, firmness, and cell diameter.

	Coded Levels			Actual Levels[a]			Responses			
Runs	x_1	x_2	x_3	X_1	X_2	X_3	Color (L*)	Specific volume (ml/g)	Firmness (g_f)	Cell Diameter (mm)
1	-1	-1	-1	157	370	13	67.3	1.9	491.9	10.0
2	1	-1	-1	177	370	13	60.6	1.9	551.9	9.8
3	-1	1	-1	157	590	13	65.5	2.2	173.6	15.5
4	1	1	-1	177	590	13	63.0	2.4	153.0	15.7
5	-1	-1	1	157	370	50	67.3	1.8	499.0	11.1
6	1	-1	1	177	370	50	60.2	1.9	586.0	10.3
7	-1	1	1	157	590	50	69.0	2.3	144.0	17.7
8	1	1	1	177	590	50	64.9	2.3	130.5	17.3
9	-1.68	0	0	150	480	32	71.1	2.2	198.0	14.4
10	1.682	0	0	184	480	32	51.2	2.2	236.2	12.6
11	0	-1.68	0	167	295	32	59.5	1.5	1451.1	8.0
12	0	1.682	0	167	665	32	62.7	2.6	142.0	16.1
13	0	0	-1.68	167	480	0	61.7	2.3	296.1	11.8
14	0	0	1.682	167	480	63	61.6	2.2	220.4	14.7
15	0	0	0	167	480	32	62.7	2.2	233.4	13.2
16	0	0	0	167	480	32	62.7	2.3	180.0	14.1
17	0	0	0	167	480	32	63.2	2.2	240.3	12.8
18	0	0	0	167	480	32	63.0	2.2	223.8	13.0
19	0	0	0	167	480	32	62.7	2.2	235.1	13.5
20	0	0	0	167	480	32	62.5	2.2	232.4	12.7

[a]X_1 represents precook temperature of pea flour (°C); X_2 represents amount of water (g); and X_3 represents proof time (min).

The device was calibrated with a white tile. L*, a*, and b* values were given as output. L* represents lightness (0 = black and 100 = white) while red and green colors are indicated by the a* value (+a = red and –a = green) and the b* value indicates yellow (+b) and blue (-b) colors.

6.2.4.2. Crumb moisture

Moisture of breads were determined with material taken from the center of the crumb according to AACC International (2002b) method 44-15.02.

6.2.4.3. Loaf volume

After 24 hours, loaves were weighed and loaf volume measured by rapeseed displacement by the AACC International (2002c) method 10-05. Loaf specific volume (loaf volume [mL]/loaf weight [g]) was calculated.

6.2.4.4. Texture and shelf life of breads.

Texture Profile Analysis (TPA) (Bourne 1978) of the bread crumb was performed on two slices from each loaf of bread during the optimization stage. For the shelf life study, bread texture was analyzed on days 0, 1, 3, 5 and 7. The texture analyzer (TA-XT2, Stable Micro Systems, Godalming, United Kingdom) was equipped with a 38 mm Perspex cylinder probe along with a 50 kg load cell. TPA was carried out with a constant speed of 2.0 mm/s (applying to the pre-test speed, test speed, and post-test speed) for a distance of 10.0 mm, corresponding to 40% compression of the 25 mm slices. There was a 5-second wait time between the first and second compression cycles; the trigger force was 20.0 g. Range of values for textural attributes was extracted from the resulting curve including hardness, springiness, gumminess and chewiness (Bourne 1978).

6.2.4.5. C-Cell analysis

C-cell uses high definition imaging and controlled illumination. The bread slices were assessed for crumb grain characteristics using a C-Cell Instrument (Calibre Control International Ltd., Appleton, Warrington, United Kingdom). C-Cell uses high definition imaging and controlled illumination to obtain images. Chen and others (2007) reported that C-Cell Instrument has the capability to determine important bread crumb attributes, including average cell diameter and volume, average cell wall thickness, average crumb fineness (number of cells/cm) and slice brightness.

6.2.5. Sensory evaluation

Sensory analysis of GF bread was completed by a panel of 50 individuals, both male and female, after breads were allowed to sit for 16 hours after baking. Two replicates of the sensory evaluation were conducted. A nine-point hedonic scale was used to evaluate the overall acceptability of the optimized bread formulations and a commercial GF premix (Gluten-free Pantry, Glutino, Quebec, Canada). The panelists scored on a scale of 1 (dislike extremely) to 9 (like extremely) on appearance, flavor, texture, and overall acceptability. An example of sensory evaluation score sheet is shown in Figure 6. Analysis of variance and least significant different was used to analyze the sensory data, significant difference among samples was determined at the $P< 0.05$ confidence level.

6.2.6. Experimental design

RSM was used to predict the optimum conditions for the GF bread with pea flour. The circumscribed CCD was used in the experiment with three factors, five levels, including five replicates at the center point to fit a second order model (Box and Wilson 1951). The FDS graph was evaluated on Design-Expert 5 (Stat-Ease Corporation, Minneapolis, MN), indicated that two

Figure 6. Example of a sensory evaluation score sheet.

Sensory Evaluation of Pea Fortified Gluten Free Bread

SAMPLE NUMBER:_____

Please evaluate the bread sample for the following qualities: Flavor, Texture, Appearance and Overall Acceptability (i.e. liking). Make an X on the appropriate line. Please give comments in the space provided below each quality if desired.

APPEARANCE:
_____ like extremely
_____ like very much
_____ like moderately
_____ like slightly
_____ neither like nor dislike
_____ dislike slightly
_____ dislike moderately
_____ dislike very much
_____ dislike extremely
COMMENTS:

FLAVOR:
_____ like extremely
_____ like very much
_____ like moderately
_____ like slightly
_____ neither like nor dislike
_____ dislike slightly
_____ dislike moderately
_____ dislike very much
_____ dislike extremely
COMMENTS:

TEXTURE:
_____ like extremely
_____ like very much
_____ like moderately
_____ like slightly
_____ neither like nor dislike
_____ dislike slightly
_____ dislike moderately
_____ dislike very much
_____ dislike extremely
COMMENTS:

OVERALL ACCEPTABILITY:
_____ like extremely
_____ like very much
_____ like moderately
_____ like slightly
_____ neither like nor dislike
_____ dislike slightly
_____ dislike moderately
_____ dislike very much
_____ dislike extremely
COMMENTS:

different replicates of each CCD run was sufficient for this experiment. The extreme limits were identified during the preliminary study. The α= 1.682 in the CCD was calculated by:

$$\alpha = [2^k]^{\frac{1}{4}}$$

where the PTPF, the amount of water added and proof time were the three factors (k).

After preliminary baking tests, the upper and lower limits for the variables were established. In RSM work, it is convenient to transform the natural variables that are expressed in natural units of measurement (i.e. gram, ml, °C) to coded variables. The coded variables are defined to be dimensionless with mean zero and the same spread or standard deviation (Myers and Montgomery 1995). Coded units can be calculated based on the upper and lower limits using the following formula:

$$X_i = x_i\left(\frac{max - min}{2}\right) + \left(\frac{max - min}{2}\right)$$

where X_i is the actual unit for each factor; x_i is the coded unit; max is the maximum limit; and min is the minimum limit (Myers and Montgomery 1995). The factors and their levels, with coded and actual values, and the average responses are given in Table 12 provided above. The responses were analyzed by multiple regressions through the least squares method to fit the following equations:

$$y = b_o + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x^2_1 + b_{22}x^2_2 + b_{33}x^2_3 + b_{12}x_1x_2 + b_{23}x_2x_3 + b_{13}x_1x_3$$

where b_0 is the value of the fitted response at the center point of the design point, b_1, b_2, and b_3 are linear regression terms; b_{11}, b_{22}, b_{33} are quadratic regression terms; and b_{12}, b_{23}, and b_{13} are the cross-product regression term (Montgomery 1991). The predicted response was therefore correlated to the set of regression coefficients: the intercept, linear, interaction, and quadratic coefficients.

Model selection for each response was made on the basis of the sequential model sum of squares (SMSS), lack-of-fit tests, and the R^2 values. In SMSS the highest degree model was selected for which the F-tests indicate significant ($P < 0.05$) effects, whereas the lack-of-fit should be insignificant, and R^2 should be close to 1. Where contradictions between these three requirements existed, the best overall solution was chosen. The coefficients for all terms in the

model (all linear terms for the linear model, all linear and squared terms; and two-way interactions for the quadratic model) were tested for significance statements in this study were based on these tests. RSM was conducted on Design-Expert 5 (Stat-Ease Corporation, Minneapolis, MN).

After obtaining the models for each response, the numerical optimization was performed using the following desirability (D) function:

$$D = (d_1^{r_1} \text{ x } d_2^{r_2} \text{ x } \dots \ d_n^{r_n})^{\frac{1}{\Sigma r_i}}$$

where d_i are the desirability indices for each response ($d_i = 0$ least desirable; $d_i = 1$ most desirable) and r_i the relative importance of each response. The responses used for optimization were crumb color (maximize $r_i = 5$), loaf specific volume (maximize $r_i = 5$), crumb firmness (minimize $r_i = 5$) and number of small cell (maximize $r_i = 5$). Five baking trials were then performed for both the evaluation of the optimized conditions and for the short-term shelf-life study on the optimized formulation. Models were confirmed by comparing the average response to the prediction interval at 95% confidence level.

7. RESULTS AND DISCUSSIONS

7.1. Pasting properties of precooked pea flour

The pasting properties of raw pea flour, precooked pea flour at different temperatures, native brown rice flour, and native potato starch analyzed by RVA are presented in Table 13. The peak viscosity and setback values of brown rice flour and potato starch are significantly higher than pea flour. Pea flour has low viscosity and minimal breakdown values compared to cereal grains (Chung and others 2008), which could be due to the dilution of starch by protein, lipid and fiber (Ovando-Martínez and others 2011).

The heat treatments applied during the precooking process, resulted in reduced viscosity for precooked pea flour compared to the raw pea flour. The viscosity of the pea flour decreased as the temperature of precooking increased (Figure 7). Lowering peak viscosity and setback due to heat-moisture treatment were also reported in rice starch and pigeon pea starch (Hoover and others 1993; Maninder and others 2007). Lower viscosity and setback after heat-moisture treatment of peas could be due to thermal degradation of amylopectin and amylose (Maninder and others 2007), and decreased starch granule hydration (Donovan 1979).

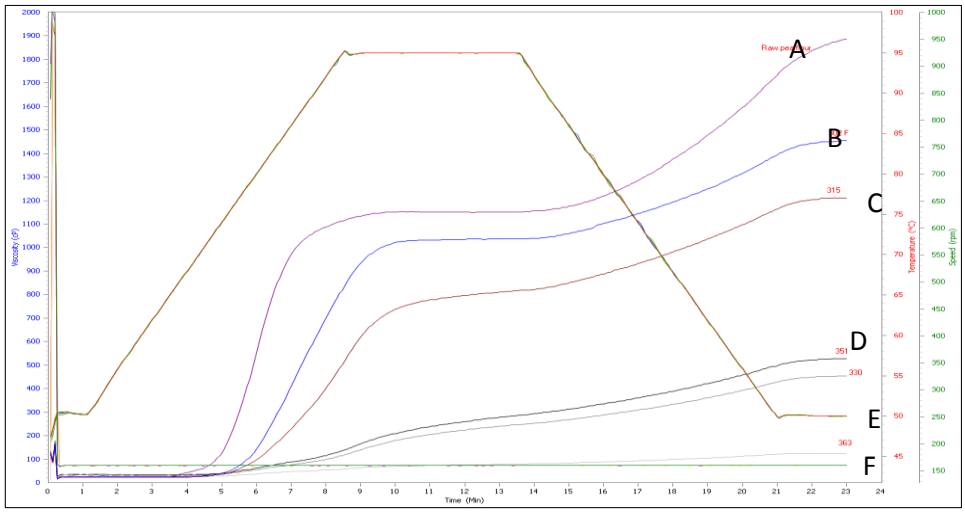

Figure 7. Viscogram of raw pea flour (A), and flours from precooked peas at 150°C (B), 157 °C (C), 177 °C (D), 167 °C (E), and 184 °C (F).

Table 13. Pasting properties of raw pea flour, precooked pea flour, brown rice flour, and potato starch as measured by Rapid Visco Analyzer.

	Peak Viscosity (cP)	Breakdown (cP)	Cold Paste Viscosity (cP)	Setback (cP)	Peak Time (Min)	Pasting Temp (°C)
PF Raw	1159.7 ± 15.3	4.0 ± 1.0	1888.7 ± 15.6	733.0 ± 4.0	11.0 ± 1.2	74.1 ± 0.0
PF 150 °C	1029.3 ± 20.6	3.0 ± 2.6	1441.7 ± 15.7	415.3 ± 7.6	11.1 ± 1.0	81.2 ± 0.4
PF 157 °C	818.0 ± 11.3	-0.5 ± 0.7	1222.5 ± 17.7	404.0 ± 7.1	13.0 ± 0.0	94.9 ± 0.0
PF 167 °C	229.0 ± 27.2	-1.0 ± 0.0	434.0 ± 53.1	204.0 ± 25.9	13.0 ± 0.0	NA ± NA
PF 177 °C	278.0 ± 6.0	-1.0 ± 0.0	524.0 ± 12.1	245.0 ± 6.2	13.0 ± 0.0	NA ± NA
PF 184 °C	75.3 ± 3.1	0.0 ± 0.0	120.0 ± 7.0	44.7 ± 4.0	12.8 ± 0.1	NA ± NA
Brown rice	4971.3 ± 26.6	2646.7 ± 25.5	5613.0 ± 232.4	3288.3 ± 218.7	9.6 ± 0.0	85.6 ± 0.0
Potato starch	15447.7 ± 595.4	13888.7 ± 767.2	3687.3 ± 1054.9	2128.3 ± 1221.6	3.8 ± 0.0	63.9 ± 0.2

7.2. Response surface optimization

7.2.1. Model quality

As indicated in the literature review (p 26), FDS validates if the number of runs were sufficient as shown in Figure 8. When one run of a complete CCD (20 runs) was analyzed, the FDS value was 0.42. Because of the low FDS value, the second CCD run was added (40 runs total) and the FDS value increased to 0.99, meaning that the FDS was capable of predicting the true average within 1 standard deviation about 99% of the time. Two separate CCD runs was therefore sufficient for this experiment.

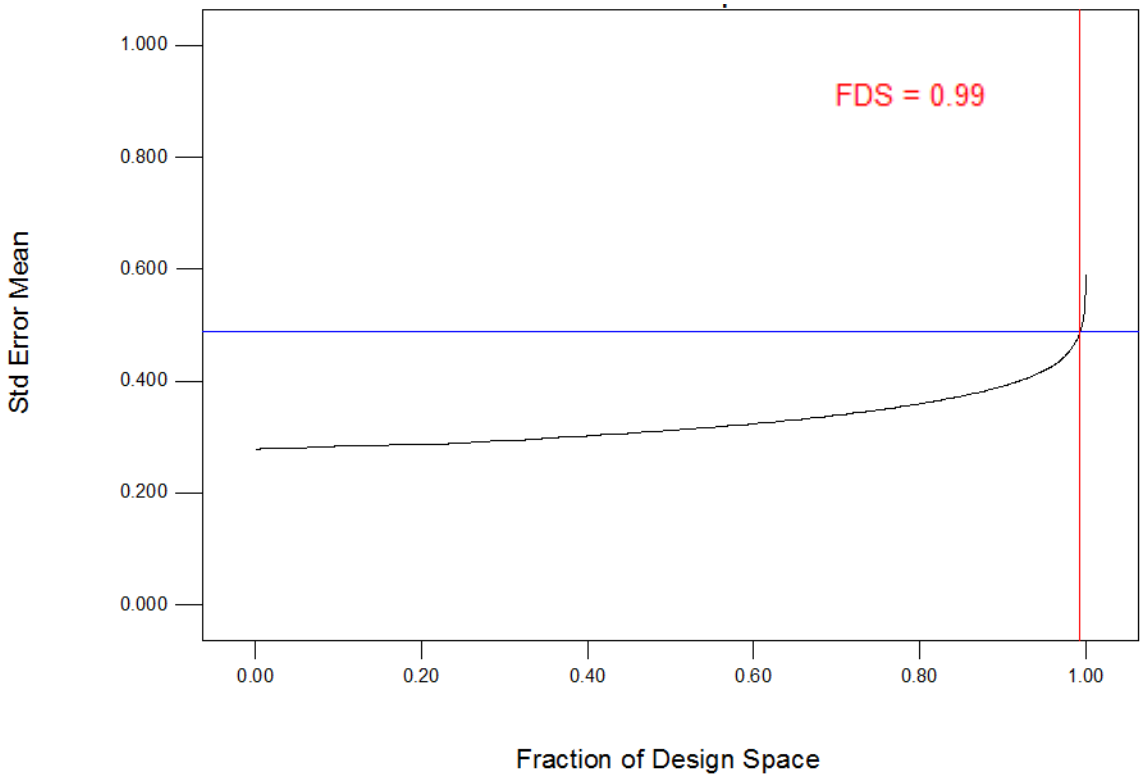

Figure 8. Fraction of design space (FDS) of two central composite design runs in the experiment.

Model summary for crumb brightness, specific volume, crumb firmness, and cell diameter responses are presented in Table 14. Linear effects were chosen to predict crumb brightness and cell diameter of GF breads. Quadratic effects were chosen to predict specific

volume and crumb firmness. Acceptable models were obtained for specific volume, crumb firmness, and cell diameter responses, as indicated by the significant SMSS, insignificant lack of fit test, and high R^2 values. However, crumb brightness model had low R^2 values, as well as a significant lack of fit p-value.

Table 14. Model summary for crumb brightness, specific volume, crumb firmness, and cell diameter.

Responses	Model Source	SMSS[1] p-value	Lack of Fit p-value	Adjusted $R^{2\dagger}$	Predicted $R^{2\dagger}$
Crumb brightness (L*)	Linear	< 0.01**	< 0.01**	0.56	0.46
Specific Volume (ml/g)	Quadratic	0.10*	0.18	0.66	0.44
Crumb Firmness (g_f)	Quadratic	< 0.01**	0.62	0.94	0.90
Cell diameter (mm)	Linear	< 0.01**	0.26	0.86	0.83

*P<0.10, **P<0.05
[1]Sequential model sum of squares
† Multiple correlation coefficients

Predicted R^2 indicates that only 46% of variation was explained by the crumb brightness model, possibly because some other factors, apart from PTPF, water and proof time, also affected the crumb brightness of the bread. Even though the linear model had significant lack of fit test, the quadratic model for crumb brightness could not be used because the SMSS was not significant (P > 0.10).

Predicted R^2 of specific volume was low (0.44) because the quadratic model SMSS P-value was 0.10. The linear model cannot be used due to the significant lack of fit test (P > 0.10) despite the SMSS P-value of <0.0001. For that reason, the quadratic model for specific volume was selected because of the insignificant lack of fit test and significant SMSS P-value (P<0.10).

7.2.2. Model prediction for each response

7.2.2.1 Crumb brightness

The results indicate that the linear effects or the main effects of PTPF were significant, and the amount of water and proof time do not significantly affect the crumb brightness of the breads (Table 15). Second order terms (interaction and quadratic effects) did not significantly affect crumb brightness.

Table 15. ANOVA results for crumb brightness in terms of coded factors after removal of non-significant second order term.

Factor[a]	Coefficient Estimate	Degrees of Freedom	Mean Squares	F Value	
Model		3	148.9	17.6	**
Intercept	63.1	1			
A	-3.9	1	424.6	50.2	**
B	0.9	1	18.7	2.2	
C	0.4	1	3.5	0.4	
Residual		36	8.5		
Lack of Fit		11	17.0	3.6	**
Pure Error		25	4.7		
Corr Total*		39			

[a]A= precook temperature of pea flour , B= water ,C= Proof time.
* Sum of squares total corrected for the mean.
**P<0.05

The color of pea flour directly relates to the crumb color of the breads. The correlation between PTPF and crumb brightness was observed (Figure 9). The crumb color responses from two CCD runs were plotted against PTPF, which were negatively correlated (i.e. correlation coefficient -0.752). As shown in figure 9, within the same PTPF, the crumb brightness varied because the amount of water and proof time varied between treatments according to the CCD runs. Crumb brightness increased as the PTPF decreased, as illustrated in Figure 10. This was expected because Maillard reaction occurred during precooking of pea flour, which resulted in

the darkening of pea flour. Maillard reaction was also the main cause of the darkening effects in pasta and noodle products that contained pea flour (Comer 2012; Pinarli and others 2004).

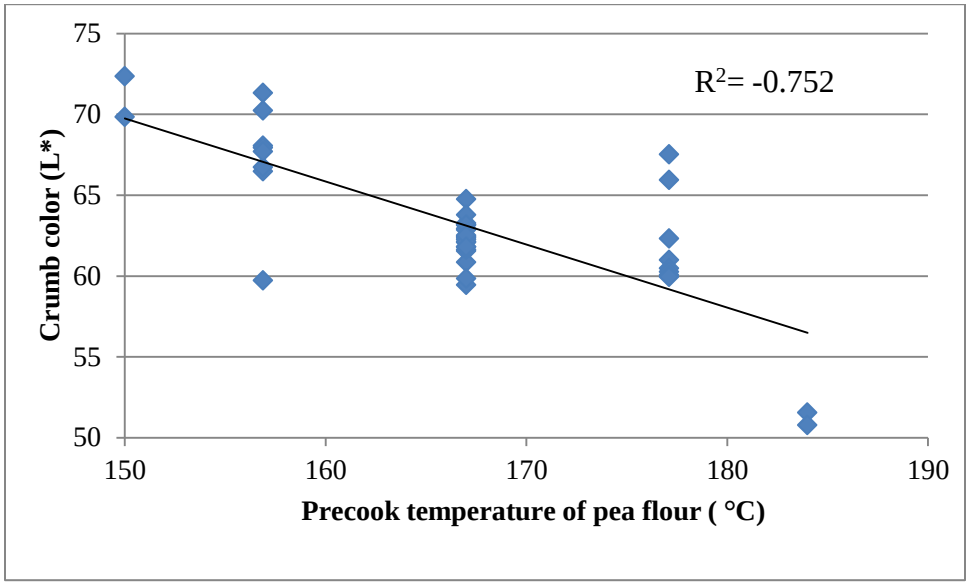

Figure 9. Correlation between crumb color and precook temperature used during preparation of pea flours.

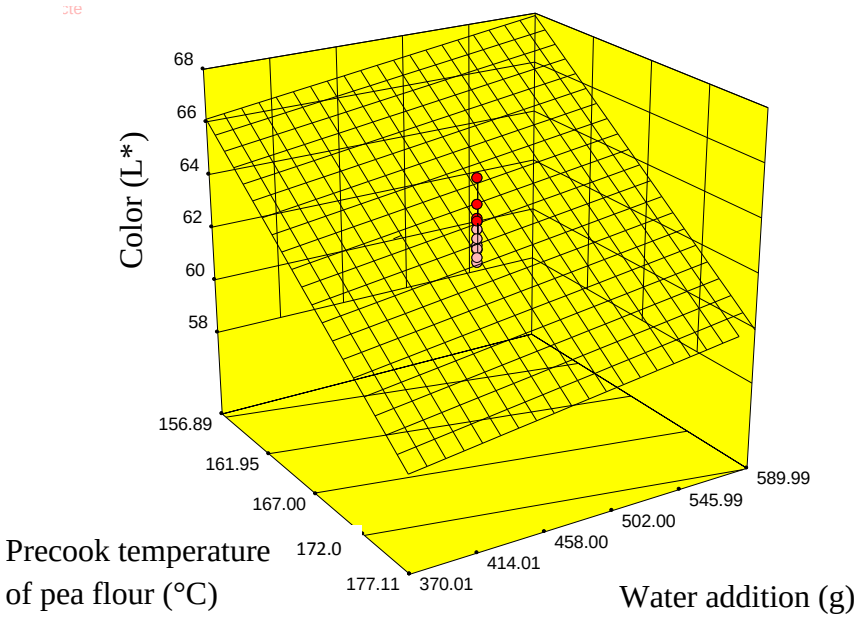

Figure 10. Effect of precook temperature and amount of water addition on crumb color.

7.2.2.2. Specific volume

The linear and quadratic terms of water addition significantly ($P \leq 0.10$) affected the specific volume (Table 16). Furthermore, the coefficient values indicate the linear term of water addition had the greatest impact on specific volume compared to other independent variables.

Table 16. ANOVA results for specific volume in terms of coded factors.

Factor[a]	Coefficient Estimate	Degrees of Freedom	Mean Squares	F Value	
Model		9	0.2	9.3	**
Intercept	2.2	1			
A	0.0	1	0.0	0.3	
B	0.3	1	1.6	80.7	**
C	0.0	1	0.0	0.3	
AB	0.0	1	0.0	0.0	
AC	0.0	1	0.0	0.4	
BC	0.0	1	0.0	0.0	
A^2	0.0	1	0.0	0.9	
B^2	-0.1	1	0.1	6.2	**
C^2	0.0	1	0.0	0.1	
Residual		30	0.0		
Lack of Fit		5	0.0	1.7	
Pure Error		25	0.0		
Cor Total		39			

[a]A= precook temperature of pea flour , B= water ,C= Proof time.

* Sum of squares total corrected for the mean.

**P<0.05

According to the coefficient estimate, an increase in the amount of water increased the specific volume of the GF bread. This agrees with the findings on rice bread by Nishita et al (1976) and Haque and Morris (1994), both of whom reported loaves with large volume when high amount of water was used. Significant quadratic term also indicated that addition of water, at certain levels, can decrease the specific volume. This is expected since the mechanical and rheological behavior of dough is influenced mainly by the amount of water added (Ablett and

others 1986). Water is the main plasticizer in GF bread. Gallagher and others (2003b) reported addition of water to GF bread can increased loaf volume with increasing water addition. However, excessive amounts of water decreases the energy require to rupture gas cells (Gan and others 1995), decreases gas retention capacity, promotes structure weakening of the bread, and ultimately affect the loaf volume (Arendt and others 2008). The effects of water addition and PTPF on specific volume were represented in Figure 11.

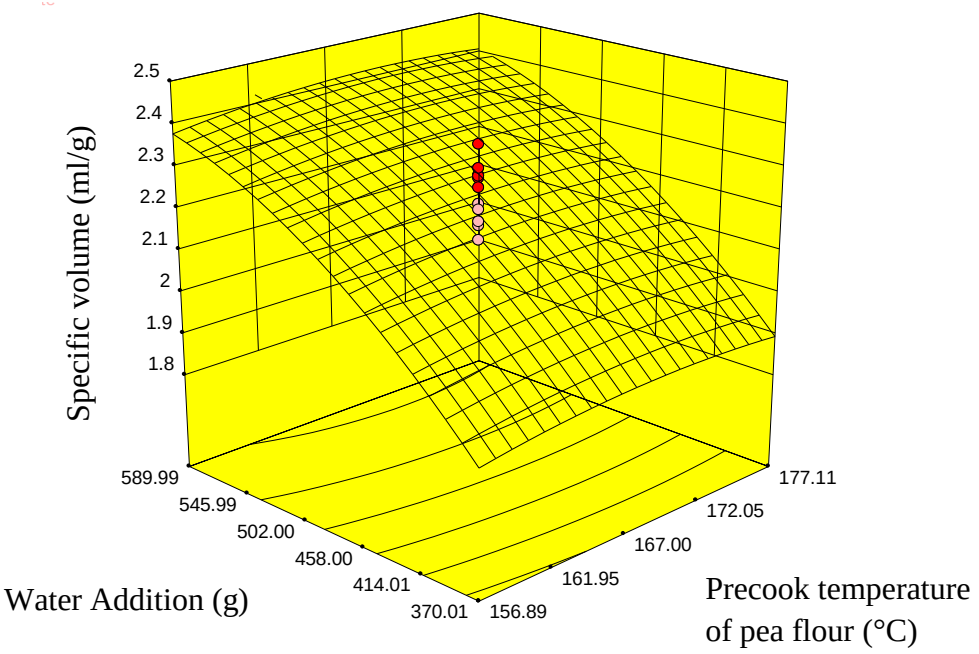

Figure 11. Effect of water addition and precook temperature on breads specific volume.

7.2.2.3. Crumb firmness

Interaction between PTPF, and quadratic terms of water and proof time significantly ($P \leq 0.05$) affect the crumb firmness of GF breads (Table 17). Coefficient estimate on water addition indicates that linear and quadratic water terms had the greatest effects on crumb firmness, and that the addition of water decreased crumb firmness. Nishita and Bean (1979) reported that the setback parameters that relate to the retrogradation of starch, was found to be the most important parameters in predicting GF rice bread characteristics. Higher setback values indicates higher

tendency for starch retrogradation (Kim and others 1997), and consequently affect crumb firmness. However, the setback values, influenced by the degree of precooking of pea flour, did not significantly affect the crumb firmness of breads in this study (P= 0.11). The crumb firmness was significantly affected by the interaction between water and PTPF.

Table 17. ANOVA results for crumb firmness in terms of coded factors

Factor[a]	Coefficient Estimate	Degrees of Freedom	Mean Squares	F Value	
Model		9	108604.2	64.1	**
Intercept	222.5	1			
A	13.0	1	4594.3	2.71 (P= 0.11)	
B	-191.8	1	890356.9	525.4	**
C	-10.1	1	2794.6	1.7	
AB	-22.6	1	8200.7	4.8	**
AC	4.3	1	289.8	0.2	
BC	-11.7	1	2171.8	1.3	
A^2	2.4	1	161.5	0.1	
B^2	90.9	1	176568.3	104.2	**
C^2	16.9	1	8289.7	4.9	**
Residual		30	1694.6		
Lack of Fit		5	1278.0	0.7	
Pure Error		25	1777.9		
Cor Total		39			

[a]A= precook temperature of pea flour , B= water , C= Proof time.
* Sum of squares total corrected for the mean.
**P<0.05

When the PTPF increased (lower setback values), the crumb firmness increased (Figure 12). This result was different than the finding in rice bread (Nishita and Bean 1979). However, it could be explained that when pea flour was cooked at high temperature, starch granule hydration decreased, while amylose and amylopectin degradation increased (Hoover and Manuel 1996b). When the precooked pea flour was incorporated into the GF breads, starch underwent less crosslinking, swelling, and water uptake, resulting in lower viscosity and water uptake by the batter. This in turn caused increased firmness observed in the finished GF breads.

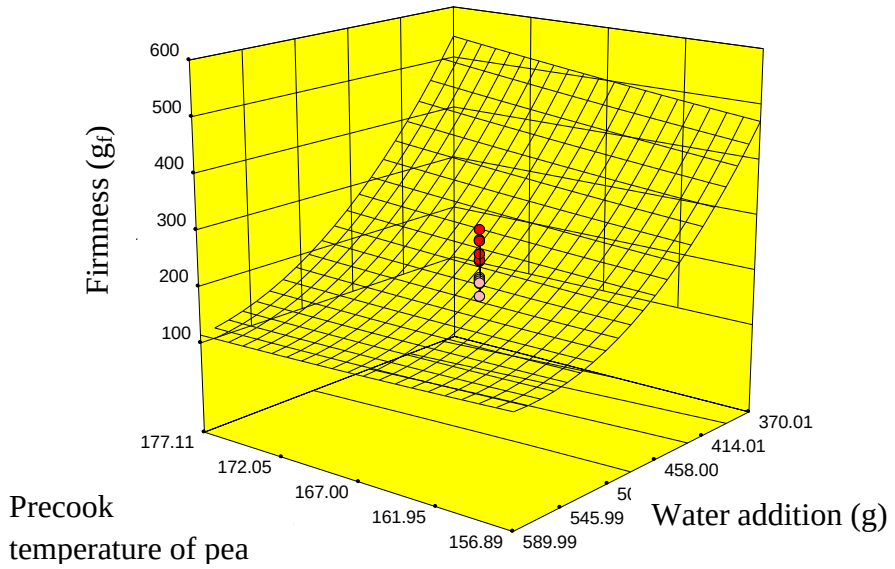

Figure 12. Effects of water addition and precook temperature on crumb firmness.

7.2.2.4. Cell diameter

The linear terms of water and proof time significantly ($P \leq 0.05$) affect the cell diameter (Table 18). According to the estimated coefficient, water had the dominating effect on cell diameter.

Table 18. ANOVA results for cell diameter in terms of coded factors after removal of non-significant second order terms.

Factor[a]	Coefficient Estimate	Degrees of Freedom	Mean Squares	F Value
Model		3	71.9	78.6 **
Intercept	13.2	1		
A	-0.3	1	2.5	2.7 (P= 0.11)
B	2.8	1	197.8	216.4 **
C	0.7	1	15.3	16.8 **
Residual		36	0.9	
Lack of Fit		11	1.1	1.3
Pure Error		25	0.8	
Cor Total		39		

[a]A= precook temperature of pea flour , B= water , C= Proof time.
* Sum of squares total corrected for the mean.
**P<0.05

The response surface analysis predicted that the cell diameter increased as the water addition increased (Figure 13). This was also observed in breads with varying amounts of added water (Figure 14). Because of the lack of gluten, addition of hydrocolloids is recognized as an important factor for stabilizing gas cell in GF breads (Schober and others 2007). Gas cells in breads are formed during mixing and later expand with carbon dioxide produced by yeast during proofing (Gan and others 1995). Prolong proofing cause the gas cell to increase in size, and eventually rupture. High levels of water decreases the energy required to ruptured gas cells (Gan and others 1995) resulting in increased gas cell diameters in the bread crumbs. Therefore, as the proof time and the amount of water added to the bread increased, the cell diameter also increased.

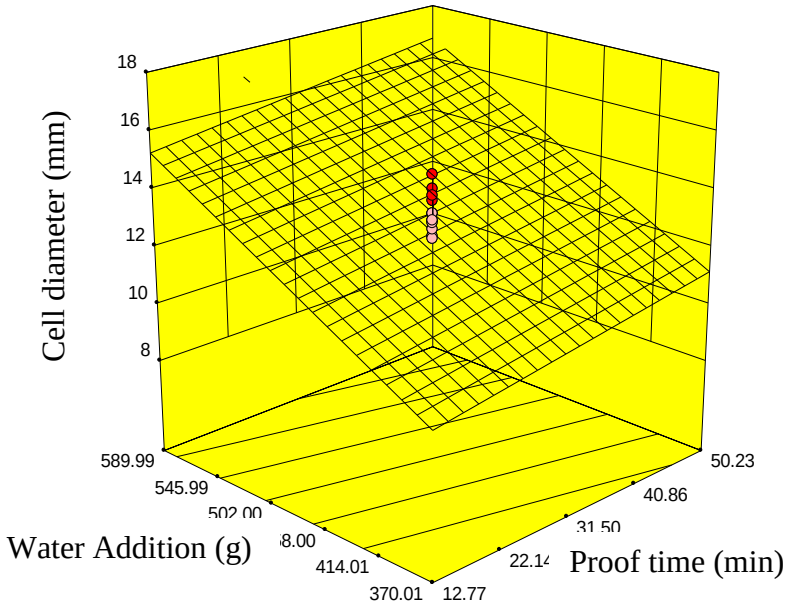

Figure 13. Effects of water and proof time on cell diameter.

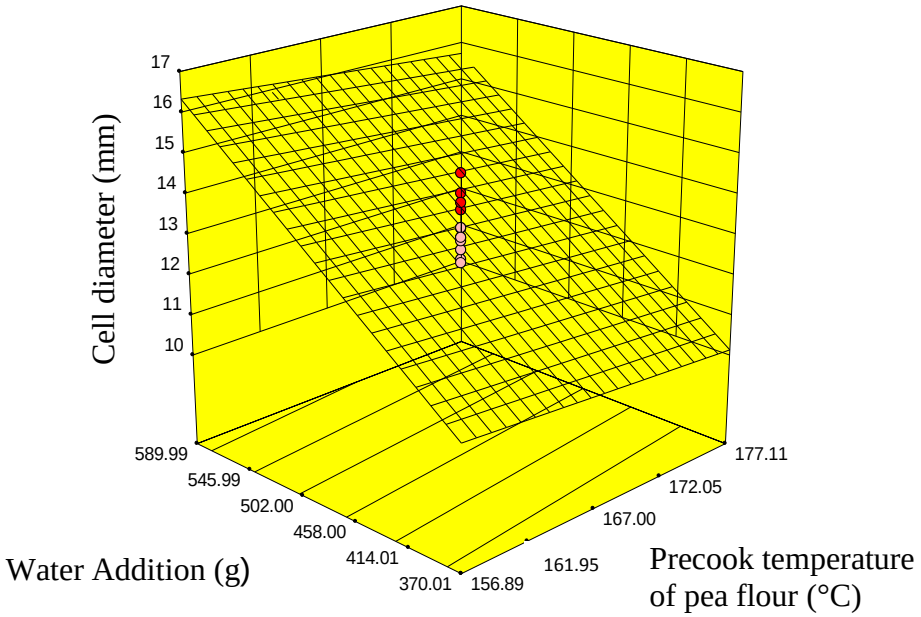

Figure 14. Effects of water and precook temperature on cell diameter.

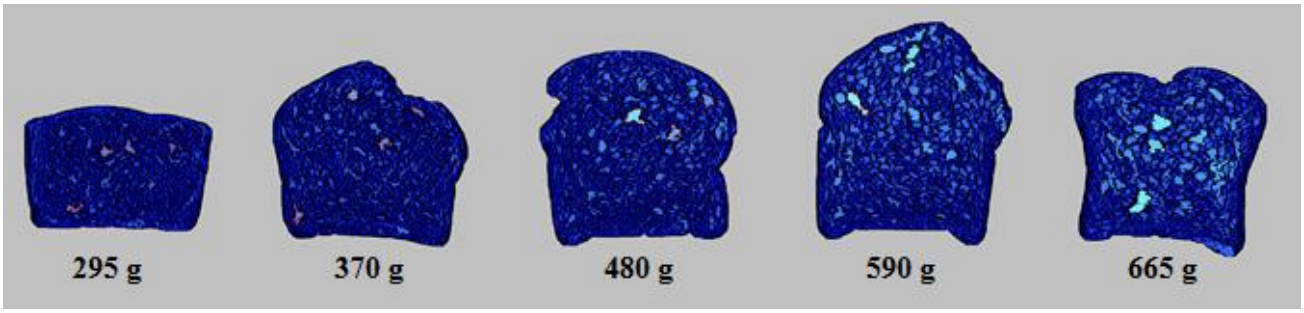

Figure 15. The effect of water addition on the cell diameter of gluten-free yellow pea bread analyzed by C-Cell.

7.2.3. Comparison between predicted and measured values

The optimized parameters for PTPF, water, and proof time were 156.9 °C, 523.8 g, and 18.0 min, respectively. The optimized bread had brightness (L* value), specific volume, crumb firmness, and cell diameter of 68.2, 2.6 ml/g, 174.2 g_f, and 3.81 mm, respectively. Overall, the measured responses fell within 95% confidence interval of the predicted values (Table 19), except for specific volume. Specific volume was measured at 2.6 ml/g, higher than the high limit interval of 2.4 ml/g. Inaccuracy was expected since the predicted R^2 value of the specific volume

was 0.44, meaning that only 44% of the variables in the data can be explained by the model for specific volume. Although the measured specific volume is higher than the predicted values, it is not a concern because high specific volume is a desirable quality characteristic.

The specific volume in this study was comparable to the specific volumes of 2.57 and 2.53 ml/g observed in wheat starch and corn starch GF breads, respectively (Gallagher and others 2003a; Lopez and others 2004). GF bread made with sorghum and rice flour had lower specific volumes at 1.71 and 1.92 ml/g, respectively. McCarthy and others (2005a) reported specific volume of 3.03 ml/g for optimized rice and potato starch based GF breads utilizing RSM.

Table 19. Comparison between crumb brightness, specific volume, crumb firmness, and cell diameter of optimized gluten-free bread and predicted values.

Responses	Predicted Value	95% CI* low	95% CI* high	Measured Value	STDEV**
Crumb brightness (L*)	67.1	65.4	68.9	68.2	0.7
Specific Volume (ml/g)	2.3	2.2	2.4	2.6	0.1
Crumb Firmness (g_f)	181.4	148.8	214.1	174.2	12.8
Cell diameter (mm)	3.73	3.60	3.89	3.81	0.23

* Confidence interval
** Standard Deviation

The optimized formulation produced softer crumb than GF breads made from wheat starch (350 g_f), rice and potato starch (313 g_f), sorghum (479-826 g_f), rice flour (858-1,500 g_f), and corn- soy blend (836-1012 g_f), and traditional wheat bread (677.1 g_f) (Gallagher and others 2003a; McCarthy and others 2005; Schober and others 2005; Sciarini and others 2010). Although the base flour significantly affects the final product quality, it is important to note that the specific volume and crumb hardness can also be influenced by the overall formulations and the processing methods.

Bread crumb brightness (L* value) measured at 68.2 were comparable to crumb brightness of GF breads made with sorghum flour (54.8-62.9), rice flour (67.70-79.33), and corn flour (57.11). Higher crumb brightness (86) was found in rice and potato starch (50:50) combination (Schober and others 2005). Wheat bread had lighter crumb (74.4) brightness (Lopez and others 2004; McCarthy and others 2005; Schober and others 2005) than the optimized product.

Limited GF bread studies provide data on the cell diameter, even fewer numbers of studies use C-cell image analyzer. The bread from the optimized formula had cell diameter of 3.81mm. Sorghum bread had cell diameters between 2.07 and 2.68 mm (Sabanis and Tzia 2009). Cell diameter of rice and potato starch-based products varied largely between 1.7 and 13.0 mm (Schober and others 2007) due to the amount of water addition, gum addition, and proof time.

7.3. Sensory evaluation of the optimized bread formula

Sensory evaluation of the optimized yellow pea GF bread (OPT) and commercial premix GF bread (COM) were compared. Panelists were asked to score products based on appearance, flavor, texture and overall acceptability from 1 (dislike extremely) to 9 (like extremely). The results indicated that the appearances of both OPT and COM was not significantly differently ($P< 0.05$) (Table 20). The flavor, texture, and overall acceptability of OPT were rated lower than COM. The main negative comment on the OPT was the distinct pea flavor. The average scores for the COM were 6.4, 6.7 and 6.6 for flavor, texture, and overall acceptability, respectively. This indicates that the product averaged “like slightly” (score of 6). OPT average scores for flavor, texture, and overall acceptability were 5.1, 6.0 and 5.8, indicating that product averaged between “neither like nor dislike” (score of 5), and “like slightly” (score of 6).

Table 20. Sensory comparison between optimized formula and commercial premix. Sensory score ranged from 1 (dislike extremely) to 9 (like extremely).

Sensory Parameter	OPT*	COM*	Pr > F
Appearance	6.4 a	6.7 a	0.0516
Flavor	5.1 a	6.4 b	0.0001
Texture	6.0 a	6.7 b	0.0016
Overall Acceptability	5.8 a	6.6 b	0.0001

*OPT: optimized yellow pea GF bread formula, and COM: commercial GF bread premix. Values followed by different letters within the same row are significantly different.

It is important to note that the panelist were a mixture of celiac and non-celiac panelists. For non-celiac panelists, their expectations of bread would be based entirely on products made from wheat flour (McCarthy and others 2005), and the flavor of GF bread would be unfamiliar to them (Haque and Morris 1994). This may account partly for the low scores. Using a panel of CD patients would be more appropriate since they would be more familiar with the organoleptic properties of starch-based products (McCarthy and others 2005); however, it was too difficult to find enough panelist if the sensory was limited to people with CD.

7.4. Shelf life of optimized gluten-free bread

During storage, bread loses its freshness and stales, the crumb becomes more firm and less elastic. Water migrates from crumb to crust and leads to a glass to rubber transition of the two components. During aging, amylose and amylopectin reorganize, and starch network becomes more rigid. Migration of water and amylopectin retrogradation, in particular the formation of double helical structures and crystalline regions, are responsible for the staling of breads (McCarthy and others 2005; Nishita and others 1976). The crumb hardness of optimized GF formula (OPT) was much lower than the commercial premix (COM) breads from day 1 (Figure 15). The crumb hardness of OPT formula at day 7 was 211.9 gf, much lower than GF breads from other studies, >600 gf. Crumb firming and an increase in crumbliness have a

negative impact on the eating quality of bread (Keetels and others 1996a; Keetels and others 1996b). Crumb resilience and springiness decreased over the storage period (Figures 16-17). The decrease in crumb springiness indicates an increase in brittleness (Kadan and others 2001; Moore and others 2004). Increased crumb firmness and decreased resilience during storage suggests the onset of starch retrogradation (Kadan and others 2001). Similar crumb resilience and springiness values were observed between OPT, COM, and other studies (Gallagher and others 2003b; McCarthy and others 2005; Ronda and Roos 2011).

Mold was observed on COM bread from day 5, while no mold was observed on OPT during the 7-day period. The measured values during the storage was limited to 5 and 7 days. One reason for the lack of mold may be related to the use of apple cider vinegar and calcium proprionate in the OPT product. Acetic acid from apple cider vingar, and proprionic acid from calcium proprionate are known antimicrobial agents (Belitz and others 2004), therefore delaying the mold growth during the 7-day period.

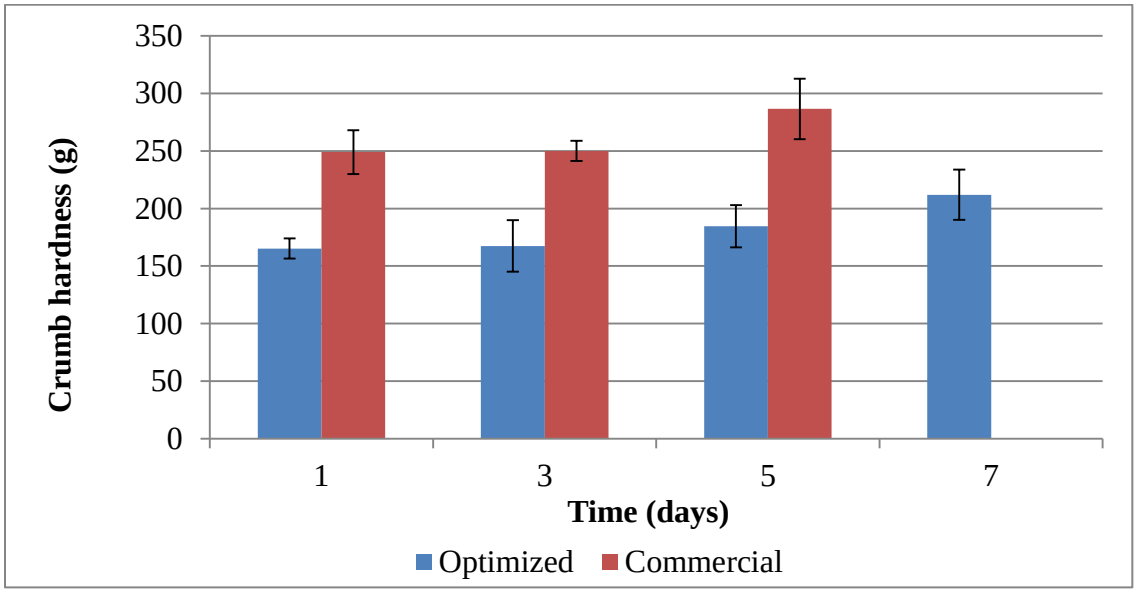

Figure 16. Changes in crumb firmness of optimized and commercial gluten-free bread over storage period of seven days. Bars represent standard deviation. No data for was obtained on the commercial bread on day 7 due to mold growth.

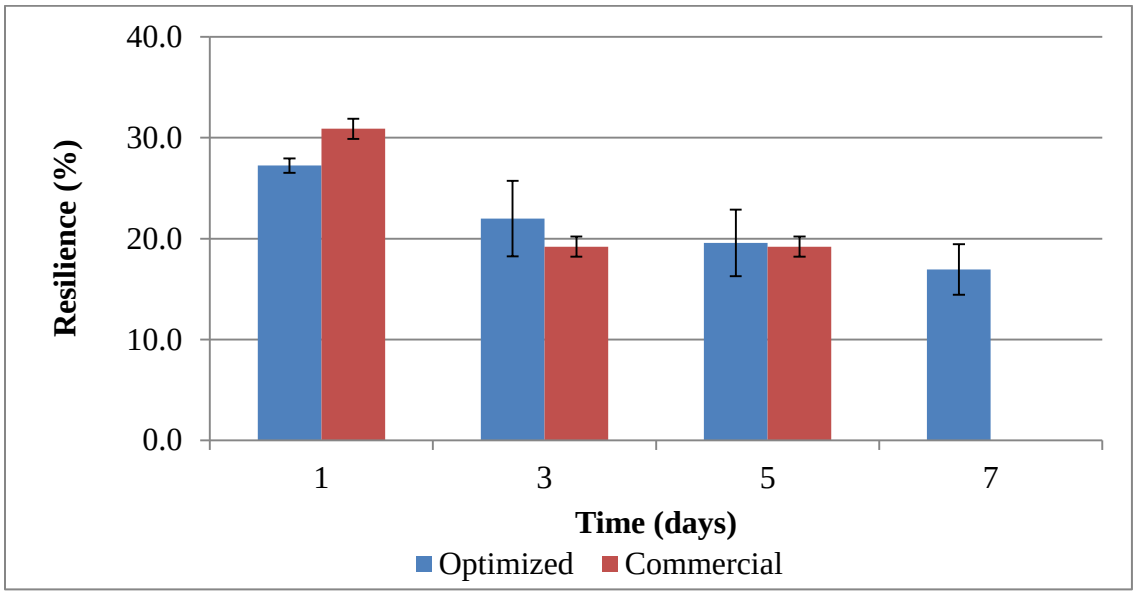

Figure 17. Changes in crumb resilience of optimized and commercial gluten-free bread over storage period of seven days. Bars represent standard deviation. No data for was obtained on the commercial bread on day 7 due to mold growth.

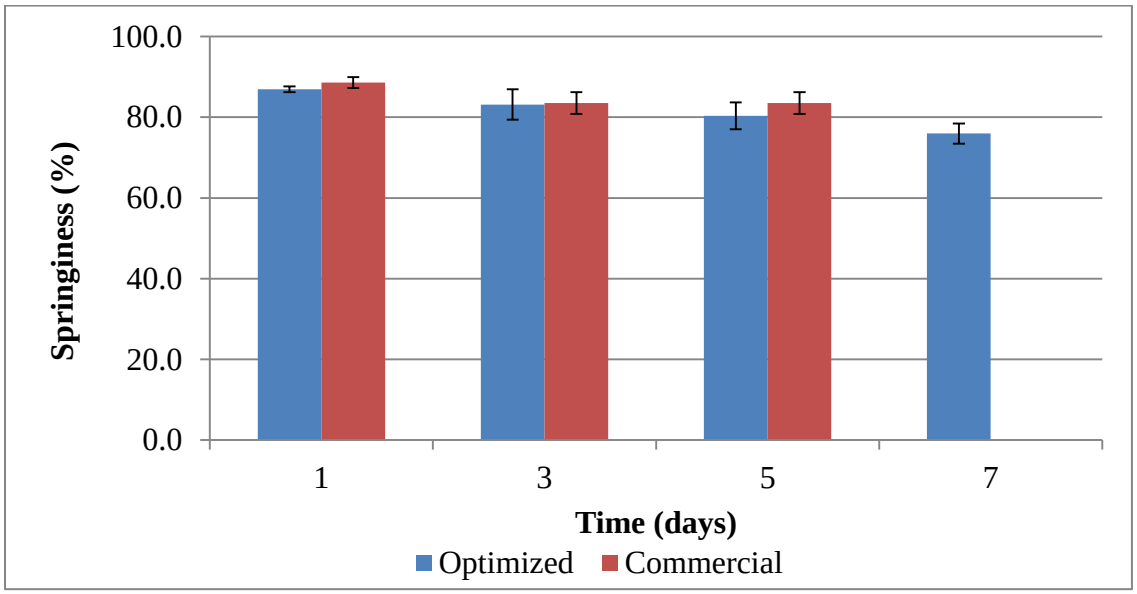

Figure 18. Changes in springiness of optimized and commercial gluten-free bread over storage period of seven days. Bars represent standard deviation. No data for was obtained on the commercial bread on day 7 due to mold growth.

8. CONCLUSIONS

This research presents a value-added GF application of yellow pea flour. The results in this study supported that by optimizing PTPF, water addition and proof time; yellow pea flour can be used to fortify GF bread with acceptable sensory and shelf life characteristics. Even though the OPT sensory rated lower than COM, the result was acceptable. OPT had the overall acceptability of 5.8 out of 9 while COM scored 6.6 out of 9. The shelf life of OPT was longer than COM in terms of texture and mold growth.

Yellow pea flour fortified GF bread was optimized successfully with high loaf specific volume (2.6 ml/g), soft crumb (174.2 g_f), bright crumb color (L* value =68.2), and small cell diameter (3.81mm) using PTPF (156.9 °C), water addition (523.8 g) and proof time (18.0 min) as the factors. Water addition had the greatest effects on the quality of GF breads, affecting loaf volume and cell diameters. Loaf volume and cell diameter increased with increasing of water additions. PTPF had the greatest effect on the crumb color. Crumb brightness decreased as the PTPF increased.

9. FUTURE DIRECTION

Acceptable precooked pea flour was achieved by the moisture-heat treatment used in this study. It might be worthwhile to further optimize the time and temperature combinations for precooking the pea flour. Other treatments for producing pea flour, such as extrusion, fractionations, and autoclaving, should be compared to identify the best processing method for GF bread application to increase the sensory scores of the product.

This study was not intended to make the best possible GF bread product. The formulations, especially the ratio of pea flour, potato starch, and brown rice flour, should be further optimized by using RSM. Nutritional quality and cost analysis of the GF bread with yellow pea flour added should also be evaluated.

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11. APPENDIX

Figure A-1. Effects of types of gums on gluten-free breads fortified with yellow pea flour.

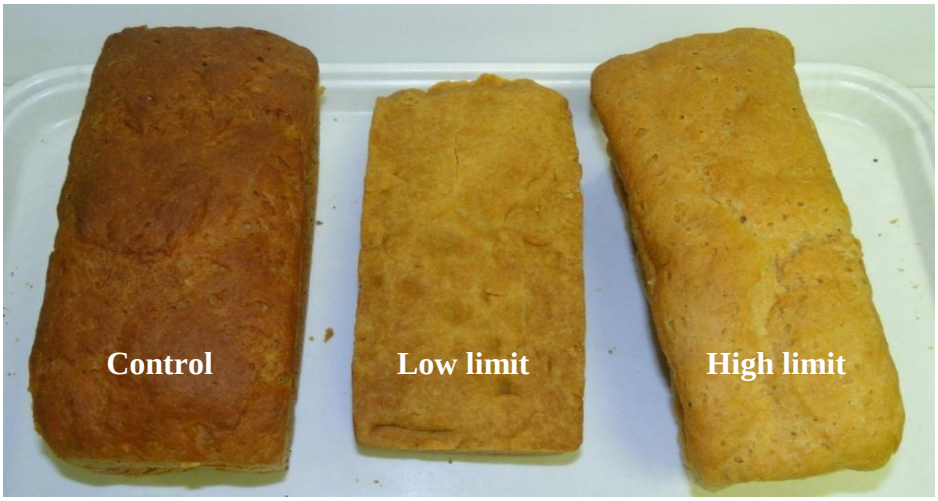

Figure A-2. Effects of water absorption on gluten-free breads fortified with yellow pea flour

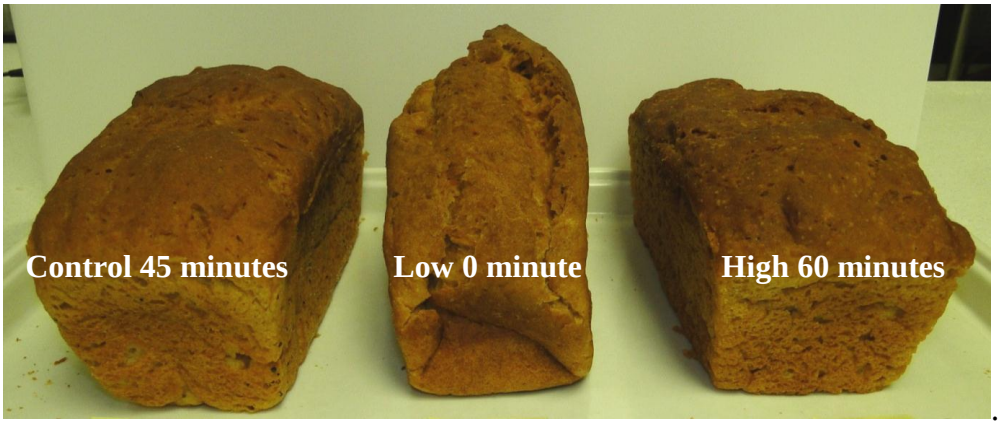

Figure A-3. Effects of proof time on gluten-free breads fortified with yellow pea flour.