Development of gluten-free pasta using amaranth flour and pea protein flour

by

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The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis globally accessible and will not permit alterations after a degree is conferred.

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NOMENCLATURE

AF	Amaranth Flour
ANOVA	One-Way Analysis of Variance
CAGR	Compound Annual Growth Rate
CD	Celiac disease
CL	Cooking Loss
CPW	Cooked Pasta Weight
DPW	Dried Pasta Weight
DSF	Defatted Soy Flour
GF	Gluten-free
GFD	Gluten-free Diet
HLA	Human Leucocyte Antigen
LSD	Least Significant Differences
MC	Moisture Content
NCGS	Non-Celiac Gluten Sensitivity
OCT	Optimal Cooking Time
OPW	Original Pasta Weight
OPW	Oven Dried Cooked Pasta Weight
PF	Pumpkin Flour
PPF	Pea Protein Flour

SPC	Soy Protein Concentrate
Td	Denaturation Temperatures
tTG	Tissue Transglutaminase
UD	Unit Density
USD	United States Dollar
WA	Wheat Allergy
WAC	Water Absorption Capacity

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ABSTRACT

About 40 million people in U.S. are suffering from gluten-related diseases, resulting in the increased demand for gluten-free products. However, the challenge in developing a gluten-free product is to produce a viscoelastic network in the absence of gluten. The present study aimed to develop a gluten-free pasta using pea protein flour (PPF) and amaranth flour (AF).

The effect of the addition of pea protein flour at concentrations of 5%, 10%, 15% and 20% to 95%, 90%, 85% and 80% AF respectively were studied. The effect of extruder screw speeds 110 rpm, 450 rpm, and 900 rpm was studied as well. As it has been reported that high-temperature drying has a positive effect on wheat pasta, gluten-free pasta was also studied at various temperature-time combinations. The effect of drying temperatures of 60°C, 80°C, and 100°C for both 12hr and 24hr was studied. To observe the effect of all the parameters, tests such as water absorption capacity, cooking loss, unit density, and color were conducted on the obtained products.

The addition of PPF at 20% concentration to AF increased the protein content from 17.3g to 25.6g. For 20% addition of pea protein flour, cooking losses decreased by 31% and firmness increased by 74.34%.

As drying temperature increased from 60°C to 100°C, water absorption capacity increased by 16.46% and cooking loss decreased by 59.09%. However, with an increase in drying temperature to 100°C, L* (lightness) value and b* (yellowness) value decreased by 32.35% and 18.69%, respectively, while a* (redness) value increased by 20.54% resulting in a darker product. Negative impact on product unit density was observed as well as it decreased by 32.35% with increase in temperature from 60°C to 100°C.

The significant negative effect of high screw speed was observed on pasta unit density, water absorption capacity and cooking loss. High screw speeds resulted in unacceptable puffed

products resulting from the flash off process. Water absorption decreased by 90% and cooking loss increased by 110.9% with an increase in screw speed from 110 to 900 rpm. Only, pasta prepared at 110 rpm was unpuffed (acceptable).

According to this study, we conclude that high protein content and drying temperature have a positive effect on pasta firmness, cooking properties, and unit density . On the other hand, pasta of low density was obtained at a very high screw speed. Overall, results showed that firmer pasta with compact structure was obtained when formulated using 80% AF and 20% PPF, drying at 80°C for 12 hrs and extruding at 110 rpm.

Even though various aspects of pasta manufacturing were studied, the pasta characteristics were still found to be inferior to the benchmark (Barilla gluten-free pasta. Further studies with emulsifier addition should be done to improve the pasta texture. To avoid the puffing of the product, an extruder with controlled barrel temperature should also be used in the future to study the effect of screw speed. To obtain pasta of a better appearance, the effect of drying pasta at various temperatures should be studied, instead of drying at a constant temperature for the whole drying cycle.

CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

Wheat is widely used in the food industry to produce products like bread, pasta, and cookies (Pena 2007). Its wide use is attributed to gluten, which is responsible for the viscoelastic behavior of dough. Gluten is referred to as a complex formed between water, wheat prolamins (gliadins) and glutenin when energy is provided through mixing (Ludvigsson et al., 2013). The prolamins fraction of gluten is known for its large amount of glutamine (35%) and proline (15%) amino acids (Balakireva & Andrey, 2016; Stern et al., 2001). Prolamins of rye (secalins) and barley (hordeins) (Vader et al., 2003) have similarly high concentration of these two amino acids, therefore can also be referred to as gluten (Hill et al., 2016).

The ingestion of grains containing gluten triggers gluten-related disorders in individuals who are genetically and/or immunologically susceptible to gluten (Fasano et al., 2015). The most known gluten-related diseases are celiac disease (CD) and wheat allergy (WA). Another gluten-related disease that has recently gained attention is non-celiac gluten sensitivity (NCGS). Traditionally gluten-free diet (GFD) were used to treat people suffering from gluten intolerance or gluten allergies. However, nowadays many people are shifting to GFD even without such intolerances to achieve a healthier lifestyle and for weight loss (Silvester et al., 2016).

Celiac disease is a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals (Ludvigsson et al., 2013). This disease causes both malabsorption and an abnormal immune reaction to gluten (Fasano & Catassi, 2001). Celiac disease is associated with deamination of gluten peptides by the enzyme tissue transglutaminase (tTG) which allows for high-affinity binding to human leucocyte antigen (HLA) DQ2 and HLA DQ8 molecules subsequently triggering an inflammatory reaction (Green & Jabri, 2003; Ludvigsson et al., 2013) and mucosa damage (Biesiekierski, 2017). On the other hand, wheat

allergy is related to the cross-linking of immunoglobulin (Ig)E by repeat sequences in gluten peptides which results in the activation of mast cells and the release of inflammatory mediators (Sapone et al., 2012; Tanabe, 2008). Conversely, individuals suffering from NCGS do not suffer from small intestine damage; they experience discomfort when they ingest food containing gluten (Sapone et al., 2012). However, gluten-free (GF) diets improved these symptoms among patients. Even though all these gluten-related diseases have been studied significantly by researchers to prevent and cure its manifestation, a strict gluten-free diet is currently the only treatment for these diseases (Fasano & Catassi, 2001; Lohi et al., 2007; Mayer et al., 1991; Niewinski, 2008; Sapone et al., 2012; Wahab, Meijer & Mulder, 2002).

Most of the foods available in the market such as pasta, bread, and biscuits are made of gluten-containing grains. But, as the prevalence of gluten-related diseases increases, the demand for gluten-free products also rises (Curiel et al., 2014; Sapone et al., 2012). For example, in the U.S. alone, over 7% of the population should avoid gluten for medical reasons, which includes 1% of people with celiac disease, 6% who have non-celiac gluten sensitivity, and 0.1% who are allergic to wheat (Hensel, 2015). Additionally, global gluten-free products market size was estimated at United States Dollar (USD) 17.59 billion in 2018 and is anticipated to expand at a compound annual growth rate (CAGR) of 9.1% from 2019 to 2025 (grandviewresearch.com).

Moreover, studies have shown that gluten-free foods are not nutritionally rich which leads to an unbalanced intake of macro and micronutrients such as more intake of carbohydrates and fats, while low intake of proteins and vitamins (Bardella et al., 2000; Mariani et al., 1998; Thompson et al., 2005). Hence, gluten-free grains with balanced nutritional composition should be used to develop gluten-free products.

Gluten-Free Grains

Several gluten-free grains that have been studied with an increased prevalence of gluten-related diseases. Some of them are maize, sorghum, rice, and teff. The other widely studied grains are amaranth, buckwheat, and quinoa which are also known as pseudocereals.

Rice

Rice flour is one of the most popular ingredients used for developing GF products, due to its bland flavor, low levels of sodium, white color, easy digestibility, and hypoallergenic proteins (Cornejo & Rosell 2015). Its high amylose content has been found to have a positive effect on water absorption and volume expansion of the product (Juliano, 2016). Rice can be used to make noodles due to its high amylose content, which helps in creating a network (Kohlwey, Kendall, & Mohindra, 1995). However, rice has no vitamin A, C, or D. It also forms a poor protein network when mixed with water due to low levels of prolamines (He and Hoseney, 1991).

Maize

Maize is a grain that is devoid of gluten and is available in abundance and has a reasonable price. Additionally, Tam et al. (2004) found that maize can be used to produce Bihon type noodles due to its high amylose content.

Sorghum

Sorghum is consumed by almost half a billion people in at least thirty countries (FAO, 2012; Ferreira et al., 2016), however its use in gluten free products is limited. It is also considered being the fifth most important cereal (FAOSTAT 2009). Sorghum is nutritionally equivalent to most cereals (Mokrane et al., 2010). Therefore, sorghum has a lot of potential to enhance the nutritional quality of GF products.

Teff

Teff is a minor crop that is small seeded, falls into the group of millet (Tatham et al., 1996). It is nutritionally very rich and contains high levels of minerals such as calcium, magnesium and iron and folate. Additionally, teff has protein content of 11%, in comparison to 8%, and 5 % for wheat and rice flour respectively. As most of the gluten-free food available in the market have low protein, teff can be used as an alternative to improve the protein of GF foods (Hager et al., 2012).

Pseudocereals

Pseudocereal is the term used for amaranth, buckwheat, and quinoa. These are dicotyledonous plants in contrast to monocotyledonous cereal grains. But these are considered as pseudo-cereals because they are similar to cereal grains in their composition and function.

These are of great importance and are high in dietary fiber, vitamins, minerals and good quality fat (Alvarez-Jubete et al., 2010). Their protein content has been reported to be 16.5% ,14.5% and 12.5% respectively for amaranth, quinoa and buckwheat, which is higher than wheat (12%) (Alvarez-Jubete et al., 2009). Additionally, their protein is high in lysine and low in glutamic acid and proline (Gorinstein et al., 2002; Kozioł 1992; Li and Zhang 2001). Also, amaranth followed by quinoa and buckwheat has shown an exceptionally high level of calcium, magnesium, zinc, and iron (Alvarez-Jubete et al., 2009), with high levels of vitamin E (Alvarez-Jubete et al., 2009). Furthermore, they are also rich in unsaturated fatty acids, specifically linolenic acid followed by oleic (Jahaniaval et al., 2000; Mazza, 1988; Navruz-Varli, & Sanlier 2016).

As aforementioned GF diets are generally low in nutrition, thus the use of pseudocereals can help to combat this problem.

Legumes

Legumes are another alternative, that are gaining popularity in gluten free industry because of its functional and nutritional profiles. It consists of 11 classes including dry peas, broad beans, chickpeas etc. They are rich in essential amino acids such as phenylalanine, isoleucine and leucine (Melini et al., 2017). However, studies reporting the effect of legumes on gluten free pasta is limited. One study reported that addition of legume flour results in yellower and firmer pasta (Bouasla, Wójtowicz, & Zidoune, 2017). The enhanced nutrition content and cooking qualities by adding bean flour has also been reported (Giuberti et al., 2015). Hence, legumes such as pea flour, chickpea flour and broad beans have a lot of scope and maybe used to produce gluten free pasta; very little work has been done in this area and needs to be investigated.

Gluten-Free Pasta

Pasta is one product that is most demanded by people with celiac disease (CD) (Zandonadi et al., 2012). In 2017, the gluten-free pasta market valued $909.8 million globally and is projected to reach $1,289.2 million by 2025. It has also been reported that, in 2017, about 40 million people were known to be gluten-sensitive or gluten intolerant in the U.S., whereas approximately 70 million of the population was known to suffer from wheat intolerance in Europe (Deshmukh & Thomas, 2019). There is significant research interest in the development of gluten-free pasta.

Pasta is traditionally prepared with wheat products, as it contains gluten, which is responsible for the elasticity and consistency of the dough. Hence, it is important to find alternatives that can replicate the properties of gluten for higher acceptance of the product. It will also enhance the supply of gluten-free products. (Araujo & Araujo, 2011; Zandonadi et al., 2009; Zarcadas & Case, 2005). According to USDA (A-A-20062F, 2015), the required pasta

characteristics are smooth surface with characteristic yellow color and does not break on cooking with firm texture.

Alternative pasta formulations are summarized in Table 1.1, which provides an extensive summary of gluten-free pasta efforts. Here flours of most interest are reviewed.

Pasta using teff flour or oat flour along with the addition of egg white powder and emulsifiers was formulated by Hager et al. (2012). The range of flours and additives tested to produce pasta and concluded optimum formulation are listed in Table 1.1. Enhanced protein content was reported with the use of egg white powder and the use of teff flour. With the use of oat flour stickiness similar to wheat pasta was found as well. In contrast, teff pasta was reported to have significantly lower stickiness value. This study indicated the potential use of teff and oat in GF pasta production. However, the sensorial aspects of these pastas were not reported in this study.

Zandonadi et al. (2012) reported the potential use of green banana flour to develop GF pasta. The pasta was stated to be low in protein and fiber content. However, it was suggested that green banana flour consists of 50% resistant starch, which acts like a fiber and makes up for low fiber content. Furthermore, other positive effects such as higher water absorption and high yield of banana flour were discussed as well. Even though modified pasta was less firm and stickier as compared to the standard pasta, it didn't affect its acceptance, indicating the benefits of using flour rich in resistant starch. However, this study did not compare it to any other available pastas.

Flour of sweet potato has also been used to develop gluten-free pasta by Limroongreungrat & Huang (2007). Sweet potato flour was reported to be treated with sodium hydroxide to produce pasta by an extrusion process. Moreover, the positive effects of fortification using defatted soy flour (DSF) and soy protein concentrate (SPC) were also discussed in the same study. The addition of DSF and SPC was observed to decrease cohesiveness, firmness, and springiness and increase

the lightness of the tested samples (Table 1.1). It was concluded in the study that, 15 g/100 g DSF or 15 g/100 g SPC enhanced the protein content of the developed pasta by 5 times as compared to pasta made from the only alkaline treated sweet potato flour indicating fortification as a good method to enhance the overall quality of the end product.

Researchers have also tried using legume flour for the nutritional improvement of gluten-free pasta products. Gluten-free pasta was developed using rice flour and white-seeded low phytic acid and lectin-free bean flour (ws+lpa+lf) in the study conducted by Giuberti et al. (2015). Modified product was observed to have increased protein, ash and dietary fiber contents, while total starch content decreased with the inclusion of new white-seeded low phytic acid and lectin free bean flour (ws+lpa+lf), because legume grains are characterized by lower starch and higher protein, dietary fiber and mineral contents compared to cereals (Tharanathan & Mahadevamma, 2003). With respect to the control pasta, modified pasta was reported to have increased water absorption capacity due to greater access to protein polar groups (Alonso, Aguirre, & Marzo, 2000), with no effect on cooking loss and texture properties. However, the negative impact of bean flour addition on the pasta color was reported, as the lightness (L* value) decreased with addition of bean flour.

The mixture of sorghum, rice, corn flour, and potato starch has also been studied for the development of gluten-free pasta (Ferreira et al., 2016). The samples containing a higher proportion of sorghum flour and/or corn flour were found to be bitter due to phenolic acids explaining its limited use in GF products. It was stated that when the formulation contained a higher content of potato flour, quality indicators, such as cooking time, yield and density showed improved results. The formulations tested, and the optimum formulation can be seen from Table 1.1.

The effect of partial replacement of corn flour with non-traditional flours such as pumpkin flour (PF) and durian seed flour on the characteristics of gluten-free pasta have also been investigated by Mirhosseini et al. (2015). The ash content of gluten-free pasta was found to increase as the percentage of durian seed flour or PF in the formulation was increased. Additionally, a positive effect on cooking yield with partial replacement of corn flour with durian seed flour and PF were also reported due to the formation of stable polymeric networks. Pasta developed by using durian seed flour was observed to have higher hardness and lower adhesiveness and firmness than the control which confirmed some positive effect of durian seed flour on texture properties of gluten-free pasta. However, pasta was not found to be desirable in terms of color, aroma and overall acceptability due to induced brownish color and bitter taste.

Amaranth has been found to be used widely by many researchers to develop gluten-free pasta. Bostos et al. (2015) used amaranth flour with dried potato pulp, extruded potato pulp, and fresh egg to develop pasta. It was observed that a high amount of extruded potato pulp and amaranth flour resulted in lower solid losses. Similar results were reported by Fiorda et al. (2013) where amaranth was used as one of the main ingredients, to develop GF pasta. Amaranth flour has also been reported to increase the firmness and decrease the stickiness of the pasta (Fiorda et al., 2013). The only concern that has been reported for using amaranth flour was the development of a darker color product (Islas-Rubio et al., 2014).

Schoenlechner et al. (2010) developed pasta using all three pseudocereals amaranth, quinoa, and buckwheat at various concentrations. Negative effects of amaranth on texture firmness, cooking weight, and cooking loss were discussed in this study. Buckwheat was reported to be best suitable for gluten-free noodle production, as it had a positive effect on firmness and cooking weight cooking loss when combined with the other two flours. The importance of using

emulsifier distilled monoglyceride to improve dough matrix, texture, and reduce cooking loss was discussed in detail as well.

Even though numerous studies have reported the development of pasta using different raw materials, only one study was found reporting the effect of drying temperature on gluten-free pasta. D'Amico et al. (2015) reported the effect of using drying temperatures 60, 80, and 100 °C on GF pasta prepared using amaranth, quinoa, and buckwheat flour and another pasta using millet flour and white bean flour. A positive effect of higher drying temperature was reported due to the modification of protein and starch structures.

Conclusions

Regardless of the great efforts to produce high-quality gluten-free pasta, the pasta similar to wheat pasta has still not been developed. No study has reported being able to develop gluten-free pasta of similar sensory and texture characteristics as of traditional wheat pasta. Most studies have only focused on using different raw materials and have tried developing pasta by just varying raw material concentrations. Little efforts have been made to understand the effect of processing conditions on gluten-free pasta characteristics. Additionally, the modification that raw material goes through at each processing step and how it can contribute to pasta with required characteristics such as low cooking loss , high water absorption capacity and high firmness has not been investigated as well. Hence, more research is needed to find the right blend and study the effects of pasta processing conditions on the gluten-free raw materials to obtain the desired high-quality gluten-free pasta.

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Table 1.1 Properties of gluten free pasta developed by other scientists*

	Pasta Formulation				Independent Variables							
	Flour (%)	**Water**	**Additives**	**Emulsifiers**	**Total energy value (kcal)**	**Moisture(g/ 100 g)**	**Protein (g/100 g)**	**Carbohydrate (g/100 g)**	**Lipid (g/100 g)**	**Ash (g/100 g)**	**Total dietary fibre (g/100 g)**	**References**
Range of Exprimented Formulations	Pre-Gelatinised Flour:Cassava Starch:Amaranth Flour 10-40:50-70:10-30	10g/100g flour	Annatto dye powder 2g + fresh whole egg 48g)/ 100g flour									Fiorda et al.,2013
Optimum Formulation	Pre-Gelatinised Flour:.Cassava Starch:Amaranth Flour 10:60:30	10g/100g flour	Annatto dye powder 2g + fresh whole egg 48g)/ 100g flour		338.72 ± 3.63	12.64 ± 0.65	10.40 ± 0.05	70.31 ±0.53	5.26 ± 0.31	1.39 ± 0.01	9.37 ±1.36	
Range of Exprimented Formulations	Amaranth:Quinoa: Buckwheat 0-50:0-55:0-100	30%-33%	Egg white powder (0%-12% of flour)	Distilled monoglycerides 0-6% of flour								Schoen-lechner et al.,2010
Optimum Formulation	Amaranth:Quinoa: Buckwheat 20:20:60	30%	Egg white powder (up to 6% of flour)	Distilled monoglycerides (1.2% of flour)								
Range of Experimented Formulations	Wheat 100	31.46% - 38.54% /100 % wheat flour	Egg white powder 3.96% -11.04% / 100 % wheat flour	0%-2.41% /100 % wheat flour								Hager et al.,2012
Optimum Formulation	Wheat 69.6g	22.8 g	Egg white powder 7g	0.6g		32.86 ± 0.18	13.45 ± 0.11	50.65		0.74 ± 0.01	<0.1	
Range of Experimented Formulations	Oat 100	35.43% - 49.57% /100 % oat flour	Egg white powder 11.46% - 18.54% /100 % oat flour	0.00% - 2.41% /100 % oat flour								
Optimum Formulation	Oat 64.7g	24.3g	Egg white powder 9.7g	1.3g		32.01 ± 1.58	13.02 ± 0.25	48.49		0.81 ± 0.07	1.00 ± 0.49	
Range of Experimented Formulations	Teff 100	35.43% - 49.57% /100 % teff flour	Egg white powder 11.46% - 18.53% /100 % teff flour	0.00% - 2.41% /100 % teff flour								
Optimum Formulation	Teff 62.8g	25.1g	Egg white powder 11g	1.1g		31.59 ± 0.06	17.85 ± 0.11	46.88		1.64 ± 0.04	0.55 ± 0.14	

*The rows which are next to each and are highlighted in same color, are the experimented and optimum formulation of the same study

Table 1.1 Continued

	Pasta Formulation	**Dependent Variables**										
	Flour (%)	**Firmness (N)**	**Stickiness (N)**	**Cooking Loss (%)**	**Optimum Cooking Time (Min)**	**Mass Increase (%)**	**Water Absorption capacity (%)**	**Volume Increase (%)**	**L**	**a***	**b***	**References**
Range of Experimented Formulations	Pre-Gelatinized Flour: Cassava Starch: Amaranth Flour 10-40:50-70:10-30	3-4.5	3.2-15.1	0.4 -1.4	2.5-5	80-180			54-70	10.-18	34-46	Fiorda et al.,2013
Optimum Formulation	Pre-Gelatinized Flour: Cassava Starch: Amaranth Flour 10:60:30	4.4 ± 0.1	3.2 ± 0.3	0.6	3.0 ± 0.29	101.5 ± 0.11			58	16	44	
Range of Experimented Formulations	Amaranth: Quinoa: Buckwheat 0-50:0-55:0-100	2.37-4.44		5.31-7.45	6.8-11		237.65-297.46					Schoenlechner et al.,2010
Optimum Formulation	Amaranth: Quinoa: Buckwheat	2.99±0.137		6.63±0.156								
Range of Experimented Formulations	Wheat 100	2.6-6.2		2.39-4.46								Hager et al.,2012
Optimum Formulation	Wheat 69.6g	5.98±0.20	0.209 ± 0.059	3.27 ± 0.40								
Range of Experimented Formulations	Oat 100	2.21-4.98		2.50-3.43								
Optimum Formulation	Oat 64.7g	4.16 ± 0.15	0.207 ± 0.030	3.27 ± 0.04								
Range of Experimented Formulations	Teff 100	2.12 -4.53		2.97-5.65								
Optimum Formulation	Teff 62.8g	4.52 ± 0.21	0.075 ± 0.018	4.06 ± 0.31								

Table 1.1 Continued

					Independent Variable							
	Flour (%)	**Water**	**Additives**	**Emulsifiers**	**Total energy value (kcal)**	**Moisture (g/100 g)**	**Protein (g/100 g)**	**Carbohydrate (g/100 g)**	**Lipid (g/100 g)**	**Ash (g/100 g)**	**Total dietary fiber (g/100 g)**	**References**
Range of Experimented Formulations	Sorghum: corn: rice: potato 40-60:10-40:10-30:10-40		Per 250g flour eggs 50 g + soybean oil 4 mL		377.89 - 378.38	10.84 - 11.43	7.86 - 8.85	74.16-75.90	4.41 -4.86	0.75 - 0.87		Ferreira et al., 2016
Optimum Formulation	Sorghum: rice: potato flour 40:20:40		Per 250g flour eggs 50 g + soybean oil 4 mL		377.89	11.08	7.86	75.9	4.41	0.75		
Range of Experimented Formulations	Rice flour: bean flour 60-100:20-40	50 ml for 100 g of flour				8.6-9.1	Umcooked-9-16.2 Cooked-7.8-14.3		Uncooked-1.1-1.7 Cooked-0.7-0.9	Uncooked-0.5-2.2 Cooked - 0.4-1.4	Uncooked-1.2-8.9 Cooked-2.8-11.5	Giuberti et al., 2015
Optimum Formulation	Rice flour: bean flour 60:40	50 ml for 100 g of flour				8.6	Umcooked-16.2 Cooked-14.3		Uncooked-1.7 Cooked-0.9	Uncooked-2.2 Cooked - 1.4	Uncooked-8.9 Cooked-11.5	
Range of Experimented Formulations	Alkaline-treated sweet potato flour: defatted soy flour 85-55:15-45	50 g/100 g				10.5-12.2	8.9-25.5					Limroon-greungrat & Huang , 2007
Optimum Formulation	Alkaline-treated sweet potato flour: defatted soy flour 85:15	50 g/100 g				10.5	8.9					
Range of Experimented Formulations	Alkaline-treated sweet potato flour: soy protein concentrates 85-55:15-45	50 g/100 g				11.0-11.4	10.4-30.7					
Optimum Formulation	Alkaline-treated sweet potato flour: soy protein concentrates 85:15	50 g/100 g				11.1	10.4					

Table 1.1 Continued

		Dependent Variable										
	Flour (%)	**Firmness (N)**	**Stickiness (N)**	**Hardness (g)**	**Cooking loss (%)**	**Optimum cooking time (min)**	**Water absorption Capacity (%)**	**Volume increase (%)**	**L**	**a***	**b***	**References**
Range of Experimented Formulations	Sorghum: Corn: Rice: Potato 40-60:10-40:10-30:10-40		2.53-3.90	2.14-4.12	0.85 - 1.1	11-15		256.47 - 286.00				Ferreira et al., 2016
Optimum Formulation	Sorghum: Rice: Potato Flour 40:20:40		3.38	3.01	1.1	11		286				
Range of Experimented Formulations	Rice Flour: Bean Flour 60-100:20-40				12.9-14.8	7.0-11.1	70.00-109.3		Uncooked: 97.7 -99.2 Cooked:92.3-94.5	Uncooked: - (-0.2) - (-0.3) Cooked:(-0.3) - (-0.4)	Uncooked: 5.00 -15.7 Cooked: 4.1-10.3	Giuberti et al., 2015
Optimum Formulation	Rice Flour: Bean Flour 60:40				14.8	11.1	109.3		Uncooked: 97.7 Cooked:92.3	Uncooked:-(-0.2) Cooked:(-0.3)	Uncooked: 15.7 Cooked: 10.3	
Range of Experimented Formulations	Alkaline-Treated Sweet Potato Flour: Defatted Soy Flour 85-55:15-45	0.4-1.8	4.1-7.8		9.9-15.8				41.9-48.9	18.7-19.6	35.0-35.6	Limroon-greungrat & Huang, 2007
Optimum Formulation	Alkaline-Treated Sweet Potato Flour: Defatted Soy Flour 85:15	1	7.5		11.2				41.9	18.7	35	
Range of Experimented Formulations	Alkaline-Treated Sweet Potato Flour: Soy Protein Concentrate 85-55:15-45	0.4-1.8	3.3-7.8		9.9-16.6				42.0-47.9	15.2-19.5	35.6-38.4	
Optimum Formulation	Alkaline-Treated Sweet Potato Flour: Soy Protein Concentrate 85:15	1.1	6.2		11.5				42	19.5	35.6	

Table 1.1 Continued

					Independent Variable							
	Flour (%)	**Water**	**Additives**	**Emulsifiers**	**Total energy value (kcal)**	**Moisture (g/100 g)**	**Protein (g/100 g)**	**Carbohydrate (g/100 g)**	**Lipid (g/100 g)**	**Ash (g/100 g)**	**Total dietary fiber (g/100 g)**	**References**
Range of Experimented Formulations	Corn Starch and Corn Flour (4:1), (40.5g-27g)/100g+ Durian Seed Flour (0g-27g)/100g+ Pumpkin Flour (0g-27g)/100g		Salt 1 g/100g + sunflower oil 3 g/100g+ dry egg powder 5 g/100g			49.76 - 57.80				1.03 - 1.38		Mirhosseini et al., 2015
Optimum Formulation	Corn Starch and Corn Flour (4:1), 40.5g/100g+ Pumpkin Flour 13.5g/100g		Salt 1 g/100g + sunflower oil 3 g/100g+ dry egg powder 5 g/100g			49.76 ± 0.19				1.03 ± 0.05		
Range of Experimented Formulations twin-screw extruder Temperature (C):100-120 Screw Speed (rpm): 80-120	Brown Rice Flour 100 G	12%	Soy protein isolate 8.0 g	monoglyceride 1.0 g + guar gum 0.4 g + sodium alginate 0.4g + xanthan gum 0.4 g						1.85 ± 0.07a		Wang et al., 2016
Optimum Temperature (C):120 Screw Speed (rpm): 120	Brown Rice Flour 100 G	12%	Soy protein isolate 8.0 g	monoglyceride 1.0 g + guar gum 0.4 g + sodium alginate 0.4g + xanthan gum 0.4 g			14.46 ± 0.20a		0.68 ± 0.1a		Soluble:2.39 ± 0.04b Insoluble: 3.64 ± 0.09a	

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Table 1.1 Continued

		Dependent Variables										
	Flour (%)	**Firmness (N)**	**Stickiness (N)**	**Hardness (G)**	**Cooking Loss (%)**	**Optimum Cooking Time (Min)**	**Water Absorption Capacity (%)**	**Volume Increase (%)**	**L**	**A***	**B***	**References**
Range of Experimented Formulations	Corn Starch and Corn Flour (4:1), (40.5g-27g)/100g+ Durian Seed Flour (0g-27g)/100g+ Pumpkin Flour (0g-27g)/100g			Uncooked: 3534-5276 Cooked:704.8-945.5					49.48-70.13	0.94-11.44	15.73-54.01	Mirhosseini et al., 2015
Optimum Formulation	Corn Starch and Corn Flour (4:1), 40.5g/100g+ Pumpkin Flour 13.5g/100g			Uncooked: 5276 ± 218 Cooked: 710.5 ± 165.0					68.28 ± 0.83	1.35 ± 0.41	52.01 ± 0.062	
Range of Experimented Formulations twin-screw extruder Temperature (C):100-120 Screw Speed (rpm): 80-120	Brown Rice Flour 100 G			1836.0 - 2387.2	6.7-11.2		110.7-124.2					Wang et al., 2016
Optimum Temperature (C):120 Screw Speed (rpm): 120	Brown Rice Flour 100 G			2387.2 ± 13.72	6.7 ± 0.2		112.4 ± 1.0					

Table 1.1 Continued

					Independent Variable							
	Flour (%)	**Water**	**Additives**	**Emulsifiers**	**Total energy value (kcal)**	**Moisture (g/100 g)**	**Protein (g/100 g)**	**Carbohydrate (g/100 g)**	**Lipid (g/100 g)**	**Ash (g/100 g)**	**Total dietary fibre (g/100 g)**	**References**
Range of Experimented Formulations	Dried Potato Pulp 65-80g + Extruded Potato Pulp 7-17g + Amaranth Flour 10-25g	18.8 mL per 100 g mixture	Fresh egg 56 g per 100 g mixture									Bastos et al., 2015
Optimum Formulation	Dried Potato Pulp 65g + Extruded Potato Pulp 10 g + Amaranth Flour 25 g	18.8 mL per 100 g mixture	Fresh egg 56 g per 100 g mixture									
Range of Experimented Formulations	Semolina: Amaranth 0-100:0-100	89.2/280g flour	Distilled monoglycerides 1.2 g/100 g+ egg white powder 9 g/100 g			6.4-7	15.2-17.1	69.1-75.9		1.3-2.6		Islas-Rubio et al., 2014
Optimum Formulation	Raw: Popped (90:10) Amaranth Flour Blend 100g	89.2/280g flour	Distilled monoglycerides 1.2 g/100 g+ egg white powder 9 g/100 g			6.4	17.1	69.1		2.6		
Optimum Formulation (No Information About Trials)	Green Banana Flour 47.0	16.40%	Egg whites 31.5%	guar gum 2.5% +xanthan gum 2.5%		11.77 ±0.67	9.30 ±0.10	74.10± 0.98	0.00 ±0.00	2.75 ±0.03	2.08 ±0.212	Zandonadi et al., 2012
Range of Experimented Formulations	Rice: Quinoa: Amaranth 15-85:10-50:5-35	33%-39%	Egg whites10%-18%			9.8-11.9						Makdoud and Rosentrater, 2017
Optimum Formulation	Rice: Quinoa: Amaranth 50:40:10	39	Egg whites 18%			11.83						

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Table 1.1 Continued

		Dependent Variable										
	Flour (%)	**Firmness (N)**	**Stickiness (N)**	**Hardness (g)**	**Cooking Loss (%)**	**Optimum Cooking Time (Min)**	**Water Absorption Capacity (%)**	**Volume Increase (%)**	**L**	**a***	**b***	**References**
Range of Experimented Formulations	Dried Potato Pulp 65g-80g + Extruded Potato Pulp 7g-17 G + Amaranth Flour 10g-25 G				3.8 - 34.8	2 -4 min	32.7- 111.5			2.90 - 5.36	41.6-48.9.	Bastos et al., 2015
Optimum Formulation	Dried Potato Pulp 65g + Extruded Potato Pulp 10 G + Amaranth Flour 25 G				3.8	2	96.2 ± 1.3		60.3 ± 1.2	4.4 ± 0.1	41.6 ± 0.6	
Range of experimented Formulations	Semolina: Amaranth 0-100:0-100	0.84-1.49		8.0-11.5	4.0-5.5				Fresh 59.2-74.5 Dried 54.9-65.6 Cooked 53.1-69.9	Fresh - 3.7-1.7 Dried - 2.2-1.9 Cooked - 3.6- 2.3	Fresh 30.4-33.4 Dried 29.6-32.8 Cooked 29.6-33.4	Islas-Rubio et al., 2014
Optimum Temperature	Raw: Popped (90:10) Amaranth Flour Blend 100g	0.84		11.5	4				Fresh 60.7 Dried 54.9 Cooked 53.1	Fresh 1.7 Dried 1.9 Cooked 2.1	Fresh 33.4 Dried 32.2 Cooked 32.9	
Optimum Formulation (No Trials)	Green Banana Flour 47.0	530.00±20.00	250.00 ±60.00		12.75±1.98		401.7 ±18.86	452.38±19.02				Zandonadi et al., 2012
Range of Experimented Formulations	Rice: Quinoa: Amaranth 15-85:10-50:5-35			14-28			108-190		60-74.5			Makdoud & Rosentrater, 2017
Optimum Formulation	Rice: Quinoa: Amaranth 50:40:10			14	7		161		65.32	3.18	17.23	

CHAPTER 2. OBJECTIVES AND HYPOTHESES

FDA (21CFR139.150, 2019) has not provided any standards for pasta quality or any attributes. According to USDA (A-A-20062F, 2015), however, gluten free pasta is referred to as non-standardized product. They have explained some requirements of characteristics in wheat pasta, such as it should have a smooth surface with characteristic yellow color and not break upon cooking with firm texture. Thus, the objective of this study was to develop gluten free pasta which could attain the characteristics mentioned above

Objectives

1. Develop a gluten-free pasta using blends of pea protein flour and amaranth flour
2. Study the effect of drying time and temperature for developed gluten-free pasta
3. Study the effect of extruder screw speed on the developed gluten-free pasta

Hypotheses

HO_1 = There is no effect of the addition of pea protein flour on the overall quality of GF pasta

HA_1= The addition of pea protein flour will significantly affect the overall quality of the obtained product

HO_1= There is no effect of different drying time and temperature combinations on the final quality of developed pasta

HA_2 = The pasta dried at different drying time and temperature combination will be different from each other in terms of the final quality

HO_1 = There is no effect of different screw speeds on the overall quality of the gluten-free pasta

HA_3 = The gluten-free pasta extruded at different screw speeds will be different from each other.

Organization of Thesis

Chapter 3 represents the first experimental study and focused on developing the gluten-free pasta and to study the effect of combination of pea protein flour and amaranth flour at different concentrations on the required characteristics of the pasta

Chapter 4 represents the second experimental study. It focuses on studying the effect of different drying time and temperature combinations on the attributes of developed pasta

Chapter 5 represents the third experimental study. It was focused on analyzing the effect of different screw speed on the quality of developed pasta.

CHAPTER 3. DEVELOPMENT OF GLUTEN-FREE PASTA USING AMARANTH FLOUR AND PEA PROTEIN FLOUR

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Modified from a manuscript to be submitted to Journal of Food Research

Abstract

Due to the increased prevalence of gluten-related diseases and changes in dietary preferences, there is an increase in demand for gluten-free products. The goal of the present study was to develop gluten-free pasta using blends of amaranth flour (AF) and pea protein flour (PPF) ranging from 80 to 100 % and 5 to 20% respectively. The major challenge of alternate flour is to build a viscoelastic network without the presence of gluten. Resulting quality was evaluated by conducting tests analyzing color, cooking time, unit density, firmness, cooking loss, and water absorption capacity. Based on ANOVA, no significant differences in a* value, b* value, water absorption capacity, or cooking loss were observed between the treatments. A slight decrease in optimal cooking time was observed as PPF was added. Moreover, a substantial increase in protein content from 17.3g to 25.6g was observed with the addition of 20% PPF. An increase in protein content also had a significant effect on pasta firmness, as it increased by 289.65% with the addition of 20% PPF.

Introduction

Gluten is a complex formed between water and wheat prolamins (glutenins and gliadins) when they are mixed (Ludvigsson et al., 2013). The prolamins fraction of gluten is known for its large amount of glutamine (35%) and proline (15%) amino acids (Balakireva & Andrey, 2016; Stern et al., 2001). Prolamins of rye (secalins) and barley (hordeins) (Vader et al., 2003) have a

similarly high concentration of these two amino acids and therefore can be referred to as gluten (Hill et al., 2016).

Ingestion of gluten-containing grains triggers gluten-related disorders in individuals who are genetically and/or immunologically susceptible to gluten (Fasano et al., 2015). The most well-known gluten-related diseases are wheat allergy (WA) and celiac disease (CD). Another gluten-related disease that has recently gained attention is non-celiac gluten sensitivity (NCGS).

Wheat allergy is related to the cross-linking of immunoglobulin (Ig)E by repeat sequences in gluten peptides which results in activation of mast cells and the release of inflammatory mediators (Sapone et al., 2012; Tanabe, 2008). Additionally, celiac disease is a chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals (Ludvigsson et al., 2013). This disease causes both malabsorption and an abnormal immune reaction to gluten (Fasano & Catassi, 2001). Conversely, individuals suffering from NCGS do not suffer from small intestine damage; they experience discomfort when they ingest food containing gluten (Sapone et al., 2012). However, gluten-free (GF) diets improved these observations among patients. Even though all these gluten-related diseases have been studied significantly by researchers to prevent and cure its manifestation, a strict gluten-free diet is currently the only treatment for these diseases (Fasano & Catassi, 2001; Lohi et al., 2007; Mayer et al., 1991; Niewinski, 2008; Sapone et al., 2012; Wahab, Meijer & Mulder, 2002).

Most of the foods available in the market such as pasta, bread, and biscuits are made of gluten containing grains. But, as prevalence of the gluten-related diseases increases, the demand for gluten-free products also rises (Curiel et al., 2014; Sapone et al., 2012). For example, in the U.S. alone, over 7% of the population should avoid gluten for medical reasons, which includes 1%

of people with celiac disease, 6% who have non-celiac gluten sensitivity, and 0.1% who are allergic to wheat (Hensel, 2015).

While there are many GF products available, there is still a high demand for pasta by people with celiac disease (Zandonadi et al., 2012). Pasta is also one of the simplest food products traditionally prepared by mixing semolina and water (Marti, & Pagani, 2013). Typically, durum wheat proteins, when mixed with water, are known to form a complex matrix responsible for the typical viscoelastic behavior that allows the formation of good quality pasta (Mariotti et al., 2011). However, absence of gluten creates a challenge to produce quality pasta. Studies show that gluten-free foods are not nutritionally rich which leads to unbalanced intake of macro- and micronutrients such as high intake of fat and carbohydrates and low intake of vitamins and proteins (Bardella et al., 2000; Mariani et al., 1998; Thompson et al., 2005). Thus, it is important to use raw materials of good nutritional quality to provide some health benefits.

According to Aguilar et al. (2015), amaranth, which is a dicotyledon, has a protein content of 16%, which is higher than regularly consumed cereals such as wheat (12-14 %) and rice (7-10%). Additionally, its protein is considered of very good quality because of its high lysine levels (Bressani, 1994). Moreover, it is rich in minerals (calcium, magnesium, and iron) (Alvarez-Jubete et al., 2009), vitamins (e.g., folic acid), essential amino acids, and essential fatty acids (Becker et al., 1981). High amounts of unsaturated fatty acids, with a very high level of linoleic acid has also been reported (Alemayehu et al., 2015; Berghofer & Schoenlechner, 2002). Thus, amaranth can be used as a good raw material to produce GF products due to its high nutritional composition. To further enhance the nutritional quality of gluten free pasta pea protein flour can also be used. Flours from legumes are considered to have a positive effect on glycemic response and sensory qualities (Pellegrini & Agostoni, 2015). Furthermore, pea has high dietary fibre and good emulsifying

properties contributing to lower dough adhesiveness to press elements, facilitate dough flow through a chamber and a die, and lower protein network structure destruction (Tömösközi et al., 2001). Thus, the combination of pea protein flour and amaranth flour can be used to develop gluten free pasta.

The objective of this study was to develop gluten-free pasta using amaranth and pea protein flour and to study resulting quality parameters of the pasta by conducting tests analyzing water absorption capacity, cooking loss, cooking time, color, unit density, moisture content, and firmness.

Materials and Methods

Raw Materials

Amaranth grains were provided by Nutricity LLC (Scottsdale, Arizona, United States). Pea protein flour was obtained from AGT Foods (Regina, SK, Canada). Amaranth grains were milled into the flour with a particle size of 0.4 mm or less, using hammer mill (Viking).

Pasta Production and Experimental Design

The pasta dough was prepared by combining pea protein flour, water, and amaranth flour. The water was slowly added to the flour, as it is one of the very crucial parts for proper dough development. If water is too high the dough becomes sticky and if it is too low it is very difficult to make a cohesive dough and extrude it (Makdoud and Rosentrater, 2017; Schoenlencher et al., 2010).

The pea flour at concentration of 0%, 5%, 10%, 15%, and 20% was added to 100%, 95%, 90%, 85%, and 80% amaranth flour respectively, making up to 100%. The water content (70 g/100 g) was kept constant to study the impact of protein on pasta. The pasta dough was prepared by mixing the flours and water in a laboratory scale mixer (KitchenAid KSM75WH) for 5 min at a

speed of 8. The dough was then kneaded by hand for one minute and stored in airtight bags at room temperature for a day. It allows a uniform moisture distribution (Rosentrater et al., 2005).

After one day the dough gets properly hydrated and a little soft. The dough was then extruded using laboratory scale extruder (Kitchen Aid KPEXTA) using a Stand-Mixer Pasta-Extruder Attachment with a speed of 8. The diameter of the holes of the disc die was ≈ 1.65 mm. Extruded pastas were immediately dried for 24h at 60°C in the oven (Heratherm General Protocol Ovens, ThermoFisher Scientific, Waltham, Massachusetts, United States), then they were stored in airtight bags (Makdoud and Rosentrater, 2017). Each formulation was prepared one time, but replicates were conducted for each parameter studied.

Peak Viscosity

Peak viscosity of flour samples which were 100% AF, 80% AF and 20% PPF, 50% AF and 50% PPF, and 100% PPF was measured using Rapid Visco Analyzer (RVA-4) using the method given in the manual (Newport Scientific Pty. Ltd.). Four grams of sample was added to canister along with 25 ml of water. It was then heated to 50°C, then to 95°C and again cooled down to 50°C.

Proximate Analysis

Protein, ash, fat, and fibre analysis on flour blends were done by a third-party laboratory (Servi-Tech Laboratories, Hastings, Nebraska) following official AOAC standard methods.

Moisture Content

AACC method 44-19 (AACC, 2000) was used to determine the moisture content of the pasta samples. Two grams of dried pasta sample was weighed out and kept in the oven (Heratherm General Protocol Ovens, ThermoFisher Scientific, Waltham, Massachusetts, United States) at

135°C for 2 h. The initial weight and the final weights were noted. The difference between the two gives the moisture content of the sample. The test was performed in triplicate.

$$MC \text{ (\% dry basis)} = ((OPW - DPW)/DPW) \times 100 \quad (3.1)$$

Where, MC = moisture content; DPW = dried pasta weight (g) (before cooking); OPW = original (wet) pasta weight (g)

Unit Density

To measure the unit density, the method described by Rosentrater et al. (2005) was used. The length (L) and the diameter (D) of the sample were measured using Traceable Electronic Digital Caliper (Fisher Scientific, Pittsburg, Pennsylvania 15275, United States). The pasta was assumed to be a cylinder, so the following formula was used to calculate the volume:

$$V = \pi \times D^2/4 \times L \quad (3.2)$$

Where, V= volume (m^3); D= diameter (m); L= length (m)

The mass of the pasta strand was the measured and unit density was calculated according to the below mentioned formula

$$\text{Unit Density} = m/V \quad (3.3)$$

Where, m= mass (kg); V= volume (m^3)

Color Analysis

To determine the color of the pasta, the method described by Makdoud and Rosentrater (2017) was used. The pasta samples were spread in a petri dish and analyzed for L*, a* and b* values using Chroma Meter CR-410 colorimeter (Konica Minolta Optics, Inc. Chroma meter, Ramsey, New Jersey, USA) equipped with a xenon lamp.

The functional properties of pasta include cooking time, water absorption and cooking loss. All methods were carried out according to the guidelines of AACC 66-50.01 (2004). All the tests were performed in triplicate.

Optimal cooking time

Optimal coking time was measures according to the guidelines of AACC 66-50. The same method has also been used by Bouasla, Wójtowicz, & Zidoune (2017) and Makdoud & Rosentrater (2017). To determine the optimal cooking time (OCT), 5 g of dried pasta sample was weighed. It was then boiled in 200 mL of distilled water. Every 30 sec, a pasta strand was removed from boiling water and squeezed between two pieces of Plexiglas. When the center core disappeared, pastas were considered cooked.

Water absorption capacity

Water absorption capacity was measured according to the guidelines of AACC 66-50. For water absorption, 10 g of dried pasta sample was pre-weighed and boiled in 300 mL of water at previously determined cooking time. Then, pasta was removed and weighed. The same method has also been used by Bouasla, Wójtowicz, & Zidoune (2017) and Makdoud & Rosentrater (2017). The weight difference between dried pasta and cooked pasta was used to calculate the water absorption.

$$WAC\ (\%) = ((CPW - DPW)/DPW)\ X100 \qquad (3.4)$$

Where, WAC= water absorption capacity (%); CPW= cooked (wet) pasta weight (g) and DPW = dried pasta weight (g) (before cooking)

Cooking loss

Cooking loss was measured according to the guidelines of AACC 66-50. The same method has also been used by Bouasla, Wójtowicz, & Zidoune (2017) and Makdoud & Rosentrater (2017).

It is an important parameter to determine the stability of the network formed. The better the protein network is developed, the smaller is the cooking loss. CL was measured by putting cooked pasta in an oven at 50°C for 48 h (using the same units as described previously):

$$CL\ (\%) = ((DPW - ODPW)/DPW) \times 100 \quad (3.5)$$

Where, CL= cooking loss (%), ODPW = oven dried cooked pasta weight, DPW = dried pasta weight (g) (before cooking)

Firmness

The firmness of pasta samples was analyzed using a TA. XTplus texture analyzer (Stable Micro System Co. Ltd.,) equipped with a 5 kg load cell. Five strands of pasta samples were used for each analysis. They were placed at the center of the measurement plate under the knife blade. The measurement was performed at the test speed: 0.17 mm/s and distance: 3 mm. The firmness of pasta was recorded as the maximum force required to cut the sample. Each sample was analyzed 5 times.

Statistical Analysis

Results were analyzed using R studio. Data was analyzed using one-way analysis of variance (ANOVA). Least significant differences (LSD), at a 5% level of probability, were determined between treatments.

Results and Discussion

Peak Viscosity

When starch is heated in the presence of water beyond its critical temperature, it swells. As starch granules swell, viscosity increases, but as the temperature increases, they begin to rupture. The peak viscosity represents the equilibrium viscosity between swelling and rupturing of the starch granules (Acevedo et al., 2013).

The peak viscosity decreased as PPF was added at 20%, 50% and 100% concetration (Table 3.1). The peak viscosity ranged from 116 cP to 1379 cP. The sample with 100% amaranth flour had the greatest peak viscosity (1379cP) which is comparable to what was reported by Shevkani et al. (2014). Shevkani reported the peak viscosity of amaranth from different cultivars to be in the range of 1050 to 1459 cP. The peak viscosity decreased as PPF was added, possibly due to increased protein content. The high protein content results in embedment of starch in the protein matrix, thus reducing the starch swelling. Similar results have been reported by Acevedo et al. (2013), Chung et al. (2008) and Kiin-Kabari et al. (2015). Moreover, the peak viscosity of 100% AF and 80% AF with 20% PPF was found comparable to the peak viscosity of wheat flour by Bakare et al. (2015) and Choi et al. (2012), which were 1214.01 cP and 1636.8 cP, respectively. Due to the similarity of peak viscosity in the current study and in literature, it was decided to study the effects of PPF addition till 20%.

Proximate Analysis

The different flour blends prepared by mixing various concentrations of AF and PPF were analyzed for their protein, fat, fiber, and ash content. It was found that with the addition of 20% PPF, protein content increased by 47.97% (i.e., from 17.3 to 25.6%). However, slight decreases in dietary fiber and crude fat were observed with the addition of 20% PPF to AF (Table 3.2).

Moisture Content

The moisture content of the pasta is the amount of moisture present in the dry pasta. High moisture content can lead to microbial growth, which is why it is important to maintain moisture content below 12%, which is considered as the safe limit (Bustos et al., 2015; Ferreira et al., 2016).

As shown in Table 3.3, there was no significant difference in the moisture content of the samples. It ranged from 4.74 to 8.73%. All the samples were found to have moisture content less than 12%, which is considered as the safe limit.

Unit Density

Unit density is measured to ensure that pasta is dense enough that it will stay immersed in water. Moreover, it is a great parameter to study the compactness of the product structure.

The unit density value for all the tested samples is presented in Table 3.3. The unit density ranged from 1040.2 to 1111.4 kg/m^3. No significant differences were observed as the PPF was added to AF. As the unit densities of all the samples were above 1000 kg/m3, it would not float on water. Therefore, higher density also signified the lower porosity and expansion of the trial pasta (Miladinov & Hanna, 2000). High unit density could be attributed to low shear rates and low barrel temperature due to the use of a Kitchen Aid, which prevented the product puffing. Hence, a denser product was obtained due to low porosity and less expansion (Wang et al., 1999).

Color

People generally associate pasta quality with the color of the product. Thus, the L* (brightness) and b* (yellowness) values are important color attributes of the pasta which are related to its desirability (Rayas-Duarte et al., 1996).

In the undertaken study, L* values decreased significantly by 26.51% i.e. from 46.4 to 34.1, as the substitution level of pea protein flour increased to 20% (Table 3.3). No significant differences, in a* and b* values were observed. The L*, b*, and a* values ranged from 46.4 to 34.1, 22.8 to 19.48, and 5.06 to 7.6, respectively, for the tested samples. The highest L* and b* values and lowest a* value was observed for pasta formulated using 100% amaranth flour.

Even though there were no significant differences in b* value, visually yellower, brighter pasta due to the use of 100% AF was obtained (Fig 3.1), which could be due to the amount of carotenoid pigment in the amaranth (Acquistucci, 2000). The increase in b* value by the addition of amaranth flour to semolina has likewise been reported by Islas-Rubio et al. (2014).

Even though no significant decreases in b* value or increases in a* value were observed, darker pasta was obtained as PPF concentration increased; this can also be observed in Figure 3.1. The observed darker product with PPF could be due to a substantial increase in the amount of lysine, which contributes to the Maillard reaction (Wang et al., 1999). Similar results have been reported by Sudha & Leelavathi (2012).

Cooking Quality

Some of the important parameters to determine the final characteristics of the pasta are water absorption capacity, cooking loss, and optimal cooking time.

All the results are tabulated in Table 3.3. No significant differences were observed in water absorption capacity (WAC) or cooking loss (CL) of the tested samples. However, optimal cooking time (OCT) decreased significantly by 10.71% i.e. from 5.6 min to 5 min with an increase in PPF. The WAC, CL, and OCT of the tested samples ranged from 131.8 to 150.5%, 16.2 to 23.5%, and 5.00 to 5.6 min, respectively.

For all the samples, the WAC was found to be greater than 100%. According to Fiorda et al. (2013), pasta should double its mass during cooking. Thus, in the present work, the experimental pasta can be considered of acceptable quality. No significant differences in water absorption capacity were observed as the protein content of the pasta sample increased. However, a slight decrease in water absorption was observed with a 20% addition of PPF. This could be attributed to stronger protein network formation, which could have led to decreased starch swelling

(Zweifel, 2001). This finding was supported by the RVA results. Moreover, PPF is rich in hydrophobic amino acids such as alanine and leucine. Perhaps these amino acids could be a reason for the observed phenomenon (Sikorski, 2001; Stone et al., 2015; Swanson, 1990).

Cooking loss for good quality wheat pasta should be lower than 12%, but for GF pasta, 20 to 25% CL has been considered acceptable (Giuberti et al., 2015). For all the blends, cooking loss was less than 25%. The highest cooking loss was observed for the pasta prepared using 100% AF, which decreased to 16.2% with the addition of 20% pea protein flour. The cooking losses for all the samples were found to be a little higher than what was reported by Sudha and Leelavathi (2012). However, in their study, pasta was prepared using amaranth seed flour and dehydrated green pea flour as opposed to amaranth seed flour and pea protein flour, as with this study.

Firmness

Firmness is an important parameter, which is associated with the al-dente quality of pasta, which is most demanded by consumers (Zweifel, 2001).

As the concentration of amaranth flour decreased and pea protein flour increased, a significant increase in firmness was observed (Table 3.3). The firmness increased by 144.28% i.e. from 0.29 to 0.71 N with a 10% addition of PPF and by 289.65% (0.29 to 1.13N) with the addition of 20% PPF. The firmness of 100% AF pasta was found to be 0.29 N. Similar firmness was reported by Schoenlechner et al. (2010) for pasta prepared with 100% AF.

An increase in firmness with a decrease in AF concentration can be attributed to a decrease in amylopectin, which is also in accordance with the peak viscosity results (Table 3.1). AF is rich in amylopectin (Kong, Bao & Corke, 2009), which negatively impacts pasta firmness. Amylopectin results in a softer pasta structure due to high swelling power, which further decreases the pasta firmness. Gianibelli, Sissons & Batey (2005) reported similar results for spaghetti

prepared by replacing semolina starch with waxy starches of barley, wheat and maize. Another reason for the increase in pasta firmness was the increase in protein content with the increase in PPF concentration (Table 3.2). An increase in protein content results in a stable protein network formation, resulting in a firmer pasta. Similar results have been reported by Duda et al. (2019), in which firmer pasta was obtained as the protein increased by adding cricket powder to the wheat pasta.

Conclusions

The research aimed to develop pasta using AF and PPF and study the effect of PPF addition to AF. It was observed that protein content increased substantially as the PPF was added to the AF. The higher protein content showed a positive effect on cooking qualities and resulted in increased water absorption capacity and lower cooking loss. This was attributed to stronger protein network formation due to enhanced protein content. However, the product with PPF was found to be darker in color, in comparison to one which was developed using only amaranth flour. However, no statistically significant differences were observed in the yellowness of the product. Overall, it was concluded that enhanced protein content resulted in pasta of higher quality both nutritionally and structurally.

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Table 3.1 Peak viscosity of different blends

Blends	**Peak Viscosity (cP)**
100% Amaranth	1379.00
80% amaranth 20% Pea Protein Flour	834.00
50% amaranth 50% Pea Protein Flour	250.00
100% Pea Protein Flour	116.00

Table 3.2 Proximate analysis of different flour blends

Sample	**Crude Protein (%, d.b.)**	**Crude Fiber (%, d.b.)**	**Crude Fat (%, d.b.)**	**Crude Ash (%, d.b.)**
100% AF	17.3	4.5	7.0	3.4
95% AF & 5% PPF	19.1	3.9	6.8	3.4
90% AF & 10% PPF	21.4	4.2	6.5	3.6
85% AF & 15% PPF	22.6	5.1	6.4	3.7
80% AF & 20% PPF	25.6	3.8	6.1	3.8
Pea protein*	≥55.0	≤15.0	≤15.0	

* Pea protein values were retrieved from AGT Foods Website (http://agtfoods.com/products/pulseplus-protein.html)

Table 3.3 Treatment effects on pasta properties†

Formulation Blend	**Moisture Content (%, d.b.)**	**Unit Density (kg/m^3)**	**Color**			**Optimal Cooking Time (min)**	**Water Absorption Capacity (%)**	**Cooking Loss (%)**	**Firmness (N)**
			L	a	b				
100% AF	4.94(0.74) ^{a} n=3	1040.2(92.8)^{a} n=5	46.4 (5.9)^{a} n=3	5.06(2.1)^{a} n=3	22.8 (4.8)^{a} n=3	5.6 (0.29) ^{a} n=3	146.5 (16.7)^{a} n=3	23.5 (5.64)^{a} n=3	0.29(0.07)^{c} n=5
95%AF & 5% PPF	4.74(1.91)^{a} n=3	1102.1(57.8)^{a} n=5	37.7 (6.8) ^{ab} n=3	6 (2.1)^{a} n=3	19.65 (5.6)^{a} n=3	5.0 (0.0)^{b} n=3	146.8 (5.39)^{a} n=3	18.5 (2.11)^{a} n=3	0.414(0.05)^{c} n=5
90%AF & 10% PPF	6.23(1.31)^{a} n=3	1111.4(100.7)^{a} n=5	37.8 (4.5)^{ab} n=3	7.1 (1.1)^{a} n=3	21.2 (3.1)^{a} n=3	5.0(0.0) ^{b} n=3	149 (7.8)^{a} n=3	19.05 (1.41)^{a} n=3	0.71(0.08)^{b} n=5
85%AF & 15% PPF	8.73(2.99)^{a} n=3	1103.5 (66.2)^{a} n=5	35.1 (9.8)^{ab} n=3	7.3 (2.7)^{a} n=3	19.92 (7.8)^{a} n=3	5.16 (0.29)^{b} n=3	150.5 (5.2)^{a} n=3	17.6 (1.5)^{a} n=3	0.96(0.23)^{ab} n=5
80%AF & 20% PPF	8.15(3.79)^{a} n=3	1081.1 (68.5)^{a} n=5	34.1 (8.1)^{b} n=3	7.6 (2.3)^{a} n=3	19.48 (6.5)^{a} n=3	5.0 (0.0) ^{b} n=3	131.8 (16.4)^{a} n=3	16.2 (1.4) ^{a} n=3	1.13(0.22)^{a} n=5

† Results are presented as the mean (standard deviation) . Letters a–c represents significant difference at the 5% level of significance for the samples.

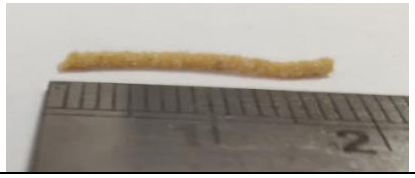
100% AF	
95% AF 5% PPF	
90% AF 10% PPF	
85% AF 15% PPF	
80% AF 20% PPF	

Figure 3.1 Pasta Samples with different raw material combinations (scale gradations are in cm)

CHAPTER 4. EFFECT OF DRYING ON GLUTEN-FREE PASTA DEVELOPED USING AMARANTH AND PEA PROTEIN FLOUR

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Abstract

High temperature drying has been widely used for wheat pasta; however, not many studies have observed its effects on gluten-free (GF) pasta. The aim of the present study was to evaluate the effect of various drying temperatures (60°C, 80°C, 100°C) and time (12 and 24 hr) combinations on GF pasta prepared using amaranth and pea protein flours. The effect of variation in drying time and temperature on formulated pasta was studied by conducting tests such as cooking loss, water absorption capacity, firmness, unit density, and color. Statistical analysis tested the significant differences. It was found that high temperature drying has a positive effect on the cooking quality of pasta. There was a substantial increase in water absorption capacity and a decrease in cooking loss by 16.06% and 59.09%, respectively, for all the samples due to stable matrix formation. However, high temperature drying resulted in a porous pasta structure, which resulted in decreased unit density by 32.35% with an increase in temperature from 60°C to 100°C. Additionally, a darker colored pasta was obtained at higher temperatures. Overall, high temperature drying both positively and negatively impacted gluten-free pasta characteristics.

Introduction

Pasta is a cereal-based food, which is consumed all over the world (Ogawa et al., 2015). It is often prepared by mixing flour and water, which is then extruded and dried. Drying is an important processing parameter of pasta which significantly affects its overall quality including appearance and cooking quality (Cubadda et al., 2007; Manthey & Schorno, 2002).

Pasta is traditionally dried at lower temperatures (40-50 ^{0}C) for longer times (up to 40 hr). However, these conditions promoted microbial growth. In order to combat this, high-temperature drying was introduced into the industry (Sensidoni, Peres, & Pollini, 1999). High temperature drying results in increased productivity by lowering drying time and reducing microbial count (Güler, Köksel, & Ng, 2002). Additionally, drying temperature has an impact on structural modification of protein (denaturation) and starch (swelling) (Petitot, Abecassis, & Micard, 2009a). For example, low-temperature drying results in a less organized protein network with no modifications in starch structure. While at higher temperatures, definite protein network formation occurs due to complete protein denaturation along with changes in starch structure (De Noni & Pagani, 2010). Complexes such as starch-protein and starch/lipids/protein are also formed at higher temperatures (De Noni & Pagani, 2010), resulting in higher quality pasta.

Many studies have researched the effects on wheat pasta high temperature drying (Cubadda et al., 2007; Manthey & Schorno, 2002; Zweifel et al., 2003). But there is limited information regarding drying temperature effects on gluten-free pasta. Compared to wheat pasta, poor texture and cooking quality have been reported in gluten-free pasta (Schoenlechner et al., 2010). Thus, focus on processing conditions such as drying conditions is required in order to obtain gluten-free pasta of higher quality. Furthermore, research has shown that pasta quality is dependent upon gluten quality rather than its content when dried at low temperatures (D'Amico et al., 2015). However, at high temperatures, protein polymerize into a protein network depends on its content rather than on its quality (Bruneel et al., 2010). Since the focus of the present study is on high temperature drying, highly nutritious, high quality amaranth (Aguilar et al., 2015; Alvarez-Jubete et al., 2009) and pea protein flour (Melini et al., 2017; Tömösközi et al., 2001) were used in this study.

The objective of this study was to determine the effects of different drying temperatures (60 ^{0}C, 80 ^{0}C, and 100 ^{0}C) and time (12 and 24 hr) combinations on the quality of gluten-free pasta obtained by mixing amaranth flour with pea protein flour. Pasta quality was determined by conducting tests such as cooking loss, cooking weight, and water absorption capacity.

Materials and Methods

Raw Materials

Amaranth grains were provided by Nutricity LLC (Scottsdale, Arizona, United States). Pea protein flour was obtained from AGT Foods (Regina, SK, Canada). Amaranth grains were milled into the flour with a particle size of 0.4 mm or less, using hammer mill (Viking).

Experimental Design

The pasta was prepared by combining pea protein flour, water, and amaranth flour. For the trials, pasta samples using 100% AF and 20% PPF with 80% AF were prepared making up to 100g of flour. The water content (70 ml/100g) was kept constant. To test the effect of drying time and temperature, they were dried at 60°C for 12 and 24 hrs, 80°C, 12 and 24 hrs, and 100°C, 12 and 24 hrs. Each formulation was prepared one time, but replicates were conducted for each parameter studied.

Pasta Manufacture

To make pasta dough, amaranth flour and pea protein flour were blended making up to 100g of flour to which 70 ml of water was added and mixed using a laboratory scale mixer (KitchenAid KSM75WH) at a speed of 4 for a duration of 5 min. Then the dough was kneaded by hand for one minute and stored at room temperature for a day in an airtight bag, to ensure uniform water distribution (Rosentrater et al., 2005). After the dough was rested for one day, it was extruded by using Stand-Mixer Pasta-Extruder Attachment (Kitchen Aid KPEXTA) at a speed of 8. The

diameter of the holes of the disc die was ≈ 1.65 mm. They were then dried at the predetermined time and temperature combinations in the oven (Heratherm General Protocol Ovens, ThermoFisher Scientific, Waltham, Massachusetts, United States).

Proximate Analysis

Protein, ash, fat, and fibre analysis on flour blends were done by a third-party laboratory (Servi-Tech Laboratories, Hastings, Nebraska) following official AOAC standard methods.

Moisture Content

Moisture content of the samples was measured using AACC method 44-19 (AACC, 2000). To determine the moisture content, 2 g of dried pasta sample was weighed out and kept in the oven (Heratherm General Protocol Ovens, ThermoFisher Scientific, Waltham, Massachusetts, United States) at 135°C for 2 hrs. The difference between the two represented the moisture content of the sample. The test was performed in triplicate.

$$MC \text{ (\% dry basis)} = ((OPW - DPW)/DPW) \times 100 \quad (4.1)$$

Where, MC = moisture content (% dry basis); DPW = dried pasta weight (g) (before cooking); OPW = original (wet) pasta weight (g)

Unit Density

The method described by Rosentrater et al. (2005) was used to measure the unit density. The pasta was assumed to be a cylinder and its length (L) and diameter (D) were measured using Traceable Electronic Digital Caliper (Fisher Scientific, Pittsburg, Pennsylvania 15275, United States). The obtained values were substituted in the given formula:

$$V = \pi \times D^2/4 \times L \quad (4.2)$$

Where, V= volume (m^3); D= diameter (m^2); L= length (m)

The mass of the pasta strand was the measured and unit density was calculated according to the below mentioned formula

$$\text{Unit Density} = m/V \tag{4.3}$$

Where, m= mass (kg), V= volume (m^3)

Color Analysis

The method described by Makdoud and Rosentrater (2017) was used for color determination. L*, a* and b* values were analyzed using Chroma Meter CR-410 colorimeter (Konica Minolta Optics, Inc. Chroma meter, Ramsey, New Jersey, USA) equipped with a xenon lamp.

Optimal cooking time

Optimal cooking time was measured according to the guidelines of AACC 66-50.01 (2004) Five grams of dried pasta sample was weighed and boiled in 200 mL of distilled water. A pasta strand was removed in every 30 s from boiling water and squeezed between two pieces of Plexiglas. When the center core disappeared, pasta was considered cooked. The test was performed in triplicate.

Water absorption capacity

Water absorption capacity was measured in triplicate according to the guidelines of AACC 66-50.01 (2004). The same method has also been used by Bouasla, Wójtowicz, & Zidoune (2017) and Makdoud & Rosentrater (2017). Ten grams of dried pasta sample was pre-weighed and boiled in 300 mL of water at previously determined cooking time. The cooked pasta was then removed and weighed. Water absorption capacity was then measured using given formula:

$$WAC\ (\%) = ((CPW - DPW)\ /DPW) * 100 \quad (4.4)$$

Where, WAC= water absorption capacity (%); CPW= cooked (wet) pasta weight (g) and DPW = dried pasta weight (g) (before cooking)

Cooking loss

Cooking loss was measured in triplicate, according to the guidelines of AACC 66-50.01 (2004). The same method has also been used by Bouasla, Wójtowicz, & Zidoune (2017) and Makdoud & Rosentrater (2017). The cooked pasta was kept in an oven at 50°C for 48 hrs (using the same units as described previously):

$$CL\ (\%) = ((DPW - ODPW)\ /DPW) \times 100 \quad (4.5)$$

Where, CL= Cooking loss (%), ODPW = oven dried cooked pasta weight, DPW = dried pasta weight (g) (before cooking)

Firmness

To measure the firmness, 5 cooked strands of pasta were placed at the center of the measurement plate under the knife edge blade of TA.XTplus texture analyzer (Stable Micro System Co. Ltd.,) equipped with a 5 kg load cell. The measurement was performed at the test speed: 0.17 mm/s and distance: 3 mm. The firmness of pasta was recorded as the maximum force required to cut the sample. Each sample was analyzed 5 times

Statistical Analysis

Results were analyzed using R studio. The replicates of data obtained from each treatment were analyzed using factorial ANOVA. Least significant differences (LSD), at a 5% level of probability, were determined between treatments.

Results and Discussion

Proximate Analysis

The 20% addition of pea protein flour to amaranth flour resulted in an increase of protein content by 47.9% (17.3 to 25.6%) and a decrease in fiber and fat content from 4.5 to 3.8% and 7.0 to 6.1%, respectively. Table 4.1 shows these findings.

Moisture Content

According to USDA (A-A-20062F, 2015), moisture content of the pasta should below 12%. As shown in Table 4.2, no significant effect of temperature, time of drying and protein content was observed on the moisture content of the pasta. The moisture content ranged from 5.3 to 7.2% (Table 4.4) for all the tested samples. All the samples were found to have moisture content less than 12% which is considered as the safe limit. This ensures that pasta is safe for consumption with a low possibility of microbial growth (Bustos et al., 2015; Ferreira et al., 2016).

Our results were different from what was reported by Anese et al. (1999). However, the difference could be due to the temperature used for drying. As 50 °C was used as low temperature in the reported study, it could be a reason for the difference. The temperature used in the present study were all higher than 50°C and the pasta was dried for a longer time. Perhaps, the difference in results was observed.

Unit Density

Unit density was measured to ensure that pasta doesn’t float on water. Moreover, it is a great parameter to study the compactness of the product structure.

The main treatment effect of each independent variable on the unit density values of the pasta is presented in Table 4.2. Increasing the temperature from 60 to 80°C and 60 to 100 °C resulted in a significant decrease in unit density by 13.56% and 32.35%, respectively. The

addition of PPF at 20% concentration also resulted in a significant decrease in unit density by 7.25 %, respectively. Although significant interactions between time and temperature were observed, we noticed that time main effect itself was insignificant. Furthermore, treatment combination effects were also observed (Table 4.4). The unit density value ranged from 866.4 to 1131.4 kg/$m^{3.}$ The pasta with maximum unit density (1131.40 kg/m^3) was found at the treatment combination 100% AF, 60 °C and 12 hr, the lowest (866.4kg/m^3) was for the 80% AF with 20% PPF, 100 °C and 12 hr and 450 rpm.

The decrease in unit density with an increase in temperature can be attributed to the case hardening. Higher temperature for drying results in rapid formation of a rigid outer layer which in turn results in the porous structure. Porous structure results in an increase in volume hence, a decrease in unit density (Wang& Brennan, 1995).

Color

The color of the pasta is considered as an important factor in determining consumer acceptance (Song et al., 2013). Bright yellow color pasta is generally most desired by the consumers (Petitot et al., 2010).

Significant effect of increase in temperature, drying time and protein content was observed on brightness (L*) and yellowness (b*) and redness (a*) of the tested samples. The main treatment effects due to changing the drying time, temperature, and PPF addition are listed in Table 4.2. As the pea protein flour was added at 20% concentration L* value and b* value decreased by 25.63% and 22.47%, while a* value increased by 30.12%. With the increase in temperature from 60 to 80 °C L* value decreased by 13.56% and a* value increased by 9.21%. However, no significant effect was observed on b* value. On the other hand, increase in temperature from 60°C to 100°C L* value and b* value decreased by 32.35% and 18.69%, respectively, while a* value increased by

20.54%. L* value, a* value, and b* value decreased by 14.04%, 6.98% and 17.57%, respectively as the drying time increased from 12 to 24 hr. Interactions between the independent variable were significant as well (Table 4.3). Treatment combination effects are shown in Table 4.4. L* value ranged from 23.53 to 51.25, a* value ranged from 4.83 to 10.21 and b* value ranged from 13.15 to 23.86.

The yellow color is associated with amount of pigment, whereas non-enzymatic browning can be related to redness of the pasta (Acquistucci, 2000). Increase in redness (a* value) with increase in in temperature and protein content can be attributed to the Maillard reaction. Differences in color can also be observed in figure 4.1. Higher temperature and increased protein content favor the Maillard reaction (Karseno et al., 2018). Maillard reaction occurs between carbonyl group of sugars and amine of proteins. Hence as the PPF was added, the protein content increased (Table 4.1), more amino groups were available for reaction, favoring the reaction. Similar results were reported by Piwińska (2016).

Furthermore, brightness (L*) and yellowness (b*) values were found to decrease significantly with an increase in temperature, protein content, and drying time. As discussed above, a decrease in yellowness could be attributed to the Maillard reaction. Another reason could be degradation of carotenoid pigments (lutein and zeaxanthin) (Tang et al., 2016) due to thermal degradation at high temperatures (Shi and Chen 1997). Comparable results have also been reported by Islas-Rubio et al. (2014). The browning of durum wheat pasta due to Maillard reaction and formation of red melanoidins with an increase in drying temperature from 55 to 100°C has also been reported by Zweifel et al. (2003)

Cooking Quality

During extrusion processing, heating results in the modification of protein structure by protein denaturation. Protein denaturation results in the unfolding of protein molecules and the exposition of its functional groups. Hence, the protein network is formed by protein-protein and protein-water interactions (Matsumura and Mori 1996). Attraction forces which result in the above-mentioned interactions include noncovalent (hydrophobic, electrostatic, and hydrogen bonds) and covalent interactions (disulfide bonds) (Avanza et al., 2005). The stability of the protein matrix depends on the heating temperature and the protein fractions of the raw material used.

In the present study, pasta samples were prepared using amaranth and pea protein flour. Amaranth consist of different protein fractions with different denaturation temperatures (Td): albumin-1 (Td = 64 °C), albumin-2 (Td = 94 °C), two globulin fractions (vicilin and amarantin) (Td 75 °C and Td = 94 °C) and two glutelin fractions (Td = 70 °C and 96 °C) (Martınez& An˜o´ n, 1996). Pea protein consists primarily of globulins (>80%) and a small fraction of albumins (13- 14%). Globulin fraction consists of legumin (11S protein), vicilin and convicilin (7S proteins) (Barać et al., 2011). The thermal denaturation temperature form all the protein fractions in pea protein has been reported to be in the range of 75-85 °C (Sun & Arntfield, 2010). In this study we did not use an industrial extruder which did no resulted in protein denaturation during the processing period. However, this potentially could be a problem during drying process.

The cooking performances of the pasta samples were investigated in terms of optimum cooking time, cooking loss and water absorption.

Table 4.2 summarizes the main treatment effects on pasta cooking quality due to independent variables. The increase in temperature from 60 to 100°C resulted in a significant positive impact on pasta cooking quality. As the drying temperature was increased from 60 °C to

80 °C, pasta water absorption capacity (WAC) and optimal cooking time (OCT) significantly increased by 12.39 % and 7.24%, respectively, while cooking loss decreased by 30.93%. With further increase in temperature from 80°C to 100°C WAC and OCT significantly increased by 3.62% and 21.31%, respectively while cooking loss (CL) decreased by 31.02%. Pea protein flour addition also had a significant effect on pasta WAC, CL and OCT. OCT increased by 20.15%, while WAC and CL decreased by 8.36% and 14.30%, respectively. Furthermore, with increase in drying time from 12 to 24 hr OCT and WAC increased by 7.19% and 34.87%, respectively, while CL decreased by 9.55%. Interactions between the independent variable were significant (Table 4.3). Moreover, results showed that the treatment combination effects were significant (Table 4.4). Pasta cooking loss ranged from 10.70 to 37.00%, optimal cooking time ranged from 4.5 to 8.1 min and water absorption capacity ranged from 145 to 182.6%.

The observed decrease in WAC with addition of pea protein flour could be attributed to decrease in amylopectin content. As amaranth is rich in amylopectin (Stone & Lorenz, 1984; Zhu, 2017), the pasta prepared with 100% amaranth has more WAC then the one in which PPF was added, as amylopectin favors water absorption (Tester & Morrison, 1990). The observed decrease in WAC with increase PPF could be attributed to increased protein denaturation. PPF is rich in hydrophobic amino acids such as alanine, valine, and leucine and its exposure increase as the protein denatures (Sikorski, 2001; Stone et al., 2015; Swanson, 1990), resulting in decreased water absorption capacity.

Cooking loss values theoretically reflect the quantity of starch and other solid matter that is released from the pasta protein matrix and subsequently lost to the cooking medium (Cole, 1991). A decrease in cooking loss was observed with an increase in temperature. It could be due to case hardening. Solidified outer surface results in slow mass transfer, results in less cooking

loss. Decrease of cooking loss accompanied by an increase in drying temperature was also reported by Güler et al. (2002) and Johnston (2001). Furthermore, Zweifel (2001) reported that cooking loss decreases of samples dried at a high temperature due to a greater extent of protein denaturation and a stronger protein network. Moreover, it is known that heating modifies the three-dimensional structure of globular proteins, thus causing exposition of the SH groups and the consequent production of S-S bonds between adjacent protein chains. This also promotes the exposition of hydrophobic group which may lead to hydrophobic interactions during drying (Perez-Gago & Krochta, 2001), resulting in more stable pasta structure and less cooking loss. The cooking loss also decreased with an increase in protein content. As protein concentration was increased, protein-protein interactions increased due to the higher number of molecules in the sample, resulting in a more compact matrix and less cooking loss (Avanza et al., 2005).

The positive effect of an increase in drying time, temperature and pea protein addition were also observed on OCT. This can be attributed to the formation of stable protein matrix with the increase in time, temperature and protein content.

Firmness

Pasta firmness is an important texture parameter for consumer acceptance. The pasta should be firm with low stickiness to be acceptable (USDA, 2015).

The effect of each independent variable on firmness is illustrated in Table 4.2. It was observed that pasta firmness increases with the increase in temperature from 60 to 100°C and pea protein flour addition by 86.79 and 76.34%, respectively. Moreover, drying time also had a significant effect on pasta firmness. Firmness increased by 35.38% with the increase in drying time from 12 to 24 hr. Interaction between independent variables was significant as well (Table 4.3). Additionally, results show that the treatment combination effects were significant (Table 4.4).

In our study firmness value ranged from 0.29 to 1.25 N; the highest value was observed for treatment combination of 20% PPF, 100°C and 24 hr.

The increase in pasta firmness with increase in temperature can be attributed to increased protein denaturation. As the denaturation temperature of the protein fraction of AF and PPF is high, hence at higher temperatures the protein denatured and polymerized to form a stronger network. Thus, due to enhanced protein- protein interactions and protein- starch interactions, firmer pasta was obtained (Matsumura and Mori 1996). Similar results were reported by D'Amico et al. (2015) and Padalino et al. (2016) where higher firmness was observed as the temperature increased from 60 to 100°C and 50 to 90°C respectively .

The observed positive effect of PPF addition on pasta firmness can be attributed to enhanced protein content. As the PPF was mixed with AF, a substantial increase in protein content was observed (Table 4.1) resulting in increased availability of protein for the reaction, thus exhibiting a positive effect on pasta firmness. An increase in pasta firmness with an increase in protein content by adding cricket powder to durum wheat pasta has also been reported by Duda et al. (2019).

Additional Testing

After the study was completed, four other treatments were done to replicate the industrial process, i.e, drying at higher temperature for shorter time. The results can be observed in Table 4.5. The testing was also done at 60°C for 6 hours. However, the pasta was not at all dried. Hence, no further testing was done on that. The samples dried at temperatures 80°C and 100°C for 6 hours and 80°C and 100°C for 12 and 24 hours were found to be similar. As pasta is dried in various stages in industry, it can be concluded that yellower pasta with higher unit density can be obtained by drying it at higher temperature for a shorter time.

Conclusions

This study was conducted to analyze the effect of different drying time-temperature combinations on the gluten-free pasta. This was accomplished by varying the drying time and temperature and studying its effect on obtained pasta. The effects of addition of pea protein flour were studied as well. Changing the drying time and temperature and addition of PPF significantly affected unit density, cooking quality, color and firmness. It was observed that increase in temperature and addition of PPF resulted in pasta with improved cooking quality. It could be attributed to enhanced protein unfolding resulting in better network formation as the temperature was increased. However, high temperature drying also had some negative impact on pasta. It was observed that higher drying time and temperature resulted in less dense products due to enhanced porosity. Also, a darker product was obtained at higher temperature due to enhanced Maillard reaction. In order to replicate the industrial process, the product was also analyzed at all temperatures for 6 hours. It was observed that both 80 °C and 100 °C were found to be enough to dry for 6 hours. Overall, the pasta dried at 80 °C for 12 hours was found to be firmer with low cooking losses. Further studies on the effect of the use of multiple drying temperature for the same drying cycle instead of just using one temperature for the whole drying cycle needs to be done to further improve the pasta quality.

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Table 4.1 Proximate analysis of blends*

Sample	**Crude Protein (%, d.b.)**	**Crude Fiber (%, d.b.)**	**Crude Fat (%, d.b.)**	**Crude Ash (%, d.b.)**
100% AF	17.3	4.5	7.0	3.4
80% AF & 20% PPF	25.6	3.8	6.1	3.8
PPF	≥55.0	≤15.0	≤15.0	-

*Pea protein values were retrieved from AGT Foods Website (http://agtfoods.com/products/pulseplus-protein.html)

Table 4.2 Main treatment effects on pasta physical properties†

Parameters	**Levels**	**Moisture Content (%, d.b.)**	**Unit Density (kg/m3)**	**Color**			**Optimal Cooking time (min)**	**Water Absorption Capacity (%)**	**Cooking Loss (%)**	**Firmness (N)**
				L*	**a***	**b***				
Protein Flour (%)	0	5.3(0.0) a	1005.30(108.23) a	43.27(7.54) a	6.84(1.66) b	20.29(2.76) a	5.36(0.59) b	170.83(15.06) a	17.83(6.43) a	0.55(0.23) b
	20	6.2(2.2) a	932.38(80.09) b	32.18(6.66) b	8.90(0.82) a	15.73(4.62) b	6.44 (1.25) a	156.55(10.11) b	15.28(4.57) b	0.97(0.26) a
Temperature(°C)	60	6.23(2.28) a	1046.70(96.63) a	44.54 (7.68) a	7.16(2.35) c	19.21(2.89) a	5.25(0.58) c	149.33(5.60) c	22.92 (3.87) a	0.53(0.24) c
	80	5.75(1.69) a	942.51(89.97) b	38.5(7.51) b	7.82(1.32) b	19.21(3.88) a	5.63(0.91) b	167.83(14.68) b	15.83(2.29) b	0.75(0.29) b
	100	5.26(0.00) a	917.32(67.31) b	30.13(5.26) c	8.63(0.54) a	15.62(5.38) b	6.83(1.11) a	173.91(8.41) a	10.92(1.38) c	0.99(0.27) a
Time (hr)	12	5.91(1.89) a	976.48(119.70) a	40.58(7.99) a	8.16(1.75) a	19.75(4.13) a	5.70(0.97) b	160.89(14.33) b	17.38(5.45) a	0.65(0.31) b
	24	5.59(1.38) a	961.20(80.19) a	34.88(9.23) b	7.59(1.57) b	16.28(4.07) b	6.11(1.22) a	166.5(14.66) a	15.72(5.88) b	0.88(0.30) a

† Means (standard deviation) followed by similar letters within each independent variable are not significantly different at α=0.05, LSD, for that dependent variable

Table 4.3 Interaction results for pea protein, temperature and time on pasta physical properties (p-values) †

Interactions	**Moisture Content (%, d.b.)**	**Unit Density (kg/m^3)**	**Color**			**Optimal Cooking Time (min)**	**Water Absorption Capacity (%)**	**Cooking Loss (%)**	**Firmness (N)**
			L*	a*	b*				
PPF	0.0961	0.0003598	< 2.2e-16	2.06E-15	6.00E-15	3.18E-16	2.90E-10	1.04E-06	< 2.2e-16
Temperature	0.3827	2.08E-06	< 2.2e-16	1.1E-09	2.13E-11	< 2.2e-16	6.05E-13	< 2.2e-16	< 2.2e-16
Time	0.5691	0.4249326	1.06E-13	4.695E-05	2.19E-12	9.70E-08	0.0004801	0.001182	1.32E-14
PPF:Temperature	0.3827	0.5458031	8.90E-06	2.499E-14	1.28E-14	1.00E-10	7.16E-06	5.81E-06	0.037362
PPF:Time	0.5691	0.4070144	0.0003234	0.022829	0.0011546	0.01965	0.6081125	0.16255	0.419234
Temperature:Time	0.7198	0.0113996	0.0781507	0.002455	0.0001291	8.33E-05	0.3105085	0.216389	1.65E-07
PPF:Temperature:Time	0.7198	0.3727942	2.60E-06	0.009948	6.06E-07	1.40E-14	0.0078483	3.87E-06	0.004126

†Where, PPF=Pea Protein Flour

Table 4.4 Treatment combination effects of pea protein and screw speed on pasta physical properties (Standard deviation is shown in parenthesis) †

Parameters	**Temperature (°C)**											
	60				**80**				**100**			
	Time (hr)											
	12		**24**		**12**		**24**		**12**		**24**	
	Pea Protein Flour (%)											
	0	**20**	**0**	**20**	**0**	**20**	**0**	**20**	**0**	**20**	**0**	**20**
MC (%, d.b.)	5.3(0.0) a	7.2(3.4) a	5.3(0.0) a	7.2(3.4) a	5.3(0.0) a	7.2(3.4) a	5.3(0.0) a	5.3(0.0) a	5.3(0.0) a	5.3(0.0) a	5.3(0.0) a	5.3(0.0) a
UD (kg/m3)	1131.4(154.0) a	1027.5(42.1) ab	1023.7(70.6) abc	1004.1(38.9) abc	1031.3(72.7) ab	902.3(53.2) bc	956.0(96.7) bc	880.3(63.3) bc	899.8(18.03) bc	866. (59.6) c	989.4(53.3) abc	913.6(68.2) bc
L*	51.25(0.49) a	44.37(0.06) cd	49.9(0.76) ab	32.65(0.81) fg	47.26(1.19) bc	34.19(1.35) ef	43.15(1.95) d	29.41(1.9) gh	37.49(0.79) e	28.37(0.49) h	30.61(1.05) gh	23.53(1.16) i
a*	5.19(0.05) e	10.21(0.02) a	4.83(0.12) e	8.38(0.24) bc	6.71(0.14) d	8.94(0.53) b	6.52(0.23) d	9.1(0.66) b	9.06(0.21) b	8.8(0.10) bc	8.74(0.43) bc	7.93(0.53) c
b*	18.17(0.19) b	23.86(0.03) a	18.13(0.38) b	16.67(0.62) bc	23.27(0.50) a	16.87(1.03) bc	22.13(0.99) a	14.56(1.53) cd	23.15(0.68) a	13.15(0.34) d	16.86(0.93) bc	9.29(0.96) e
OCT (min)	4.5(0.0) h	5(0.0) g	5.5(0.0) f	6(0.0) de	4.8(0.28) ef	5(0.0) g	5.6(0.28) gh	7(0.0) c	5.5(0.0) f	7.5(0.0) b	6.1(0.28) d	8.1(0.28) a
WAC (%)	146.6(4.9) d	145(1) d	156.6(3.7) cd	149(3.6) d	179(3) a	148.3(3.5) d	182.6(3.2) a	161.3(1.5) bc	177.3(2.1) a	165(2.6) bc	182.6(10.2) a	170.6(2.5) ab
CL (%)	32.8(2.68) ab	29.34(2.54) b	37.00(1.88) a	20.97(0.85) c	22.98(2.30) c	18.6(2.14) cd	18.56(1.14) de	15.40(0.77) cd	13.65(1.30) de	11.96(1.9) e	12.8(1.95) e	10.70(0.70) e
Firmness (N)	0.290(0.04) de	0.61(0.038) cd	0.37(0.03) bc	0.89(0.05) b	0.38(0.04) e	0.72(0.06) bc	0.77(0.13) bc	1.14(0.14) a	0.68(0.04) bc	1.24(0.054) a	0.83(0.13) b	1.25(0.06) a

† Means (standard deviation) followed by similar letters within each independent variable are not significantly different at α=0.05, LSD, for that dependent variable.MC = Moisture Content, UD= Unit Density, OCT= optimal cooking time, WAC= Water Absorption Capacity, CL= Cooking Loss

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Table 4.5 Additional Testing Results (Standard deviation is shown in parenthesis) †

Formulation	**Temperature (°C)**	**Time (hr)**	**Unit Density (kg/m^3)**	**Color**			**Optimal Cooking Time (min)**	**Water Absorption Capacity (%)**	**Cooking Loss (%)**
				L*	a*	b*			
100% AF	80	6	1011.66(53.10)	43.7(3.1)	6.2(0.4)	17.6(1.5)	4(0.0)	163.7(9.9) n	20.02(1.67)
80% AF 20% PPF	80	6	893.64(46.66)	31.4(2.7)	6.8(0.6)	13.9(1.8)	3.5(0.0)	129(1)	18.14(2.14)
100% AF	100	6	882.26(61.63)	41.34(0.8)	7.3(0.3)	21.3(0.6)	3.5(0.0)	169.7(4.7)	19.06(1.42)
80% AF 20% PPF	100	6	824.06(103.77)	29.9(1.03)	8.4(0.4)	13.9(0.8)	7.1(0.28)	174.7(5.7)	20.97(0.85)

†Where, AF=Amaranth Flour, PPF=Pea Protein Flour

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60 °C, 12 hour, 100% AF	80 °C, 12 hour, 100% AF	100 °C, 12 hour, 100% AF
		
60 °C, 12 hour, 80% AF 20% PPF	80 °C, 12 hour, 80% AF 20% PPF	100 °C, 12 hour, 80% AF 20% PPF
		
60 °C, 24 hour, 100% AF	80 °C, 24 hour, 100% AF	100 °C, 24hour, 100% AF
		
60 °C, 24 hour, 80% AF 20% PPF	80 °C, 24 hour, 80% AF 20% PPF	100 °C, 24 hour, 80% AF 20% PPF

Figure 4.1 Prepared pasta samples at different drying time and temperature combinations (Scale gradations are in cm)

CHAPTER 5. EFFECT OF SCREW SPEED ON GLUTEN-FREE PASTA DEVELOPED USING AMARANTH AND PEA PROTEIN FLOUR

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Abstract

The effect of screw speed on the gluten-free pasta was investigated in the present study. Pasta with 100% amaranth flour (AF) and 80% AF with 20% pea protein flour (PPF) was extruded at screw speeds 110, 450 and 900 rpm. Moisture content, firmness, fracturability, color, water absorption capacity, cooking time, unit density and cooking loss were analyzed to quantify the effects of varying screw speed and PPF addition on pasta attributes. Significant effects of screw speed were observed on pasta cooking quality. Specifically, the water absorption decreased by 90.15% and cooking loss increased by 110.92% with an increase in screw speed from 110 rpm to 900 rpm. Pasta of compact structure were obtained at 110 rpm resulting in denser and firmer pasta.

Introduction

Pasta is a traditional food product which is consumed worldwide. Traditionally pasta was made only of semolina as it contains gluten, which is responsible for its characteristic quality. However, nowadays more and more people are suffering from gluten-related diseases or shifting to gluten-free food for health purposes (Silvester et al., 2016). To address these issues, many studies have been conducted, but strict adherence to a gluten-free diet is still the only solution suggested (Mayer et al., 1991; Sapone et al., 2012; Wahab, Meijer & Mulder, 2002). Hence, consumer demand for GF food, such as GF pasta, has been increasing (Curiel et al., 2014).

The development of gluten-free pasta is a challenge, as it is difficult to produce a cohesive and uniform matrix that can withstand cooking conditions and result in a good quality product. A

lot of different studies have been done to develop gluten-free pasta with different raw materials like brown rice (Wang et al., 2016), maize flour (Padalino et al., 2011), quinoa and soy flour (Mastromatteo et al., 2011). On the other hand, there are limited studies on the effect of processing conditions on the quality of gluten-free pasta. For good quality pasta, not only high-quality raw material, but optimal processing conditions are required as well (Pagani et al., 1989). Extrusion is one such process that is used widely to produce pasta.

During extrusion, the material is fed into an extruder. It is then mixed, kneaded, and heated by the helical screws rotating in the barrel. The screw rotation then propels the material towards the die opening, which results in the food of the die shape (Elsey et al., 1997; Yeh & Jaw 1999). The extrusion conditions, such as barrel temperature and screw speed (Wang et al., 2016) play a pivotal part in determining the quality of the final product. All these processes during extrusion results in order-disorder transition in food ingredients. They result in transitions, such as starch gelatinization, protein denaturation, and the formation of complexes between lipids and amylose, finally yielding a desirable product (Ilo & Berghofer 1999). The amylose-lipid and starch-protein complex formed further stabilizes the network and prevents solubilization of a product when subjected to cooking (Amerayo et al., 2011; Marti et al., 2010).

The objective of the present study was therefore to investigate the effect of extrusion conditions (screw speed) on the quality of gluten-free pasta developed using amaranth flour and pea protein flour. Its quality was further investigated by measuring unit density, water absorption capacity, cooking loss, color and firmness of the pasta.

Materials and Methods

Raw Materials

Amaranth grains were provided by Nutricity LLC (Scottsdale, Arizona, United States). Pea protein flour was obtained from AGT Foods. Amaranth grains were milled into the flour with a particle size of 0.4 mm or less, using hammer mill (Viking).

Experimental Design

The pasta was prepared by combining 0% and 20% pea protein flour to 100% and 80% amaranth flour making up 100% in total. The water (40 ml/100g) was added and kept constant to study the impact of screw speed on pasta. For the trials, pasta blends with 0% and 20% PPF were extruded at screw speeds of 900, 450, and 110 rpm. Each formulation was prepared one time, but replicates were conducted for each parameter studied. Barilla corn and rice gluten free pasta was obtained from nearby Walmart (Ames, Iowa) and was used as a benchmark.

Pasta Manufacture

To make pasta dough, amaranth flour and pea protein flour adding up to 100g were blended with 40 ml of water using a laboratory scale mixer (KitchenAid KSM75WH) at a speed of 4 for a duration of 5 min. Then, the dough was kneaded by hand for one minute and stored at room temperature for a day in an airtight bag (Rosentrater et al., 2005). It was then extruded using a single screw extruder (Technochem International Inc., U.S.A) at predetermined screw speeds. The attachment specific to pasta was used. It was then dried at 80 °C for 12 hrs in the oven (Heratherm General Protocol Ovens, ThermoFisher Scientific, Waltham, Massachusetts, United States).

Proximate Analysis

Protein, ash, fat, and fibre analysis on flour blends were done by a third-party laboratory (Servi-Tech Laboratories, Hastings, Nebraska) following official AOAC standard methods.

Moisture Content

Moisture content was measured in triplicate, in accordance to the AACC method 44-19 (AACC, 2000). The dried pasta sample weighing 2 g was kept in the oven (Heratherm General Protocol Ovens, ThermoFisher Scientific, Waltham, Massachusetts, United States) at 135 °C for 2 hr. The initial weight and the final weight were noted to calculate the moisture content

$$MC \text{ (\% dry basis)} = ((OPW - DPW)/DPW) \times 100 \quad (5.1)$$

Where, MC = moisture content (% dry basis); DPW = dried pasta weight (g) (before cooking); OPW = original (wet) pasta weight (g)

Unit Density

Unit density was measured in accordance to the method described by Rosentrater et al. (2005). The length (L) and the diameter (D) of the samples were measured using Traceable Electronic Digital Caliper (Fisher Scientific, Pittsburg, Pennsylvania 15275, United States) and substituted in the given formula

$$V = \pi \times D^2/4 \times L \quad (5.2)$$

Where, V= volume (m^3); D= diameter (m^2); L= length (m)

$$\text{Unit Density} = m/V \quad (5.3)$$

Where, m= mass (kg), V= volume (m^3)

Color Analysis

The color of the pasta samples in accordance to the method described by Makdoud and Rosentrater (2017) by using Chroma Meter CR-410 colorimeter (Konica Minolta Optics, Inc. Chroma meter, Ramsey, New Jersey, USA) equipped with a xenon lamp.

Optimal cooking time

The optimal cooking time (OCT) was measure using the approved method AACC 66-50.01 In every 30 sec, a pasta strand was removed from boiling water and squeezed between two pieces of Plexiglas. When the center core disappeared, pasta was considered cooked.

Water absorption capacity

The water absorption capacity (WAC) was measured by using the approved method AACC 66-50.01 (2004). 10 g of dried pasta was boiled at previously determined cooking time in 300 mL of water . Then, pasta was removed and weighed, and WAC was calculated according to the given formula. The method was performed in triplicate

$$WAC\ (\%) = CPW - DPW\ / DPW * 100 \tag{5.4}$$

Where, WAC= water absorption capacity (%); CPW= cooked (wet) pasta weight (g) and DPW = dried pasta weight (g) (before cooking)

Cooking loss

Cooking loss was measured in triplicate, by using the approved method AACC 66-50.01 (2004). The cooked pasta was kept in an oven at 50°C for 48 h (using the same units as described previously):

$$CL\ (\%) = DPW - ODPW / DPW \times 100 \tag{5.5}$$

Where, CL= cooking loss (%), ODPW is oven dried cooked pasta weight, DPW = dried pasta weight (g) (before cooking)

Firmness

To measure the pasta firmness TA.XTplus texture analyzer (Stable Micro System Co. Ltd.,) equipped with a 5 kg load cell was used. Five strands of pasta samples were placed at the center of the measurement plate under the knife cut edge blade. The measurement was performed

at the test speed: 0.17 mm/s and distance: 3 mm. The firmness of pasta was recorded as the maximum force required to cut the sample. Each sample was analyzed 5 times.

Fracturability

The fracturability of the pasta was measured using a texture analyzer (TA. XT Plus, ttc). The test was performed using a TA-92 3-point bending fixture. For each measurement, a single strand of pasta of about 3 cm in length was placed in the center of the fixture (the gap between the supports was 24 mm). The following test settings were used: - pretest speed 2.0mm/sec, test speed 0.02 mm/sec and post- test speed 10.00 mm/sec. The probe movement caused deformation of the pasta sample until the sample fractured.

Statistical Analysis

Results were analyzed using R studio. The replicates of data from each treatment were analyzed using two-way ANOVA. Least significant differences (LSD), at a 5% level of probability, were determined between treatments.

Results and Discussion

Proximate Analysis

The flour blends were analyzed for their protein, fat, fiber, and ash content. It was found that protein content increased from 17.6 to 25.3 g with the addition of 20% PPF. However, a slight decrease in crude fat and crude fiber was observed with the addition of PPF. Table 5.1 shows these findings.

Barilla gluten-free pasta was found to have much lower protein content and fat in comparison to the experimental pasta (Table 5.1). However, the fiber content was found to be similar to the tested samples. Barilla gluten free pasta was composed of corn and rice which contains 10% and 4% protein (Food data central, USDA) resulting in the observed differences.

Moisture Content

Moisture content is related to the microbial safety of the pasta product. According to USDA (A-A-20062F, 2015), the moisture content of the pasta should be below 12%.

As shown in Table 5.2, moisture content decreased with the addition of PPF by 17.49%. On the other hand, no significant effects from screw speed were observed on moisture content, which ranged from 6.37 to 10.34% (Table 5.4). All the samples were found to have moisture contents less than 12%.

Unit Density

Unit density is measured to ensure that pasta is dense enough to stay immersed in water. Moreover, it is a great parameter to study the compactness of the product structure, as it is inversely proportional to expansion. As the product expands, its volume increases; thus, unit density decreases.

The main treatment effects of each independent variable on the unit density values of the pasta samples are presented in Table 5.2. Increasing the screw speed from 110 to 450 rpm resulted in a significant decrease in unit density by 26.70%, while increasing it from 110 to 900 rpm resulted in a significant decrease of 13.5%. On the other hand, no significant effects from PPF addition were observed. Furthermore, treatment combination effects were also observed (Table 5.4). The unit density value ranged from 1132.06 to 630.08 kg/m^3. Pasta with the maximum unit density (1132.06 kg/m^3) was found at the treatment combination with 20% PPF and 110 rpm, while the lowest (630.08 kg/m^3) was associated with 100% AF pasta extruded at 450 rpm.

A reason for a decrease in unit density with an increase in screw speed could be enhanced starch gelatinization. At a higher screw speed, the friction between the barrel and screws increases, causing an increase in barrel temperature and resulting in more starch being gelatinized. As starch

gelatinizes it absorbs more water (Bear & Samsa, 1943). The absorbed water is lost as the melt leaves the extruder die during the flash off process. This leads to puffing or expansion of the product, hence a lower unit density (Mesquita et al, 2013; Moraru & Kokini, 2003; Tiwari and Jha, 2017). A decrease in unit density with an increase in screw speed has also been reported by Badrie & Mellowes (1991), Korkerd et al. (2015), and Lue et al. (1991). Also, the barrel temperature was not controlled, which could have also played a major role in the puffing of the product.

The pasta with the highest unit density was obtained at a treatment combination of 20% PPF and 110 rpm. It can be attributed to increased protein content and low screw speed. As the protein content of the feed material was increased, more protein participated in network formation, resulting in reduced product expansion by limiting the bubble growth. Conversely, at low protein content, hydrogen bonds between hydroxyls from starch (Chávez-Jáuregui et al., 2000) are the main interaction forces, resulting in an expanded product.

The Barilla gluten-free pasta was also measured for unit density and was found to equal 2051.3 kg/m3, which was substantially higher than the tested pasta. This could be attributed to the high amylose content of both corn (Tam et al., 2004) and rice (Kohlwey, Kendall, & Mohindra, 1995) which helps in development of network resulting in more compact structure. Also, addition of emulsifiers mono and diglycerides is reported in Barilla GF pasta ingredient list, which might be further preventing starch swelling resulting in a more compact structure with high unit density (Lai, 2002). Also, commercially extrusion is done at lower temperature (<50°C) which prevent starch gelatinization and further expansion of the product.

Color

Consumer acceptance is directly related to the color of the product, as bright yellow pasta is most desired by consumers (Petitot et al., 2010; Song et al., 2013).

A significant effect of PPF addition and screw speed was observed on the yellowness (b*), redness (a*), and brightness (L*) of the pasta. The main treatment effects due to changing the screw speed and PPF addition are listed in Table 5.2. With the addition of 20% PPF, L* value, a* value, and b* value increased by 6.03, 21.85, and 21.01%, respectively. The increase in lightness with the addition of PPF was also observed by Sudha & Leelavathi (2011). With the increase in screw speed from 110 to 900 rpm, L* value, a* value, and b* value increased by 9.38, 12.57, and 25.62%, respectively. Treatment combination effects are shown in Table 5.4. The L* value ranged from 37.1 to 45.9, the a* value ranged from 6.26 to 9.06, and the b* value ranged from 15.60 to 23.70.

The redness of pasta can be associated with its non-enzymatic browning (Acquistucci, 2000). Hence, the observed darker product with the PPF addition could be due to a substantial increase in the amount of lysine, which contributes to the Maillard reaction (Wang et al., 1999). Another factor that resulted in a higher redness value could be high screw speed. At a higher screw speed, the increase in redness can be attributed to enhanced reactions between reducing sugars and free amine groups. During extrusion, starch breaks down into reducing sugars (Camire, 1991). Therefore, the higher the intensity of extrusion treatment (rpm), the higher the transformation of starch and proteins and the higher the interaction between them is (Kristiawan et al., 2018).

Barilla gluten-free pasta was found to be substantially different from the tested samples. It was found to be much lighter (L* value=51.45) and yellower (b* value = 44.41) than the tested samples. The a* value was found to equal 4.55, which was found to be very low in comparison to the tested sample. This difference could be attributed to variations in the raw material and processing conditions used. Generally, pasta is dried for 3-8 hours industrially, which might prevent browning of the pasta (Landi, 1995).

Cooking Quality

The cooking performance of the pasta samples was investigated in terms of optimum cooking time, cooking loss, and water absorption capacity.

Table 5.2 summarizes the main treatment effects on pasta cooking quality due to the independent variables. The increase in screw speed from 110 to 900 rpm resulted in a significant negative impact on the pasta cooking quality. Pasta water absorption capacity (WAC) and optimal cooking time (OCT) significantly decreased by 90.15 and 34.48%, respectively, while cooking loss increased by 110.92%. Pea protein flour addition also had a significant effect on the pasta WAC and CT, with WAC increasing by 104.09% and CT decreasing by 19.96%. Interactions between the independent variables were significant as well (Table 5.3). Moreover, results showed that the treatment combination effects were also significant (Table 5.4). Pasta with the lowest cooking loss and highest cooking time and water absorption capacity was obtained at the treatment combination of 110 rpm and 20% PPF, while that with the lowest WAC and highest CL was obtained at 450 rpm and 100% AF.

The observed decrease in WAC due to an increased screw speed could be due to increased damage to the starch molecules. WAC depends on the presence of relatively intact molecules, which have not lost their ability to bind water. However, high shear rates (high screw speed) results in higher or complete starch damage (Allen et al., 2007; Jin et al., 1995), resulting in decreased WAC. The positive effects of PPF addition could be attributed to enhanced protein content (Table 5.1) . As more protein unfolded, a more stable matrix was obtained in comparison to the matrix obtained with low protein content (Kristiawan et al., 2018).

As aforementioned, expanded and highly porous products were obtained at high screw speeds. Due to the product's high porosity from being prepared at 450 and 900 rpm, it disintegrated

when exposed to the cooking conditions. Expanded products are found to be stabilized by weak electrostatic forces, resulting in a product with a fragile structure, which is easily disrupted in a water environment (Arêas, 1992). This is in accordance with our results and explains the reason behind high cooking losses, which were observed with higher screw speeds. At 110 rpm, CL was less than 25% for pasta formulated using a blend of PPF and AF.

From Table 5.4, it can be concluded that the optimal cooking time of the pasta prepared at lower rpm was higher in comparison to the pasta prepared at higher rpm. This finding can be attributed to the development of a more stable matrix at lower shear rates.

For Barilla pasta, water absorption and cooking loss were found to be 161.15 and 11.3%, respectively, while cooking time lasted 11.83 min. WAC and CT were found to be significantly higher than that of the tested pasta, and the cooking loss was found to be significantly lower. The lower cooking losses can be attributed to the presence of emulsifier mono and diglycerides. When the emulsifier is added, starch swelling and amylose leaching are reduced when heated and thus, cooking loss is reduced (Gomez & Sarcini, 2015). Aforementioned, corn and rice are both rich in amylose which further results in stable network formation further resulting in low cooking loss. Higher water absorption could be attributed to lower starch damage as extrusion is carried out at low temperatures in industries.

Firmness

Firmness is one of the major textural attribute which affects the eating quality. Pasta with a firm texture is desired by the consumers (Phongthai et al., 2017).

The samples prepared at 110 rpm were only tested for firmness. No significant differences were observed between the sample firmness. The firmness of the sample with 100% AF, 80% AF and Barilla pasta was found to be 2.58, 3.06, and 2.6 N, respectively.

In the present study, the firmness of 100% amaranth was found to be 2.58 N, which is much higher than the firmness reported by Schoenlechner et al. (2010) for 100% amaranth pasta, which was 0.31 N. Thus, it can be concluded that screw speed had a positive impact on pasta firmness. Furthermore, Wójtowicz (2012) and Wang et al. (2016) have reported that screw speed in-between 100 to 120 rpm has a positive effect on pasta firmness. The shear at these screw speeds denatures protein and forms a stable protein matrix, resulting in enhanced firmness, which is in accordance with our study.

The addition of PPF further enhanced the pasta firmness. This can be attributed to the participation of more protein in matrix formation, as protein content was increase with the addition of PPF. Enhanced firmness by increasing protein content has also been reported by Phongthai et al. (2017).

Fracturability

Fracturability indicates the breakage susceptibility and brittleness of the dry pasta. On the other hand, breaking stress is the force required per unit area and fracturability distance is the distance it can be deformed to before breaking. All these parameters are used to understand the resistance of pasta to fracture for packaging and transportation (Martinez et al., 2014). According to USDA (A-A-20062F, 2015), not more than 11% of the pasta should be broken when transported

A significant effect of change in screw speed and pea protein flour addition was observed on the breaking stress and fracturability of the tested pasta. However, no significant effect was observed on the fracturability distance. Main treatment effects are listed in Table 5.2. The breaking stress and fracturability increased by 107.14% and 35.49% when PPF was added. On the other hand, with the increase in screw speed from 110 to 450 rpm the breaking stress decreased by 6.98%, while the fracturability increased by 44.96%. Treatment combination effects are shown in

Table 5.4. Breaking stress ranged from 0.28-0.58 N/mm^2, fracturability distance ranged from 0.10mm - 0.21mm and fracturability ranged from 1.75 N – 3.79 N. Pasta with the lowest breaking stress and fracturability was obtained at the treatment combination of 110 rpm and 100% AF, while highest breaking stress and the fracturability was obtained at 450 rpm and 20% PPF and 80% AF.

The increase in breaking stress and fracturability with an increase in PPF addition can be attributed to increased protein content. As the protein content was increased with the addition of PPF, more protein was available to interact with protein and starch molecules, resulting in increased cohesiveness and decreased breakage susceptibility of the pasta. Although statistically significant differences were observed in pasta fracturability, but magnitude of those differences was small, which might not result in any differences when produced on larger scale.

For the Barilla gluten-free spaghetti breaking stress, fracturability distance and fracturability were found to be 0.83 N/mm^2, 0.64 mm and 3.73 N, respectively. This indicates that Barilla GF pasta can be deformed to a greater extent before breaking as fracturability distance was much more in comparison to the tested samples.

Conclusions

The present study aimed to investigate the effects of extruder screw speed on AF and PPF pasta qualities. Findings suggested that screw speed significantly affected the pasta characteristics such as unit density, color, and cooking quality. At high screw speeds (900 to 450 rpm) puffed pasta was obtained irrespective of the protein content. The pasta prepared at these screw speeds could not handle the cooking process and disintegrated in water. The optimal screw speed for the tested samples was found to be 110 rpm, at which pasta with high unit density and compact structure was obtained. Additionally, the pasta prepared at 110 rpm was able to handle the cooking process; its density was found to be higher than the other tested pasta as well, reflecting its more

compact structure. Therefore, processing conditions has an impact gluten free pasta structure, calling for a specific need to control the screw speed at which pasta is obtained.

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Table 5.1 Proximate analysis of flour blends *

Sample	**Crude Protein (%, d.b.)**	**Crude Fiber (%, d.b.)**	**Crude Fat (%, d.b.)**	**Crude Ash (%, d.b.)**
Barilla gluten-free pasta	7.05	3.5	1.7	
100% AF	17.3	4.5	7.0	3.4
80% AF & 20% PPF	25.6	3.8	6.1	3.8
Pea Protein	≥55.0	≤15.0	≤15.0	

*Pea protein values were retrieved from AGT Foods Website (http://agtfoods.com/products/pulseplus-protein.htm

Table 5.2 Main treatment effects on pasta physical properties (Standard deviation is shown in parenthesis) †

Parameters	**Levels**	**Moisture Content (%, d.b.)**	**Unit Density (kg/m^3)**	**Color**			**Optimal Cooking Time (min)**	**Water Absorption Capacity (%)**	**Cooking Loss (%)**	**Breaking Stress (N/mm^2)**	**Fracturability Distance (mm)**	**Fracturability (N)**
				L*	a*	b*						
Protein Flour (%)	0	9.26(1.92) a	833.31(212.41) a	39.33(1.01) b	6.82(0.60) b	16.71(1.09) b	6.11(0.96) a	22.72(40.50) b	45.35(16.37) a	0.28(0.16) b	0.10(0.06) a	2.62(1.17) b
	20	7.64(1.81) b	868.90(243.38) a	41.70(3.91) a	8.31(0.44) a	20.22(3.19) a	4.89(1.86) b	46.37(33.41) a	40.63(17.79) a	0.58(0.14) a	0.18(0.06) a	3.55(0.97) a
Rpm	110	9.49(2.22) a	1113.61(55.11) a	38.81(1.90) c	7.16(0.98) c	16.04(0.55) c	7.25(0.27) a	77.76(8.48) a	24.62(5.71) b	0.46(0.19) a	0.17(0.08) a	2.58(1.01) b
	450	7.49(0.42) a	715.26(164.89) b	40.26(2.18) b	7.49(0.40) b	19.20(1.45) b	4.5(0.54) b	18.22(32.58) b	52.42(13.15) a	0.43(0.19) b	0.16(0.08) a	3.74(.89) a
	900	8.36(2.44) a	724.43(135.13) b	42.45(3.82) a	8.06(1.11) a	20.15(3.98) a	4.75(1.57) b	7.66(18.77) b	51.93(11.94) a	0.54(0.15) a	0.18(0.05) a	2.92(1.26) b

† Means (standard deviation) followed by similar letters within each independent variable are not significantly different at α=0.05, LSD, for that dependent variable.

Table 5.3 Interaction results for pea protein, temperature and time on pasta physical properties (p-values)

Interactions	**Moisture Content (%, d.b.)**	**Unit Density (kg/m^3)**	**Color**			**Cooking Time (min)**	**Water Absorption Capacity (%)**	**Cooking Loss (%)**	**Breaking Stress (N/mm^2)**	**Fracturability Distance (mm)**	**Fracturability (N)**
			L*	**a***	**b***						
Pea Protein	0.04956	0.41765	5.897e-05	5.051e-10	9.624e-09	1.27E-07	0.005082	0.17591	0.0006054	0.1452	7.47E-06
Rpm	0.1292	4.63E-08	2.358e-05	4.661e-06	4.186e-08	1.67E-10	5.04E-06	1.71E-05	0.000154	0.3259	1.41E-05
Pea Protein: Rpm	0.1073	0.05491	6.705e-07	5.149e-05	8.478e-07	4.82E-07	0.021097	0.00196	0.0027925	3.49E-05	0.006112

Table 5.4 Treatment combination effects of pea protein and screw speed on pasta physical properties (Standard deviation is shown in parenthesis) †

Parameters	**Screw speed (rpm)**					
	110		**450**		**900**	
	Pea protein flour (%)					
	0	**20**	**0**	**20**	**0**	**20**
MC (%, d.b.)	10.01(1.99) a	8.98(2.75) a	7.42(0.59) a	7.55(0.29) a	10.34(1.64) a	6.37(0.54) a
UD (kg/m3)	1095.17(62.79) ab	1132.06(45.17) a	630.08(53.90) b	800.44(200.33) ba	774.67(97.09) b	674.19(159.21) b
L*	40.50(0.28) c	37.1(0.79) c	38.40(0.13) c	42.1(1.40) b	39.1(0.87) c	45.9 (0.88) a
a*	6.26(0.08) d	8.05(0.11) b	7.16(0.07) c	7.82(0.27) b	7.06(0.17) c	9.06(0.25) a
b*	17.91 (0.52) a	15.21 (1.09) b	18.72 (1.04) a	19.09 (0.51) a	17.21 (0.68) ab	17.67 (0.38) a
OCT (min)	7.16(0.29) a	7.33(0.29) a	5.00(0.0) c	4.00(0.0) d	6.17(0.29) b	3.33(0.29) e
WAC (%)	74.12(10.82) a	81.39(4.78) a	-9.69(16.9) b	46.14(5.45) ab	3.74(8.84) c	11.58(27.50) bc
CL (%)	28.24(6.02) cb	21.00(2.47) c	63.49(7.25) a	41.36(3.49) b	44.32(7.04) cb	59.54(11.56) ba
Breaking Stress (N/mm^2)	0.28(0.11) b	0.54(0.07) a	0.57(0.12) a	0.58(0.16) a	0.44(0.20) a	0.49(0.18) a
Fracturability Distance (mm)	0.10(0.03) b	0.21(0.03) a	0.18(0.07) a	0.17(0.05) a	0.19(0.09) ab	0.15(0.05) ab
Fracturability (N)	1.75(0.66) b	3.40(0.44) a	3.69(0.77) a	3.79(1.08) a	2.41(1.10) b	3.43(1.24) a

† Means (standard deviation) followed by similar letters within each independent variable are not significantly different at α=0.05, LSD, for that dependent variable.MC = Moisture Content, UD= Unit Density, OCT= Optimal cooking time, WAC= Water Absorption Capacity, CL= Cooking Loss

	
110 rpm, 100% AF	110 rpm, 80% AF 20% PPF
	
450 rpm, 100% AF	450 rpm, 80% AF 20% PPF
	
900 rpm, 100% AF	900 rpm, 80% AF 20% PPF

Figure 5.1 Pasta samples prepared at different screw speeds (scale gradations are in cm)

CHAPTER 6. OVERALL CONCLUSIONS

Pasta quality is influenced by numerous interdependent factors and their implications on pasta production are far from being fully understood. The present study aimed to understand some of these factors.

The studies presented in this thesis aimed to investigate some of the factors that effects the cooking quality, texture and density of gluten-free pasta. The factors which were studied in the research were, effect of pea protein flour (PPF) addition to amaranth flour (AF) at 5%, 10%, 15%, 20% concentration; the effect of the change in screw speed from 110 rpm to 900 rpm; and the effect of an increase in drying time and temperature from 12 to 24 hr and 60 °C to 100 °C, respectively.

Positive effects of PPF addition to AF was observed in the present study. The addition of 20% pea protein flour resulted in increased protein content by 47.9% i.e. from 17.3 g to 25.6 g. It was found that as the PPF concentration was increased, the cooking qualities improved which could be attributed to the stronger protein network formation due to the increased protein content. The cooking loss decreased by 31.06% and firmness increased by 74.34% (0.29 N to 1.13 N) as the 20% PPF was added.

An increase in drying temperature from 60 °C to 100 °C showed both positive and negative effects on pasta quality. It was observed that at high drying temperatures, pasta with improved cooking quality was obtained. The water absorption capacity increased by 16.46% and cooking loss decreased by 59.09% with an increase in temperature to 100 °C. This improvement in pasta cooking quality was attributed to increased protein unfolding at higher temperatures, which resulted in better crosslinking of protein. However, a* value also increased by 20.54% with

an increase in temperature resulting in darker pasta. Also, rapid solidification of the outer surface at higher temperatures resulted in a decrease in unit density by 32.35%.

Significant negative effects of high screw speed were observed on the formulated pasta. It was found that very high rpm did not work well for pasta produced using AF and PPF. Instead, it resulted in the product puffing due to the flash off process. A decrease in water absorption capacity by 90% and an increase in cooking loss by 110% was observed with an increase in screw speed from 110 to 900 rpm.

Overall, based on the three studies carried out, we found that both appropriate raw material quality and processing conditions are important to obtain pasta of required characteristics. An increase in protein content by PPF addition and the increase in temperature resulted in the pasta ofincreased water absorption capacity, lower cooking loss and enhanced firmness , while high screw speed impacted it negatively. In conclusion, GF pasta with compact texture and higher firmness can be obtained when formulated using 80% amaranth flour and 20% pea protein flour dried at 80 °C for 12 hours and extruded at 110 rpm.

CHAPTER 7. FUTURE RESEARCH

Although we obtained pasta with improved characteristics at the end of the research, the pasta characteristics were found to still be inferior to the benchmark (Barilla Gluten-free) pasta. The pasta cooking loss was found to be higher and firmness lower than the control pasta. In order to improve the firmness and lower the cooking losses, the effect of additives such as emulsifiers and hydrocolloids on the optimized pasta should be investigated in the future. Emulsifier acts as a lubricant making pasta production easier. Also, they help in making the pasta firmer and lowers the cooking loss (Lai 2002). Gums such as carboxy methylcellulose and chitosan have been reported to improve the mouthfeel and the firmness of the pasta due to their ability to form a gel (Padalino et al., 2011).

An increase in darkness and the decrease in unit density were also observed when pasta was dried at higher temperatures. To avoid this, the effect of drying pasta at several temperatures, rather than at a constant temperature should be investigated. Pasta should be dried at a lower temperature in the beginning, to avoid outer surface solidification to prevent the decrease in unit density with the subsequent increase in temperature to reduce the drying time.

The sensory acceptability of pasta prepared by addition of amaranth flour and dehydrated pea protein flour to wheat pasta has been found similar by Sudha & Leelavathi (2012). Also, bread prepared by using amaranth flour was found acceptable in the study conducted by Schoenlechner et al. (2010). Even though ,the products prepared by using amaranth flour and pea flour has been found acceptable in some studies, the sensory evaluation of the product developed in this study should also be done to confirm the findings.

Finally, future studies should also focus on controlling the barrel temperature along with the screw speed, to control puffing of the product during extrusion. To further understand the

changes within the pasta during each processing step, scanned electron microscopy must be used in future studies. Using this tool, three-dimensional in-focus images can be obtained at a number of microscopic depths.

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