

FEASIBILITY OF USING ZEIN IN GLUTEN-FREE BAKING

by

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Abstract

Flour is essential to bread production as it provides structure, texture, and flavor. The most common, wheat flour, is unique compared to other cereal flours as it forms gluten that is capable of forming viscoelastic dough, which retains gas produced during fermentation and helps create cohesive dough, all of which are critical to bread development. However, a certain percentage of the population has a rare autoimmune disorder, celiac disease, which is triggered by gluten. A gluten-free diet is the only remedy for celiac disease. Traditionally, in gluten-free breads, hydrocolloids, or gums have been used to mimic the behavior of gluten. However, the lack of a protein structure in breads made with hydrocolloids leads to an almost batter-like viscosity. Therefore, research has focused on gluten-free alternatives, particularly non-wheat cereal proteins that can be altered to mimic gluten's dough forming properties. For example, zein has an average molecular weight and larger peptides than gluten, which contribute to its hydrophobic behavior. In fact, zein from maize flour is an ideal alternative as it can be manipulated to behave like gluten under certain conditions. The main difference between gluten and zein is that zein does not exhibit a large disulfide-linked polymer. Zein is also more hydrophobic than gluten. However, zein has been found to exhibit viscoelastic properties similar to gluten's at temperatures higher than its glass transition. Other research has found the secondary structure of zein, in particular the β -sheet structure, increases at temperatures above its glass transition. This suggests that temperature and shear are not the only factors necessary to form and maintain the viscoelastic properties of zein; apparently, the β -sheet structures also affect viscoelasticity. Finally, differences such as maize variety and particle size also affect the properties of zein in bakery applications.

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Chapter 1 - Introduction

Gluten-Free Foods

Celiac disease is a rare disorder that damages the small intestine, which in turn can cause malabsorption of nutrients. The main health concern for celiac patients is malnourishment because atrophy of the intestinal villi reduces the absorptive surface of the intestinal tract whereupon the loss of nutrients leads to weight loss and abdominal distention (Maghaydah, Abdul-Hussain, Ajo, Tawalbeh, and Alsaydali, 2013). Untreated patients have increased risks of anemia, edema, and infertility among other health concerns (Wieser and Koehler, 2008). Once diagnosed with celiac disease, the only treatment is a strict, lifelong, gluten-free diet.

Today, almost three million Americans have been diagnosed with celiac disease (FDA, 2013). Moreover, celiac disease is prevalent in Europe and countries that have significant populations of European immigrants; however, celiac disease is being diagnosed in developing countries as well (Wieser and Koehler, 2008). Husby and Murray (2013) compared research on celiac disease from various decades and reported that the rate of diagnosis is four times that of 50 years ago. Greater rates of celiac disease diagnosis along with growing interest in gluten-free diets have led to increased demand for gluten-free products (Gallagher, Gormley, and Arendt, 2004). In the U.S., the gluten-free industry grew 44% between 2011 and 2013, and predictions are for the gluten-free food and beverage market to both increase 48% between 2013 and 2016 and to reach \$15.6 billion in sales (Mintel, 2013).

The popularity of gluten-free diets among individuals unaffected by celiac disease also is growing steadily (Mintel, 2013). While previous recommendations for healthy, balanced diets to limit fat, sugar, and sodium consumption still prevail, interest has shifted to reducing gluten intake as a possible health benefit. This may be because of the perception that gluten-free diets provide a healthier lifestyle despite the lack of scientific evidence (Mintel, 2013). Accordingly, the FDA (2013) recently issued a standard for gluten-free claims: foods that carry a gluten-free, without gluten, free of gluten, or no gluten label statement must contain < 20 ppm gluten.

One reason gluten-free diets have become more popular may be because the quality of gluten-free products has improved (Mintel, 2013). Hydrocolloids or gums have been added to gluten-free dough to mimic the behavior of gluten, namely trapping gas in the dough, increasing loaf volume of bread, and improving crumb texture of bread (Maghaydah, Abdul-Hussain, Ajo,

Tawalbeh, and Alsaydal 2013). However, even when gluten-free dough contains hydrocolloids, the lack of a protein structure results in batter-like, soft dough that must be baked in pans causing the final product to have an inadequate shape. Also the soft consistency of the dough contributes to smaller, denser loaves of breads with large holes. Often, gluten-free breads do not have the same light, airy texture of wheat breads. Moreover, using gums and starches in gluten-free breads can result in a spongy texture, an undesirable characteristic to gluten-free bread consumers (Maghaydah et al., 2013).

Inherently, gluten-free foods include vegetables, fruits, potatoes, legumes, dairy products, meats, and fish. Meanwhile, gluten-free grains include buckwheat, corn, quinoa, and rice (Table 1) (Husby, Olsson, and Ivarsson, 2014). Conversely, foods made from wheat, barley, and rye contains gluten. Furthermore, celiac patients must be careful with processed foods as gluten can be found in soups, sauces, pickles, candies, and potato chips. As the food industry has focused on gluten-free alternatives, naturally research has shifted to non-gluten cereal proteins, such as zein, which can mimic gluten's dough-forming properties. Zein was first isolated in corn, commonly called maize.

Table 1: Common gluten-free grains (Adapted from Husby, Olsson, and Ivarsson, 2014).

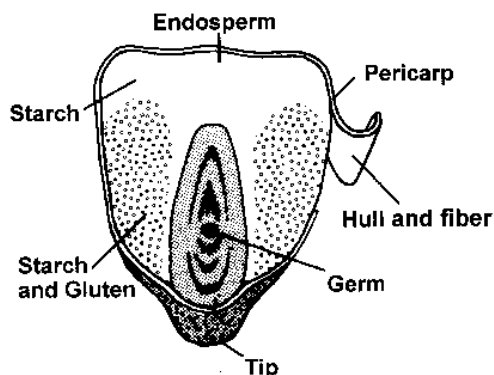
Gluten-Free Grains and Flours	Gluten-Containing Grains and Flours
Amaranth	Barley
Arrowroot	Bulgur
Buckwheat	Couscous
Corn	Dinkel
Millet	Durum
Potato Flour	Kamut
Quinoa	Rye
Rice	Semolina
Sorghum	Spelt
Tapioca	Triticale
Teff	Wheat

Corn

The U.S. is one of the largest producers of corn, producing 32% of the world's corn in 2012 (National Corn Growers Association, 2013), this includes four major varieties: field corn, sweet corn, popcorn, and ornamental corn. The major parts of the corn kernel are the endosperm and the germ (Figure 1). The germ contains most of the starch and oil, whereas the endosperm tissue contains the protein, which can vary from 6-12% dependent upon variety (Shukla and Cheryan, 2001).

Only 12% of the U.S. corn crop is used for human food, as the U.S. per capita corn consumption is 11.3 kg annually (National Corn Growers Association, 2013). However, the market for corn-containing foods has grown over the years due to the expanding Latin American population in the U.S. (USDA, 2013). Demographics show that the U.S. Hispanic population reached 50.5 million people in 2010, and predictions for 2050 indicate U.S. Hispanics will be 25% of the total population (Batis, Hernandez-Barrera, Barquera, Rivera, and Popkin, 2011).

Figure 1: Cross section of corn kernel (Shukla and Cheryan, 2001).



Nutritionally, corn is a good source of dietary fiber, vitamin C, pantothenic acid (B5), and manganese. Approximately one serving size, 227 grams of corn contain about 5 grams of protein and 125 calories, ideal amounts for low caloric needs. Also, corn consumption at average levels has been associated with releasing sugar into the bloodstream at a steady rate, thus improving blood sugar control. Therefore, the American Diabetes Association includes corn as a “best choice” among starchy vegetables and whole grains (Tufts University Health and Nutrition Letter, 2013).

Discovery of Zein

Zein was first discovered in 1821 by John Gorham (Shukla and Cheryan, 2001; Lawton, 2002; Anderson and Lamsal, 2011a) who isolated the protein, zein, from maize and described it as soft and elastic, similar to bee’s wax (Lawton, 2002). The cytoplasm of the endosperm, which is less than one nm, contains zein (Lawton, 2002). Zein is a mixture of peptides that vary in molecular size (21,000-25,000 molecular weight polypeptides and a 10,000 molecular weight peptide) and solubility (Shukla and Cheryan, 2001). The larger peptides are mostly responsible for the hydrophobicity. Lower molecular weight peptides have fewer non polar amino acids and a lower mean of hydrophobicity. Also, zein contains glutamic acid, leucine, proline, and alanine; however, it lacks basic and acidic amino acids, which is a limiting factor for solubility (Lawton, 1992).

Overall, zein is insoluble in water except in the presence of alcohol or high concentrations of urea or alkali. Zein has four subgroups - α , β , γ , and δ (Shewry and Tatham,

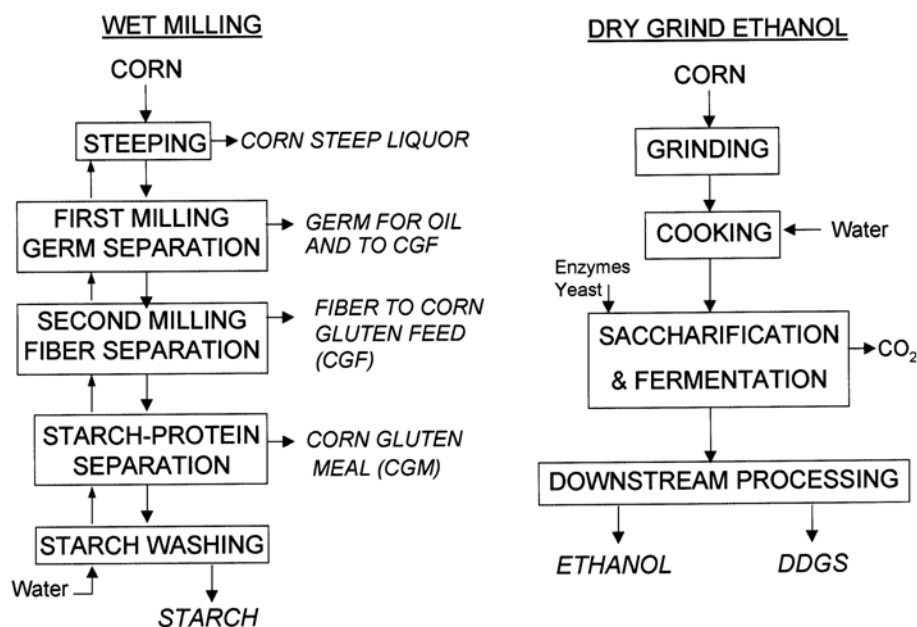
1990; Anderson and Lamsal, 2011a). Only α -zein, the predominant protein, is soluble in aqueous alcohol (Meija, Mauer, and Hamaker, 2007) as α -zein contains a majority of nonpolar residues and the polar amino acid glutamine. In 95% ethanol, α -zein represents about 80% of the total prolamin present in corn (Shukla and Cheryan, 2001). To extract β , γ , and δ zein requires a reducing agent be added to the solvent, which increases production costs (Lawton, 2002).

Corn Processing

Four different methods can be used to process corn: dry milling, alkaline processing, wet milling, and the dry grind ethanol process. Alkaline processing is used for human food products, whereas the dry grind process is used for ethanol production, and wet milling is predominantly used for industrial cornstarch and corn oil production (Figure 2) (Shukla and Cheryan, 2001) while laboratory or bench-top processes typically use dry milled corn as the starting material. However, all processes produce corn products and by-products that differ in properties and uses. For example, a by-product of wet milling is the protein-rich corn gluten meal (CGM) and corn gluten feed (CGF). CGM does not contain gluten and is safe for celiac patients. Meanwhile, the dry grind ethanol method, which involves grinding the corn and subsequent saccharification and fermentation of glucose to ethanol, produces distillers dried grains with solubles (DDGS), typically incorporated into animal feed (Shukla and Cheryan, 2001).

Although all of the by-products contain zein, commercial zein is extracted from CGM, which has 70% protein (Anderson and Lamsal, 2011b). This percentage is attributable to zein protein bodies (a layer of β - and γ -zein connected by disulfide bonds) remaining intact in corn flour despite grinding and cooking (Anderson and Lamsal, 2011b). Moreover, the β - and γ -zein layer covers a large proportion of α -zein at the protein's core (Anderson and Lamsal, 2011b).

Figure 2: Process flow diagrams for the wet milling and dry grind ethanol from corn (Shukla and Cheryan, 2001).



Corn Wet Milling - Zein Extraction

Wet milling corn can alter zein in several ways that affect extractability as well as functionality (Anderson and Lamsal, 2011a). First, corn undergoes steeping to separate the fiber and germ. A reducing agent such as sulfur dioxide is added during steeping to weaken the endosperm and allow better starch separation (Andersson and Lamsal, 2011b). Specifically, sulfur dioxide reduces disulfide bonds enhancing the extractability of zein from CGM. However, the quality of CGM depends very much on the prior steeping and drying process steps (Shukla and Cheryan, 2001). Several zein extraction processes have been developed using various solvent(s) and collection techniques. The most common commercially available solvents are ethanol and 2-propanol (Anderson and Lamsal, 2011b).

Carter and Reck developed a method that is the basis of today's commercial extraction using 88% aqueous 2-propanol with 0.25% sodium hydroxide and no reducing agents at a 1:4 solute-solvent ratio with agitation at 55-65°C for 1 hour (Shukla and Cheryan, 2001; Lawton, 2002; Anderson and Lamsal, 2011b). Next, the zein is isolated by cold precipitation, and then it is vacuum dried (Anderson and Lamsal, 2011b). To increase zein yields, the extraction process

can be repeated (Lawton, 2002). Also, solvents with higher alcohol content such as 95% (v/v) ethanol effectively extract α -zein but with low yields. Ultimately, CGM contains 36-47% α -zein while the Carter and Reck process yields ~22% α -zein. Thus, the efficiency of zein extraction is low, and the zein has a yellow hue. For food applications, zein should be a colorless, odorless product (Anderson and Lamsal, 2011b).

Then, Takahashi and Yanai (1994) developed a method to extract decolorized, odorless zein by using 70% aqueous acetone at a solute-solvent ratio of 1:5 at 40°C for 4 hours. The zein extract was then concentrated and precipitated with absolute acetone. However, the major problem with these two extraction processes is low zein yields of 22 g/g of CGM and 20.4 g/g of CGM for the Carter and Reck and the Takahashi and Yanai methods, respectively. These yields are low considering that >50% of the α -zein is not extracted (Anderson and Lamsal, 2011a).

Recently, Anderson and Lamsal (2011a) evaluated a series of solvents to improve zein yield and purity. The solvents contained more alcohol and required centrifugation to precipitate zein. Solvents tested included the following: 55% (w/w) aqueous 2 propanol; 70% (w/w/w) aqueous 2 propanol; 22.5% glycerol; 7.5% water 70% (v/v) aqueous ethanol; and 70% (v/v) aqueous ethanol followed by two cold precipitations. Additionally, reducing agents sodium hydroxide at 0.25% and sodium bisulfide at 0.5% were added to some extraction processes. Total zein protein was extracted accordingly: 70% (w/w) with aqueous 2-propanol; 55% (w/w) with aqueous 2 propanol; and 70% (v/v) with aqueous ethanol. However, the extracted zein had poor solution stability (protein precipitation) that decreased yield. Solvents that extracted the most protein with a reducing agent were as follows: 55% (w/w) aqueous 2 propanol; 70% (v/v) aqueous ethanol; and 70% (v/v) aqueous ethanol followed by two cold precipitations. The solvent, 70% (w/w) aqueous 2-propanol, disrupted β - and γ -zein from the protein body thus extracting more α -zein.

The Anderson and Lamsal (2011a) modified method extracted 30% more zein than the Carter and Reck method, and if the reducing agent was omitted, only 20% more zein was extracted than with the Carter and Reck method. Anderson and Lamsal (2011a) attributed the increased yield to the reduction of γ -zein and concluded that adding a reducing agent and centrifugation step improved zein yield.

Dry-Milled Corn- Zein Extraction

Dry-milled corn (DMC) is a good material for zein extraction because it would have had less exposure to high heat like CGM (Anderson and Lamsal, 2011b). Moreover, DMC contains 7% protein, which makes it the preferred starting material for zein extraction and isolation in the laboratory (Anderson and Lamsal, 2011b). The DMC process involves tempering the corn with water, which enables the separation of germ from corn. The corn is ground, separated with a sieve, and dried at ~ 49°C. When whole DMC is mixed with 70% (v/v) ethanol without a reducing agent, primarily α -zein is extracted with a small amount of β -zein (Anderson and Lamsal, 2011 b).

Thus, dry-milled corn is the basis for the majority of patents for zein extraction. Osborne (1891), the first to publish a U.S. patent for zein extraction from DMC, developed a method that resulted in a 5-6% yield, which commercially is not viable. However, Shukla, Cheryan, and DeVor (2000) extracted zein from DMC using ethanol and reported a zein yield of 60% containing small amounts of β -zein, which may not affect final quality. Anderson and Lamsal (2011a) suggested that defatting corn before extraction may improve zein purity.

Chapter 2 - Viscoelastic Zein

The desire of most gluten-free breads is to mimic wheat breads in appearance and texture (Moore, Schober, Dockery, and Arendt, 2004). Therefore, a better understanding of the similarities and differences between zein and gluten will provide better insight into how to transform zein so it behaves like gluten (Meija et al., 2007). Gluten is unique in that it can be both elastic and plastic at the same time, which allows it to create cohesive dough (Brown, 2000). Two proteins, gliadins and glutenin form gluten. Glutenin contributes to the elasticity of dough, and gliadin contributes to the fluidity, both important characteristics of dough rheology (Figure 4) (Pylar, 1988; Brown, 2000; Shewry and Tatham, 1997). During mixing, water is added to wheat flour, and large glutenin polymers develop through disulfide bonds creating an elastic network (Anderson and Lamsal, 2011a). Also, glutenin molecules have a large surface area and favor interactions with other proteins, which contribute to dough strength; however, in dough, excess glutenin will resist expansion during the fermentation process, which produces low volume. Compared with glutenin, gliadins are smaller, have less surface area, and interact

less with other proteins, so dough with excess gliadins will be too extensible and alter the shape and volume of the bread. Ideal dough contains a balance of gliadins and glutenin. Ultimately, gluten exhibits three properties such that it is stronger than the sum of its parts: cohesion, elasticity and a viscous flow (Pylar, 1988; Brown, 2000; Shewry and Tatham, 1997).

Dough properties are functions of ingredients, mixing, and proof time. Desirable dough rheology has high viscosity to prevent the rising of the gas cells and is extensible to prevent loss of gas cells during fermentation (Mirsaeed, Emam-Djomeh, and Mousavi, 2008). For evaluation purposes, different rheometers provide different data on dough rheology, and the most commonly used equipment in laboratories includes penetrometer, amylograph, farinograph, and mixograph (Mirsaeed et al., 2008). For example, a farinograph measures the dough resistance against mechanical shear and records the dough development time as well as the flour hydration rate, whereas a mixograph records the mixing time to peak dough development. Meanwhile, the viscoelasticity of dough has several variables such as flour type, other ingredients, temperature, water absorption, fermentation conditions, and mix time. Nevertheless, for gluten-free dough, typically wheat dough is the target.

Glass Transition Temperature

Glass transition temperature (T_g) is a critical parameter in dough rheology often used to explain the behavior of proteins during mixing. Protein polymers undergo a reversible, physical alteration of state from glassy to rubbery with the addition of heat and an uptake of plasticizer (Bugusu, Campanella, and Hamaker, 2001). The temperature at which the change of state occurs is the T_g . A protein below the T_g is in a hard, glassy state and has low molecular mobility, whereas a protein above the T_g is in a more rubbery state, which can increase volume and reactivity (Bugusu et al., 2001). During mixing, gluten absorbs water (the plasticizer) when undergoing a glass transition change, which promotes interactions with other gluten polymers to form dough (Bugusu et al., 2001). Hosene, Zeleznak, and Lai (1986) reported the T_g of gluten occurred at 21°C at 13% moisture.

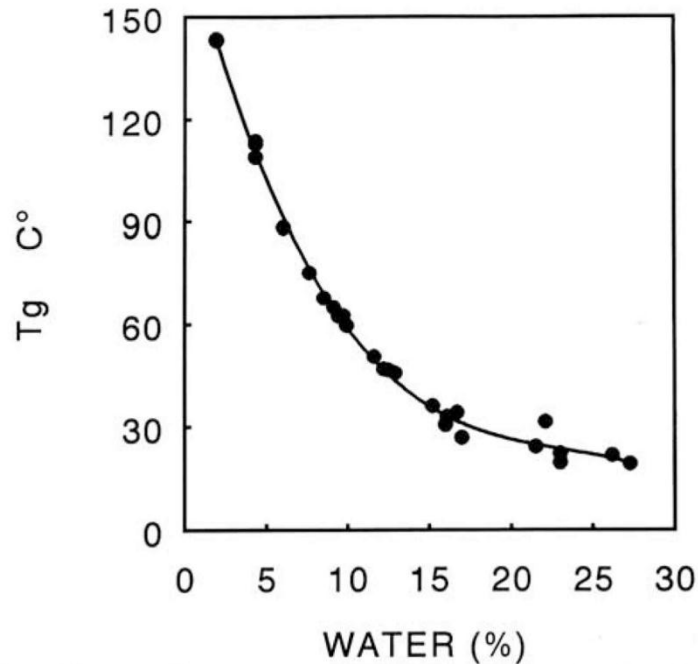
Zein without a plasticizer produced hard, brittle-like solids. Furthermore, an effective plasticizer for zein has an equal balance of polar and nonpolar groups and at the same time is compatible with zein's amino acid content. Lawton (1992) reported that effective plasticizers for zein include lactic acid, dibutyl tartrate, oleic acid, and acetanilide, whereas Andersson and

Lamsal (2001b) reported that fatty acids are effective plasticizers for zein because they interact with nonpolar amino acids like proline and leucine. While water can be a plasticizer for α -zein, if zein dough dehydrates, the dough becomes brittle, so generally, water is not used. However, Lawton (1992) and Madeka and Kokini (1996) did document the effect of water on zein Tg and found that depending on the relative humidity, zein may gain or lose moisture from the air, so this must be considered when using water as a plasticizer.

In that research, Lawton (1992) used a differential scanning calorimeter to evaluate different commercial zein samples with different moisture contents with two plasticizers: water and dibutyl tartrate. Results showed that Tg is a function of the moisture content in commercial zein plasticized by water, for Tg of zein decreased rapidly as the water content increased. At ~15% moisture, the Tg of zein plateaued at 28°C (Figure 3). Meanwhile, the Tg of hydrated, plasticized zein did not decrease below 25°C regardless of the plasticizer. In fact, the Tg of zein at a high moisture content is far below 60°C indicating that zein-starch mixtures can form dough. Lawton (1992) also reported that only one plasticizer was needed to form zein dough. This work shows that zein dough can possess viscoelastic properties similar to those of gluten-dough, but that the viscoelastic properties of zein are related to the Tg.

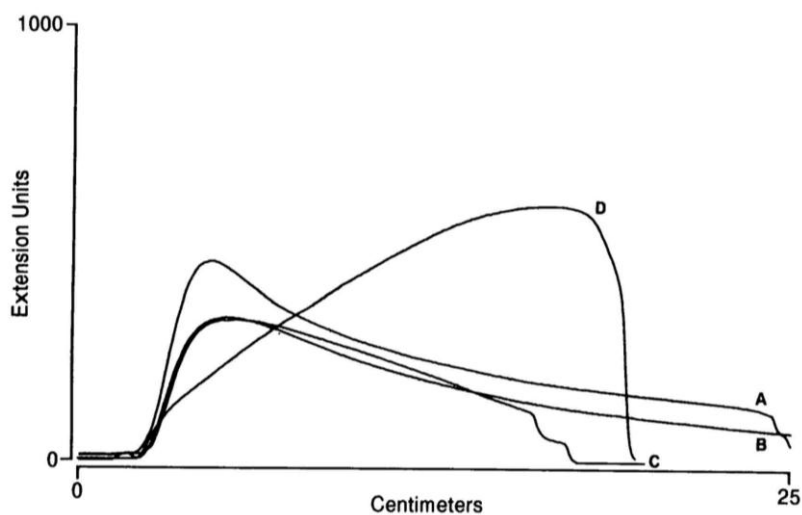
Lawton (1992) then mixed zein-starch composite flours at different temperatures. Dough mixed at 21°C did not produce viscoelastic material, but with flours mixed at 25°C, viscoelastic dough developed after 35 minutes. However, for flours mixed at 35°C, the dough exhibited viscoelastic properties at 2.5 minutes, and this dough seemed similar to wheat flour dough although less strong. Scanning electron microscopic images showed “an extensive protein fiber network” with fibers appearing to be similar to those of glutenin. Because dough was unable to develop below 25°C, the ability of the zein-starch flour to form dough clearly is related to the mixing temperature.

Figure 3: Glass transition temperature of zein as a function of moisture content (Lawton, 1992).



Finally, Lawton (1992) evaluated the mixed doughs with an extensigraph at several stages: 1) immediately after mixing, 2) after resting for 15 minutes at 28°C, and 3) after resting for 15 minutes at 25°C. Figure 4 demonstrates the differences in extensigrams of zein-starch dough compared with those of wheat dough. In wheat dough, the maximum resistance to extension increases as the dough is extended (curve D in Figure 4). However, zein-starch doughs exhibit maximum resistance to extension at the beginning of the curve and were extensible at 30 and 35°C, as the doughs stretched without breaking (curves A, B, and C in Figure 4). However, the dough temperature did affect extensibility. Particularly, zein-starch dough rested at <28°C for 15 minutes cooled, lost extensibility and viscoelasticity, and became glassy in appearance. However, dough mixed at 30 or 35°C and rested for 15 minutes at mix temperature exhibited no change in extensibility but exhibited increased resistance to extension by 100 extensigraph units. Further, dough mixed and rested at 25°C increased in resistance but decreased in extensibility. Because 25°C is close to the Tg of zein, this may be due to the “passage back through the Tg of zein” (Lawton, 1992).

Figure 4: Extensigrams of zein-starch doughs with dibutyl tartrate tested immediately after mixing: A-C) doughs mixed at 25°C, 30°C, and 35°C, respectively; D) well-developed wheat dough after a 90 minute rest (Lawton, 1992).



Secondary Structure

Meija et al. (2007) examined the secondary structures of zein and gluten using Fourier-transform infrared (FT-IR) spectroscopy. First, protein-starch doughs with viscoelastic properties were prepared at 25 and 35°C. Results showed the FT-IR amide II region of the spectra was lower in the zein-starch dough than in the gluten-starch dough with viscoelastic properties at both mixing temperatures: 25 and 35°C. A lower amide II region for zein in a viscoelastic state suggests that conformational changes occurred from protein-protein hydrophobic interactions versus protein-water interactions as happens with gluten. The amide I region from the FT-IR was used to determine the secondary structure of proteins in viscoelastic systems. This is because changes in the amide II region are more sensitive to hydration and for secondary structure determination, less reliable.

If zein was solubilized in 70% methanol at 25°C, about 65% and 30% of the protein structures were in the α -helical and β -sheet configuration, respectively. Additionally, if zein was solubilized at 35°C and done so in a viscoelastic state, β -sheet structures increased to 48% with a subsequent decrease in α -helical structures to 30%. However, when zein-starch dough

temperature decreased from 35 to 25°C, the β -sheet structure decreased to 30%, and viscoelasticity was lost. Meanwhile, zein-starch dough mixed for 5 minutes at 35°C contained a similar quantity of β -sheet structure as did gluten while at 25°C, the β -sheet content of zein decreased significantly, but the β -sheet content in gluten remained constant. These findings suggest that when shear is applied at $>28^\circ\text{C}$, zein loses its native structure and exhibits viscoelastic properties due to protein rearrangement. Also, zein in a viscoelastic system contains similar secondary structures to those of gluten but only if mixed and held at 35°C. Thus, the β -sheet content is critical to the viscoelastic properties in the zein-starch dough (Meija et al., 2007).

Zein-starch dough mixed at 35°C for three minutes had β -sheet content of 30%, which was comparable to that of the β -sheet content in native-solubilized zein. However, while gluten showed an increase in β -sheet structures to 50% after five minutes of mixing, zein contained a lower amount of α -helical structure than gluten, indicating zein has more unorganized and β -sheet structures than gluten. These findings indicate that both zein and gluten systems undergo similar changes in secondary structure during dough mixing. After three minutes of relaxation, the β -sheet structures in the zein-starch dough decreased rapidly from 50% to 30% while the β -sheet structures in gluten remained high at 50% through six minutes of relaxation; however, the α -helical structures of zein and gluten did not change during relaxation. Overall, when mixing and shear stopped at 35°C, zein exhibited a rapid change in secondary structure, but under the same conditions, gluten maintained more stable β -sheet structures within the viscoelastic network. When the temperature of the zein-starch dough was 25°C, the secondary structure changed immediately (Meija et al., 2007). This work aligns with Lawton's (1992) in demonstrating that certain temperature and shear are necessary to maintain the viscoelastic properties of zein-starch dough. However, also significant is that the polymeric β -sheet structure contributes to the viscoelastic properties of zein (Meija et al., 2007).

Non-covalent and Covalent Interactions

Disulfide bonds play a key role in the development of gluten in dough. This happens primarily via cysteine, which contains a thiol group that can form disulfide bonds between two polypeptide chains when oxidized as during dough formation. Meanwhile, the monomeric gliadins with no sulfur-containing amino acids interact via non-covalent interactions and contribute to viscosity (Shewry and Tatham, 1997). Non-covalent interactions include hydrogen bonding, van der Waals forces, and hydrophobic interactions (Smith, Bean, Selling, and Aramouni, 2014). Also, while the polymeric glutenins affect the viscoelastic properties of dough, the effect is a function of the molecular weight of the proteins, density of the covalent and non-covalent bonds, and number of disulfide bonds between the proteins (Shewry and Tatham, 1997). Shewry and Tatham (1997) reported that cleaving disulfide bonds in gluten results in loss of some viscoelastic functionality.

As water is added to wheat flour during mixing, large glutenin polymers develop through alignment of peptide chains and formation of end-to-end disulfide bonds, creating an elastic network (Pylar, 1988). Moreover, hydrophobic interactions and hydrogen bonding contribute to stabilizing gluten structure and imparting viscoelastic properties (Bugusu et al., 2001). Understanding the mechanism that drives the disulfide bond formation in gluten is extremely important in predicting functional properties in bread making (Shewry and Tatham, 1997). Disulfide bonds stabilize the folded conformations of the monomeric α - and γ -type gliadin proteins by forming a single polypeptide chain (Shewry and Tatham, 1997). These intra-chain bonds form between the cysteine residues within the polymeric glutenins and the sulfhydryl groups promote disulfide-sulfhydryl interchange that involves cleavage and reformation of disulfide bonds, which enhances dough strength (Bugusu et al., 2001). Studies suggest that the high molecular weight (HMW) subunits of glutenins, which are initially in a loop conformation, extend during the expansion of gluten to favor β -sheet structures.

Zein has different characteristics from gluten, in particular with respect to hydrophobicity. Specifically, zein contains limited cysteine residues and lacks HMW subunits linked by disulfide bonds. Consequently, zein dough lacks the large, disulfide-linked polymers that gluten has (Schober, Moreau, Bean, and Boyle, 2010; Smith et al., 2014). The lack of HMW subunits in zein may explain the β -sheet alignments that occur during the mixing of viscoelastic zein dough and why the properties are not stable. Perhaps manipulating the cross-link pattern in

zein could improve dough properties given that the pattern of disulfide bonds in wheat glutenins influences the dough strength (Meija et al., 2007).

Smith et al. (2014) evaluated non-covalent interactions of zein during formation of viscoelastic properties by changing the secondary and tertiary structures through interactions with other proteins, water, and salts. Hofmeister salts (sodium chloride and sodium thiocyanate) were added to zein–water mixtures during mixing in a farinograph at 40°C, and produced softer, more pliable zein. These findings demonstrated that zein can be manipulated through non-covalent interactions, and this may occur by disrupting hydrophobic interactions within zein or by promoting interactions between proteins (Smith et al., 2014).

To determine the significance of disulfide bonds to viscoelastic properties, Smith et al. (2014) added 2% beta-mercaptoethanol (β -ME) to wheat gluten isolate mixed in a farinograph, and reported that cleavage of disulfide bonds prevented gluten from forming viscoelastic material, as the result was a sticky paste. On the other hand, zein formed a viscoelastic material throughout the mixing period when β -ME was added, which shows zein’s unique ability to form viscoelastic material independent of disulfide linkages. Further, zein can be mixed “almost indefinitely” without breaking down because it does not rely on large polymeric disulfide-linked proteins for functionality (Smith et al., 2014).

For stretching the viscoelastic materials by hand, sodium chloride and sodium thiocyanate salts enhanced the viscoelasticity of the zein doughs, perhaps attributable to these salts promoting the zein unfolding, which can expose areas “previously buried” and engage protein-protein interactions (Smith et al., 2014). The researchers concluded that zein’s ability to form viscoelastic properties might be due to non-covalent interactions and that large disulfide protein complexes are not necessary to form viscoelastic material from zein.

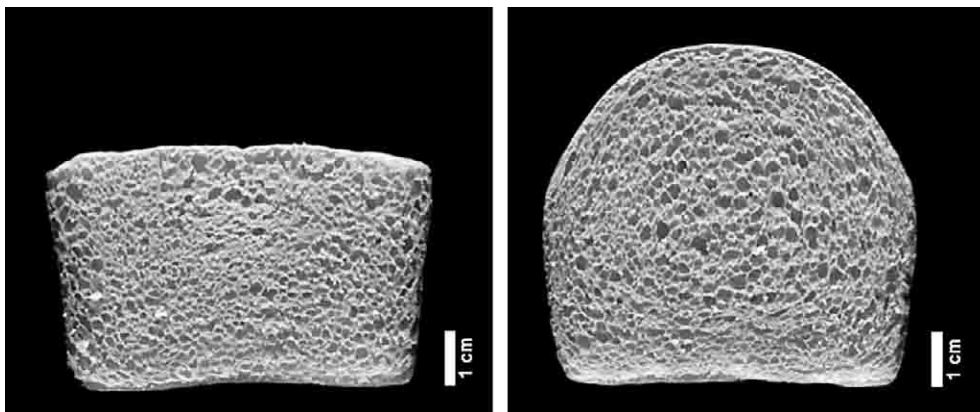
Hydrocolloids

In gluten-free breads, the lack of protein network results in breads that are crumbly and dense because the dough cannot retain the gas produced during the fermentation process (Brown, 2000, Maghaydan et al., 2013). Previous studies have shown that hydrocolloids added to gluten-free bread dough improved dough quality as hydrocolloids mimicked the properties of gluten (Schober, Bean, Boyle, and Park, 2008; Moore et al., 2004; Maghaydan et al., 2013). Common

hydrocolloids used in gluten-free foods are xanthan gum, guar gum, carrageenan gum, and pectin.

Another hydrocolloid, hydroxypropyl methylcellulose (HPMC), added to zein-starch dough for batter-based gluten-free breads, enhances water binding and thickening abilities. Schober et al. (2008) hypothesized that adding HPMC to zein dough may provide similar functionality to that of polar lipids, e.g., by stabilizing gas bubbles and increasing gas entrapment in zein strands. Zein dough with HPMC had a dough consistency similar to that of wheat dough; thus, the HPMC increased the viscosity of the dough. Conversely, zein dough without HPMC was soft, wet, and more batter-like. Moreover, bread produced from the zein dough with HPMC had a soft, elastic crumb as well as a round top while zein dough without HPMC produced bread that was aerated slightly, exhibited a flat top, and had low volume (Figure 5). To determine if the thickening abilities of the HPMC were responsible for the improved bread quality, the water content was reduced by 5% in the zein dough without HPMC. The reduction in water made the dough consistency similar to that of wheat dough, but the resulting bread had an even lower volume than zein dough without HPMC with the regular water content.

Figure 5: Images of bread slices made from zein dough without hydroxypropyl methylcellulose (HPMC) (left) and with HPMC (right) (Schober et al., 2008).



Schober et al. (2008) also replaced HPMC with different amounts of xanthan gum and monitored dough viscosity and finished bread quality. Although very similar, these gums differ

in surface-active properties. Mixing 2% HPMC in water with a high-speed mixer formed foam, whereas mixing xanthan gum formed a viscous solution with only a few bubbles. The specific volumes of the resulting breads were low, confirming that the improvements from adding HPMC to gluten-free bread dough were not due just to the viscosity increase. The favorable cell stabilization HPMC provides may explain its superior performance relative to that of other gums in gluten-free breads.

Schober et al. (2008) evaluated zein-HPMC interactions to understand if solubilized proteins might contribute to gas cell stabilization as well as HPMC, as weaker interactions of protein chains might contribute to the tendency of zein to form extensible strands upon the addition of HPMC. Extraction experiments were conducted to evaluate zein with HPMC and without HPMC to elucidate the protein contribution on a molecular level. Schober et al. (2008) found that the effect of HPMC was not detectable on the properties of zein. In the zein bread, stabilization of gas cells must be an effect of HPMC alone, without contribution of soluble proteins (Schober et al., 2008).

Maghaydan et al. (2013) investigated using hydrocolloids in rice and corn flour doughs to develop high quality gluten-free bread. Three such dough formulas were tested: 1) flour with 1% xanthan and 1% carrageenan; 2) flour with 1% xanthan and 1% pectin, and 3) flour with 1% carrageenan and 1% pectin. The moisture contents between the control (24.39%) and gluten-free breads (~40%) were significantly different, which probably can be attributed to the typical hydrophilic nature of hydrocolloids. Maghaydan et al. (2013) specified the moisture contents impacted the starch contents in rice (90%) and corn (70%) flours, which in turn influenced water holding capacity and water absorption rate.

During bread scoring, Maghaydan et al. (2013) found that the bread containing xanthan gum with pectin had the highest specific volume score due to pectin creating a network that held gas during fermentation, while the formulation containing carrageenan and pectin produced the lowest quality of bread with undesirable color, symmetry, and flavor. Meanwhile, breads without xanthan gum had low quality. Overall, recommendations to improve quality of gluten-free bread were to substitute rice and corn flours for wheat at a ratio of 1:5 and to add a combination of 1% xanthan gum and 1% carrageenan or 1% xanthan and 1% pectin.

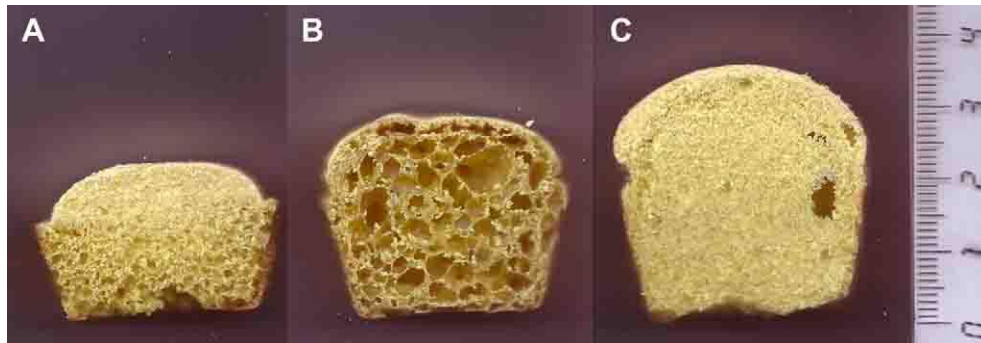
Andersson, Ohgren, Johansson, Kniola, and Stading (2011) studied adding hydrocolloids in zein-starch doughs at $>25^{\circ}\text{C}$ at three different stages: protein-water (referred to as resin),

protein-starch dough, and then the resultant bread. Zein resins were yellow and had non-sticky, rough surfaces if handled at 20°C. Using dynamic measurements on zein resins yielded a Tg of 25°C at 45% moisture. The difference between these results and past results on Tg in previous research may be attributable to different raw material sources and instruments as Andersson et al. (2011) used a rheometer but nonetheless confirmed the findings of Lawton (1992), Meija et al. (2007), and Schober et al. (2008) that zein needs to be mixed at >28°C to develop viscoelastic dough.

Next, the different protein-starch doughs were made without hydrocolloids as well as with the addition of HPMC (Andersson et al., 2011). All doughs were mixed at 40°C using a mixograph to record the mixing resistance and peak, attributes that indicate optimal dough development. All doughs showed a gradual rise followed by a gradual drop in resistance, and a maximum peak was obtained within seven minutes of mixing. However, zein dough without HPMC showed a rapid increase to resistance within three minutes of mixing along with a more prolonged breakdown. Zein dough with 2% HPMC, conversely, experienced a rapid breakdown, and the dough without hydrocolloids experienced some phase separation, which resulted in a starchy liquid that surrounded the dough after mixing. Dough with hydrocolloids did not experience the phase separation due to the improved water binding capacity from the hydrocolloids. Additionally, zein-starch doughs without hydrocolloids had sticky, smooth surfaces compared to the zein-starch doughs containing HPMC. Clearly, adding hydrocolloids affected the zein microstructure resulting in stabilized zein-starch doughs. Without hydrocolloids, zein-starch dough had high initial stiffness and rapid age-related stiffening.

Finally, the resultant bread loaves had dense crumb structures; the top was starchy whereas the bottom had more open crumb structures (Figure 6). Apparently, hydrocolloid addition improved the specific volume ($p < 0.05$, $n = 3$), and bread with HPMC provided the best dough volume during proofing as well as a loaf that had a smooth round crust and fine crumb structure. The bread that contained the β -glucan oat bran had an uneven crust, large pores, and a darker-colored crust. Ultimately, results show that pure zein-starch bread is not acceptable, but bread made from zein and hydrocolloids, which serve as a structural enhancer, may be commercially viable (Andersson et al., 2011).

Figure 6: Images of crumb structure of zein-starch breads. A) without hydrocolloids, B) with 2% beta oat glucan, and C) with 2% hydroxypropyl methylcellulose (HPMC). Scale bars in cm. (Andersson et al., 2011).



Chapter 3 - Maize Flour Characteristics

Corn variety also can play a role in the overall bread quality. Brites, Trigo, Santos, Collar, and Rosell (2010) evaluated flours produced from four different varieties of maize: fandango, pigarro, yellow hybrid, and white hybrid. They also included two different grinding techniques to produce flour: a water mill and an electric mill. Fandango and yellow hybrid produce yellow-colored maize flours, whereas pigarro and white hybrid produce white-colored maize flours.

Table 2 shows that flours from the fandango and pigarro varieties had greater protein (~9.0%) but less amylose (~29%) content than those of the hybrids, which had 8.5% protein and ~32% amylose. Also, the water mill grind produced flours with 1.48% ash, whereas the electric mill grind produced flours with 1.48% ash. Ash content of flour can affect the pH of dough during fermentation and influence other bread characteristics.

Table 2: Effects of maize variety and grind method on protein, ash, and amylose contents of maize flours (Adapted from Brites et al., 2001).

Variables		Protein % (db)	Ash % (db)	Amylose % (db)
Maize Variety	Fandango	9.5 ^a	1.50 ^b	28.6 ^b
	Pigarro	10.5 ^a	1.94 ^a	29.2 ^a
	Yellow hybrid	8.3 ^d	1.49 ^b	32.7 ^a
	White hybrid	8.8 ^d	1.39 ^b	32.2 ^a
Grind Method	Water-mill	9.3 ^a	1.48 ^b	31.3 ^a
	Electric-mill	9.3 ^a	1.48 ^a	30.3 ^a

For each variable, values followed by the same letter are not significantly different at $P \leq 0.05$

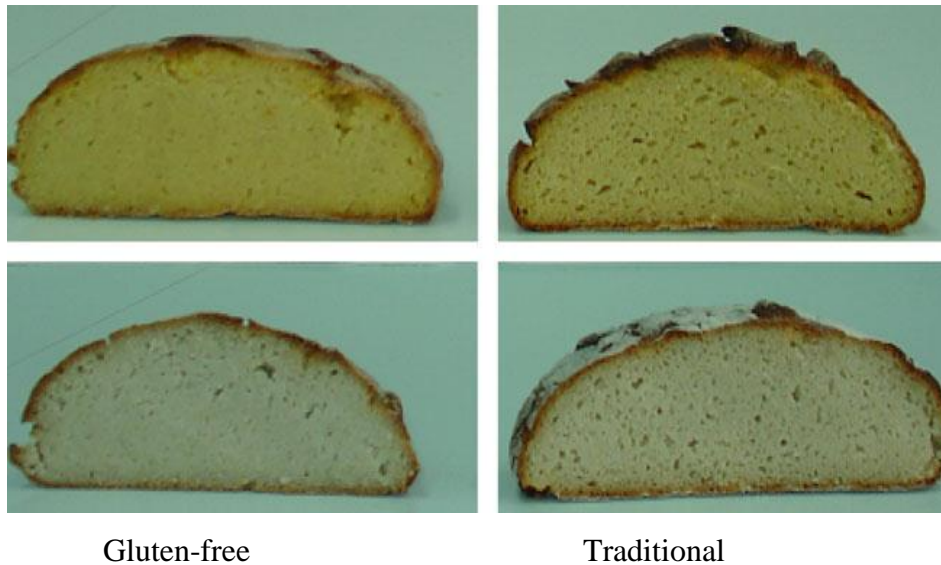
Brites et al. (2010) produced doughs with these flours and evaluated the rheology. The viscosity profiles of the maize flours obtained from a rapid viscoanalyzer showed lower maximum viscosity but overall higher final viscosity than that of wheat flour. However, the hybrid varieties showed greater viscosities than those of the regional maize flours, fandango and pigarro. Further, the water-milled maize flours had higher viscosities than did the electric-milled flours. The electric milling process probably damages the starch, decreasing its ability to absorb water.

Brites et al. (2010) studied the dough behavior during mixing and handling with a farinograph and texturometer using a broa formulation with wheat as well as a gluten-free dough. They found variety and grind did not influence adhesiveness, gumminess, or stickiness. However, when 100°C water was added to the flour and subsequently mixed, the dough had higher consistency when mixed for a shorter period than did dough mixed with 25°C water. The increase in water temperature directly increased the adhesiveness, elasticity, and stickiness of the dough. These results match reports from Lawton (1992) and Meija et al. (2007) concerning zein behavior at $>28^\circ\text{C}$. Brites et al. (2010) attributed the increase in viscosity to starch gelatinization in the flour because starch gelatinization occurs as temperatures increase. The gelatinization increases the mechanical strength of the dough, which leads to improved viscosity and consistency. To improve gluten-free breads, therefore, it may be useful to promote starch gelatinization during mixing by adding boiling water (Brites et al. 2010). Notably, Brites et al.

(2010), Lawton (1992), and Meija et al. (2007) all concluded that to use zein in gluten-free baking, mixing temperatures should exceed 25°C to produce ideal dough rheology.

Brites et al. (2010) made bread from the different maize variety flours and grind method (Figure 7) using the traditional Portuguese bread formula called broa, which is made from approximately 50% corn flour mixed with other flours such as wheat or rye. The traditional process starts with adding flours, water, yeast, and sour dough starter to form dough. Then the dough is mixed, rested, proofed, and baked. However, these test formulations are not completely gluten-free because wheat and rye contain gluten. In the broa formulation, the maize variety and grind method had no significant effects on specific volume of the bread. However, the crumb structure in the bread made from pigarro was more firm than that of the bread made from fandango. In the gluten-free formulation, maize flours from the water milling process were used because the grind method did not improve broa quality significantly. The gluten-free bread exhibited smaller volume and more dense structure than the traditional broa that contained a defined gas cell structure (Figure 7) (Brites et al., 2010). In addition, breads differed in color and texture; the crumb firmness of the gluten-free bread was higher, but the loaf volume was lower than that of traditional broa bread. Although the water mill grinding occurred at a slower rate than did the electrical mill grinding, the slower rate of grinding produced flour with lower ash content and dough with higher viscosity. However, milling choice did not affect the sensory scores of the broa breads. Overall the baking tests showed that the broa process could be applied to produce gluten-free bread.

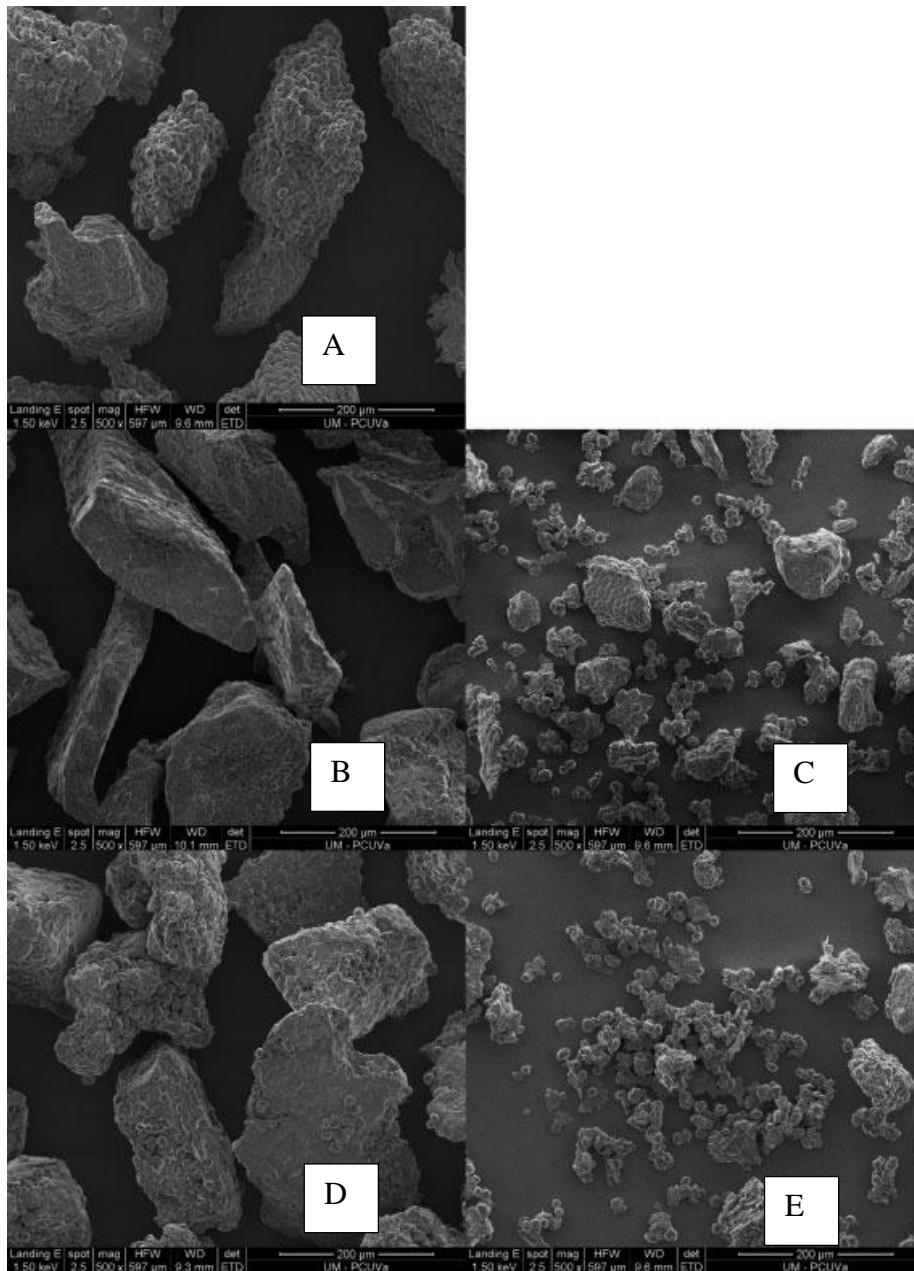
Figure 7: Crumb structure of broa bread produced with traditional and gluten-free formulations. Top: fandango variety, bottom: pigarro variety (Brites et al., 2010).



Particle Size

De la Hera, Talegon, Caballera, and Gomez (2013) studied the influence of corn flour particle size on the quality of gluten-free breads. They selected three different maize flours yellow, semolina, and white, and flours were separated with screens of 80, 106, 150, and 180 μm . Using an environmental scanning electron microscope (ESEM), differences in microstructure of the five flour fractions were observed (Figure 8). The coarser fraction of semolina flour was more condensed and looked “sheet shaped” (Figure 8B). The starch granules were inside the protein matrix and included a small number of “intergranular spaces,” which De la Hera et al. (2013) suggested contributed to the overall hardness. The finer flour fractions were similar to the coarse flour fractions, which had starch granules within the protein matrix, but the starch granules were smaller (Figure 8). Yellow maize (Figure 8A) and white flour (Figure 8D) contained starch granules that were more round and less compact compared to the semolina flour (Figure 8B). Notably, the viscosity of the slurries of the three maize flours showed the yellow flour had the lowest viscosity.

Figure 8: Environmental scanning electron microscope views of different maize flours and particle size: A) yellow-maize flour > 180 μm ; B) yellow-semolina flour >180 μm ; C) yellow-semolina flour < 80 μm ; D) white-maize flour > 150 μm ; E) white-maize flour < 106 μm . (De la Hera et al., 2008).



De la Hera et al. (2013) evaluated the effect of particle sizes of maize flour in gluten-free bread making and found that all fractions of the yellow flour had low specific volume due to the negligible increase in volume during proofing. Moreover, specific volume decreased, and particle size decreased in the breads made from the yellow and semolina flours. The coarser maize flours produced breads with more volume compared to the bread made from the finer particle-sized flours. The greatest volume bread, made from the coarser fraction of the semolina flour, was attributed to the hardness of its particles, but while the semolina flour had a higher specific volume, it had lower cohesiveness and resilience than the bread made from the yellow flour. De la Hera et al. (2010) concluded that the coarser flour produced bread with more volume than did the flours with smaller particles, as the particle size of the flour seemed to help the dough retain gas during fermentation. However, the variety of corn flour along with the milling process should be studied for optimization.

Schober et al. (2008) predicted that coarse zein particles contribute to the formation of zein strands as well as the strength of zein dough. To test this hypothesis, dry zein was milled into a fine powder (50% $<0.13 \pm 0.00$ mm, 90% $<0.35 \pm 0.00$ mm). Extension tests were performed on control dough with normal zein powder and dough made from the re-milled zein. The extension tests found that the values for peak force, extensibility, and the area under the curve were significant ($P < 0.05$) between the two samples. Schober et al. (2008) hypothesized fine zein particles formed weak zein-starch dough and larger-sized zein particles have additional starch granules, which may contribute to dough strength; but in smaller-sized zein particles, the starch interacts with the zein close to the surface, preventing zein interactions. The small particle size of the zein likely caused the weak dough, as the particles are unable to pass through the zein-starch layer at the surface even when mixed $>28^{\circ}\text{C}$. In contrast, larger-sized zein particles could pass through the zein-starch layer, interact with available zein, and form a zein network.

A research report indicates that surface-active components stabilize the liquid lamellae surrounding the gas cells thereby creating larger loaf volume (Schober et al., 2010). To test this, two different zein lots, coded (Z1) and (Z2), respectively, were evaluated. The Z1 lot was tested three ways: 1) as is; 2) defatted using hexane as the solvent; and 3) defatted using chloroform as the solvent. Lot Z2 did not undergo any defatting and resulted in a loaf volume of 4.0 ml/g first, the hexane-defatted Z1 lot produced bread with a loaf volume of 3.7ml/g compared with the non-defatted Z1 lot, which produced bread with a loaf volume of 3.3 ml/g. However, the chloroform-

defatted Z1 lot produced bread with the greatest loaf volume, 4.5 ml/g, but the bench-scale defatting process did not reduce the total lipid content, which was surprising. Accelerated solvent extraction (ASE) was the automated control method used to extract the maximum lipids from the defatted and undefatted zeins. Results showed the ASE with chloroform only decreased lipids from 8.0% to 6.1-6.6%, while Z2 had a lipid content of 3.5%. Meanwhile, ASE extraction with hexane only extracted a maximum 2.5% of lipids. In sum, HPLC analysis of the lipid composition of the ASE-hexane extract showed >10% moderately polar lipids (free fatty acids, diacylglycerols) were present, which might explain the better performance of chloroform as an extractant.

Schober et al. (2010) tested the tendency of zein to aggregate in water at 40°C with a short, intense mixing period of five seconds using Z1, Z1 defatted with hexane, Z1 defatted with chloroform, and Z2. All zein samples formed aggregates of varying cohesiveness. Aggregates were removed, stretched, and photographed as depicted in Figure 12. The Z1 sample that was not defatted fell apart during stretching because the zein particles had a thin layer of surface lipids, which prevented water absorption. This lack of water absorption could potentially inhibit the zein interactions. When hexane was the solvent, the zein strands were slightly extensible; however, when chloroform was the solvent, the zein particles interacted more and were cohesive and extensible. Z2 that was not defatted had strong, less extensible properties than the chloroform-defatted Z1. A possible explanation for this is that Z2 has larger particle size. Schober et al. (2010) concluded the solvent chloroform performed better than hexane in defatting Z1 as the modified zein was more extensible. This defatting method may also be more critical to zein dough due to the removal of the surface lipids, which improved zein aggregation. However, because chloroform is toxic, a more environmentally friendly alternative to defat zein needs to be found.

Chapter 4 - Starch

Among the numerous challenges with gluten-free baking, rapid staling and short shelf life greatly affect the quality of bread when it reaches consumers. While gluten's elasticity has been linked to slowed textural changes during starch retrogradation, the gluten-free formulation is more susceptible to staling (Ahlborn, Pike, Hendrix, Hess, and Huber, 2005). In general, staling begins as soon as the bread leaves the oven, which leads to an estimated 3 to 5% loss of baked breads sold in the U.S. (Brown, 2000). Bread staling often results in loss of flavor and aroma and changes to crust and crumb texture that results in firm and dry bread.

Staling is a complex process that can be attributed to the high starch content in bread and specifically to the amylose and amylopectin components. First, starch undergoes several transformations during the baking process. While insoluble at room temperature, starch granules are heated during baking, whereupon the hydrogen bonding sites within the molecule break and begin to swell with more water. The granules continue to swell until they have taken up the surrounding free water forming a starch gel (Pylar, 1988). After baking, while bread cools, hydrogen bonding occurs again within the amylose of the starch causing the amylose to return to a less soluble crystalline state over time. This process is called starch retrogradation and is directly related to bread staling. Several theories have been proposed to explain why starch retrogradation contributes to bread staling some of which will be discussed in this report (Ronda and Roos, 2011; Gujral, Haros, and Rosell, 2003). Methods to control the rate of bread staling include minimizing exposure to air and monitoring the time and temperature of storage.

Specifically, amylopectin has been linked to the recrystallization that contributes to bread staling. Typically, the amylopectin will recrystallize after a few days, which is coincidentally, similar to the amount of time it takes for bread to stale. Most researchers view the changes in amylopectin as the direct cause of bread staling; however, studies have shown that starch retrogradation is not the only factor that contributes to crumb staling (Ronda and Roos, 2011). Others believe that the moisture loss that occurs during bread staling is related to the firmer texture and starch recrystallization during storage. One theory is that the, "moisture levels also play an important role in the staling of bread as water moves from the center of the loaf toward

the crust” (Brown, 2000). Since gluten-free products typically have higher moisture content, the effects of staling could be more noticeable.

Schamne, Dutcosky, and Demiate (2010) studied the impact of cassava starch, rice flour, and corn flour in the development of bread and how each of the ingredients contributes to starch retrogradation, and they have a different theory than Ronda and Roos (2011). Ronda and Roos (2011) theorize the amylopectin of the starch contributes to staling because it would take the same amount of time to recrystallize as it would take for the bread to stale. On the other hand, Schamne et al. (2010) believe that the amylose in the starch molecule as opposed to the amylopectin is linked to starch retrogradation. Amylose molecules have a linear structure compared to amylopectin’s round structure, and thus the starch retrogradation could occur more readily between those molecules (Schamne et al., 2010). Therefore, Schamne et al. (2010) studied the amylose content of the corn flour and rice flour and found that the amylose content in maize starch is around 25% while in rice starch it is around 16%. This makes corn flour more ideal because the lower amylose content would delay the onset of bread staling.

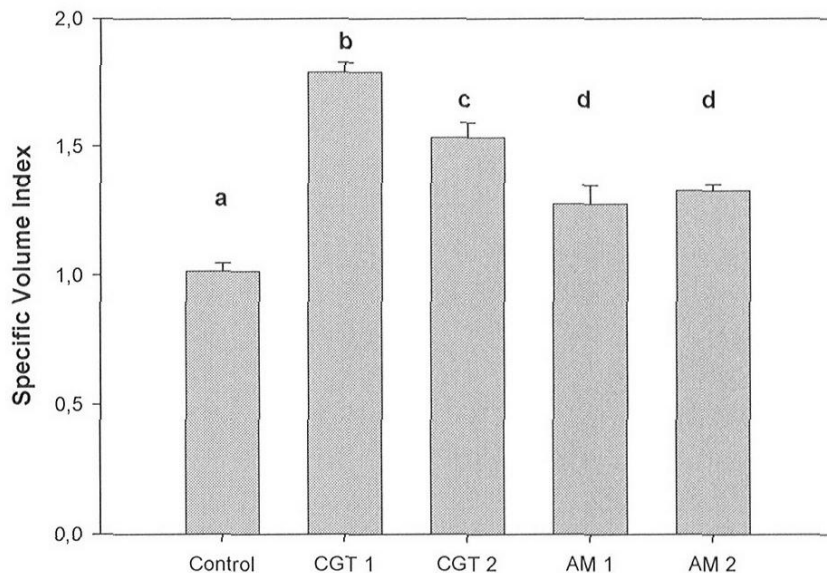
Enzymes

The development of a protein network through enzymatic addition has also been studied in gluten-free breads. Oxidizing or cross-linking enzymes have both been reported to improve dough performance and the quality of the gluten-free breads. For example, transglutaminase in rice doughs was evaluated by Gujral and Rosell (2004), who reported improved viscoelastic properties. Moore et al. (2004) also demonstrated that transglutaminase additives in protein sources such as soy powder, milk powder, and egg powder formed a network via cross-linking in gluten-free breads. However, the efficiency of adding transglutaminase directly correlates to the protein source. This is because transglutaminase forms the cross-linking network between the, “gamma carboxyamine group of the glutamine residues and the E-amino group of the lysine residues” (Renzetti, Dal Bello, and Arendt, 2008). Therefore, the amino acid content limits the cross-linking formation with transglutaminase. As expected, the low lysine level in oat, sorghum, teff, and maize showed minimal improvements by adding transglutaminase.

Other studies have investigated using starch hydrolyzing enzymes in rice bread since rice starch is more prone to starch retrogradation due to the hydrophobic nature of its proteins. This could be a viable option for other non-wheat cereal proteins to inhibit the staling effect in gluten-

free products. Additionally, Gujral and Rosell (2003) investigated cyclodextrin glycosyl transferase (CGTase), which is thought to inhibit the retrogradation of starch due to its cyclization activity (Table 3). The cyclization activity of CGTase produces cyclodextrins and can modify the interaction between starch and protein. Consequently, Gujral and Rosell (2003) discovered that the α -amylase and the CGTase produced bread with increased volume. However, the CGTase produced a significantly higher volume than the bread made with α -amylase. The authors attributed the improvements made by the CGTase to the starch hydrolyzing activity that produces fermentable sugars for the yeasts as well as the cyclization activity that forms the cyclodextrins and modifies the hydrophobicity of the surrounding environment. Gujral and Rosell (2003) hypothesized that the cyclodextrin protein complexes form better entrapment of the CO₂ produced during fermentation resulting in increased volume. Gujral and Rosell (2003) also discovered that adding CGTase produced bread that retained a soft crumb longer than that produced with α -amylase. Therefore, using starch hydrolyzing enzymes is a good approach to improving the quality of gluten-free bread and inhibiting rate of crumb firmness (Gujral and Rosell, 2003).

Table 3: Effect of different dosage of alpha-amylase (AM) and cyclodextrinase (CGT) on specific volume of rice bread (Gujral and Rosell, 2004)



Lactic Acid Bacteria

Another method to delay bread staling is to use sourdough. In wheat breads, researchers have reported that sourdough delays bread staling while improving the loaf volume and crumb structure (Moore, Juga, Schober, and Arendt, 2007). The sourdough process consists of using an acidic paste for making bread, prepared by mixing flour, water, and with a lactic acid starter culture a day before bread making. Lactic acid bacteria (LAB) are used in starter cultures for fermented foods like yogurt, cheese, and sourdough. LAB organisms undergo carbohydrate fermentation to produce LAB either alone or in combination with carbon dioxide, acetic acid, and formic acid. A starter culture can consist of pure strains of microorganisms and also pieces of already fermented dough. On the day of baking, the rest of the dough ingredients are added after which follows the same process as for straight dough. With the sourdough technique, the flavor is more pronounced, and the bread can have a better texture (Edema, 2011). The acidification of sourdough lactic acid bacteria (LAB) has been reported to improve several characteristics in bread such as texture, flavor, retarding of staling, and extension of shelf life.

Moore et al. (2007) did report in their study of LAB in gluten-free formulations that the protein particles degraded over time in the sourdough. They assumed that the LAB may digest the protein-rich particles allowing the protein to become more accessible so that it could bind with water in the bread batters. The available protein particles then might assist in sticking the other available proteins together and thus create larger aggregates and more stable microstructure. However, the microstructures of the gluten-free batters revealed no network similar to that of wheat bread.

Edema (2011) applied the Moore et al. (2007) discoveries to develop a procedure to produce sourdough bread from 100% maize meal. Typically, corn bread is considered a quick bread, which means that it does not require yeast or a starter culture (Edema, 2011). For a quick bread, all of the ingredients are added to the mixer, mixed for a short amount of time and then baked. However, most bread found in the supermarket is considered yeast bread, which means that the dough requires yeast or a starter culture to develop the flavor (Edema, 2011). For yeast breads, bakers use two different mixing methods. The first is called straight-dough where all of the ingredients are mixed together in one stage before baking (Edema, 2011). Straight-dough recipes develop all the gluten during kneading. An alternative method for making yeast bread is the sponge-dough method. The sponge-dough method involves two steps: First, a sponge or

starter culture is made and allowed to ferment for a period; next, the sponge is added to the rest of the ingredients to make the final dough. The sponge-dough method takes longer, usually around eight hours, but in the end produces more flavorful bread due to the fermentation of the sponge or starter culture.

Since prior attempts to produce gluten-free bread from corn using the straight-dough method affected the dough height (Edema, 2011), Edema (2011) created a modified mixing process that combined the straight-dough method with the sponge-dough (Figure 4-5). In the modified process, all of the ingredients were mixed in one stage and then allowed to rest for several hours just as with straight-dough. Then the batter was gently mixed again, as in the sponge and dough method, before proofing and then baking. For the sponge, Edema (2011) decided to use starter cultures of lactic acid bacteria derived from corn meal and identified and used in combinations three species of lactic acid bacteria that were isolated from 34 strains of fermented maize meal. They were *Lactobacillus plantarum*, *L. brevis*, and *L. mesenteroids* (Edema, 2009). *L. plantarum* was successful at lowering the pH of the fermented corn meal with values recorded within the pH range of 3.5-5.5 that are usually found in sourdough fermentation. Also, the corn meal fermented with *L. brevis* produced the highest amount of diacetyl, a compound important for flavor development and shelf stability of bread. Third, *L. mesenteroids* produced bread with the highest final viscosity value, which is important for baking properties like crumb texture and volume. Furthermore, the corn bread had acceptable rheological properties that were comparable to wheat dough's using a mixture of the three lactic acid bacteria. Even though corn flour lacks the gluten found in wheat, "the baking properties of corn meal were improved using the sourdough technique and a careful combination of the sponge and dough methods in two mixing stages" (Edema, 2011). The combination of starter cultures used in the study is also ideal for corn breads in which *L. mesenteroids* are used for good viscosity properties, *L. plantarum* for pH and *L. brevis* for flavor development.

Chapter 5 - Areas of Future Research

Lawton (1992), Meija et al. (2007), Schober et al. (2008), and Erickson, Campanella, and Hamaker (2012) have reported that zein possesses viscoelastic properties under certain conditions, making zein a possible alternative to wheat. Recent popular wheat substitutes include

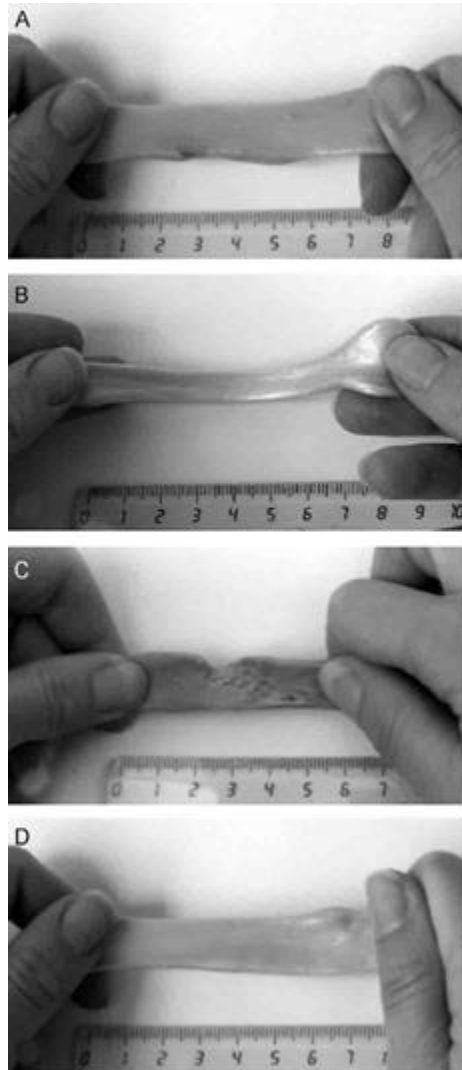
sorghum-based flours, which are readily available in most developing countries (Bugusu et al., 2001). Kafirin, the storage protein in sorghum, can be divided into subclasses α , β , and γ -kafirin based on their molecular weights, and studies have shown that sorghum flour replacement of up to 15% for wheat flour is feasible and does not affect the bread-making process negatively. However, if sorghum flour surpasses the 15%, undesirable changes in the bread such as low volume and a firmer crumb structure result (Bugusu et al., 2001). Bugusu et al. (2001) evaluated the addition of zein to sorghum-wheat bread to understand if zein would improve the bread quality.

Zein and kafirin differ from gluten as they do not form viscoelastic dough at 21°C. This is because in general, zein and kafirin are difficult to hydrate, contain hydrophobic residues, have predominantly α -helical structures, and are extractable in nonpolar solvents (Bugusu et al.; Oom, Petterson, Taylor, and Stading, 2008). These similarities suggest that kafirin may form viscoelastic properties too under the right conditions. Bugusu et al. (2001) investigated adding sorghum flour and zein to wheat flour dough and found dough development time increased while the peak height in the mixograph decreased as zein content increased in the dough. Bugusu et al. (2001) concluded that zein improved the sorghum-wheat dough due to two main factors: 1) zein was more readily available to participate in the fibril formation during dough mixing, and 2) mixing at 35°C enabled zein to form viscoelastic properties.

Oom et al. (2008) compared the viscoelastic properties of kafirin-starch and zein-starch doughs. Resin was used to define a protein-plasticizer system revealing that kafirin resin was sticky and elastic, comparable to zein resin. The Tg of both kafirin and zein resins was in the range of -4 to -3°C when using a dynamic mechanical temperature scan, which is remarkably lower than Lawton's finding (1992) who reported the Tg of zein plasticized by water was ~20°C with 25% moisture. Oom et al. (2008) attributed the change in Tg of zein to the use of oleic acid as the plasticizer. Over time, the kafirin resin became more stiff and difficult to stretch (Figure 9). Also, at a 45° phase angle, the kafirin resin had almost equal viscous and elastic contributions, but elasticity changed within 1000 seconds. At a 35° phase angle, a plateau formed, which indicates more elastic material was present than viscous material. In contrast, the zein resin remained constant for the first 1000 seconds indicating that the zein resin maintained equal viscous and elastic materials.

Oom et al. (2008) concluded that kafirin, with the addition of oleic acid, can form viscoelastic dough similar to zein. However, the kafirin resin exhibited stiffening, which was probably due to cysteine-rich γ -species. Moreover, when attempting to make kafirin-starch dough, researchers found the dough was not similar to zein-starch dough in viscoelastic properties. Further research is necessary to understand the role kafirin could have in gluten-free bread manufacturing.

Figure 9: Kafirin resin compared to zein resin. A) kafirin resin; B) zein resin; C) kafirin resin stretched immediately after mixing, and D) zein resin stretched after 2.5 hours at 21°C. (Oom, Petterson, Taylor, and Stading, 2008)



Challenges

Zein-based foods are believed to be the ideal answer for high quality, gluten-free products; as Durham (2010) stated, zein could potentially be an intermediate step toward achieving the “Holy Grail of gluten-free breads.” Consequently, research now concentrates on manipulation of zein so that it behaves and performs like gluten. However, challenges exist and include the unstable nature of zein at $< 28^{\circ}\text{C}$, the lack of stabilization of the β -sheet structure of zein, and the high cost of zein production.

Lawton (1992), Meija et al. (2007), and Schober et al. (2008) reported that zein exhibited viscoelastic properties at $>28^{\circ}\text{C}$, but discussed the unstable nature of zein at $<28^{\circ}\text{C}$. Maintaining these temperatures throughout dough handling in a commercial bakery will be a major hurdle. Studies suggest that the high molecular weight subunits of glutenins, which are initially in a loop conformation, extend during the expansion of gluten to favor β -sheet structures (Erickson et al., 2012). The lack of HMW subunits in zein may explain the β -sheet alignments that occur during the mixing of viscoelastic zein dough and why the properties are not stable. Therefore, stabilizing the β -sheet structure of zein is critical to maintain the viscoelastic nature during relaxation at $< 28^{\circ}\text{C}$ (Meija, Gonzalez, Mauer, Campanella, and Hamaker, 2012).

Schober et al. (2008) prepared zein dough at 40°C , cooled it to 20°C , reheated it to 40°C , re-kneaded it, and sheeted it before final proof. Half of the samples were mechanically treated $< 28^{\circ}\text{C}$, and the other samples were not. Also, all breads were similar (mechanical treatment did not affect bread quality), exhibiting large voids under the top crust. In actuality, these large holes were noticed when the dough were cooled below 28°C and reheated to $> 40^{\circ}\text{C}$ with a different timing schedule. Large holes in the dough result from the expanded gas held under the crust that forced the dough apart, thus creating a void, which indicates a weak bread structure.

To counter this effect, Schober et al. (2008) suggested manipulating the yeast activity in dough. Yeast produces carbon dioxide during fermentation resulting in an increase in volume during the cooling period from 40 to 20°C , which could be problematic as the fermentation of gases below 28°C creates structural weakness in dough. Nevertheless, zein dough held at 25°C for 10 minutes was baked and the resultant bread exhibited a good crumb texture and contained no large voids. Overall, it is feasible to produce zein bread in a typical bakery using ambient temperatures that are $<28^{\circ}\text{C}$ as long as the dough remains at 40°C for most of the time. Typically dough is handled at $<25^{\circ}\text{C}$ in bakeries, so increases to 40°C would require more energy input.

Erickson et al. (2009) believe that the unstable nature of zein limits its usage in gluten-free baking. However, if zein could retain the viscoelastic properties at lower temperatures, this could greatly improve its usage in bakery settings. Meija et al. (2012) investigated using co-proteins to stabilize the β -sheet structure of zein. High molecular weight glutenins found in gluten are responsible for the formation of β -sheet structures in gluten polymers that allow gluten-starch dough to hold carbon dioxide over an extended period. FT-IR, along with nuclear

magnetic resonance (NMR), show that β -sheet structure of HMW glutenin increases when the proteins are in a mixed, hydrated dough system. HMW glutenin subunits are initially in a loop conformation and become, “extended during fibril formation and form polymeric alignments in which high proportions of B-sheet structures are favored at the expense of B-turns.” Disulfide cross links in wheat glutenin also affect dough strength. Therefore, B-sheet and disulfide bonds play a key role in stabilizing viscoelastic properties during dough formation.

To monitor changes in the secondary structure by FT-IR spectroscopy of the dough systems during relaxation, zein, gluten, zein HMW glutenin, and zein casein dough systems were mixed for 5 minutes at 35°C and then allowed to relax at 25°C for up to six minutes (Meija et al., 2012). The results show that the β -sheet content of zein dough was lower ($p=0.05$) than in zein HMW-glutenin or gluten at six minutes of relaxation. However, the zein HMW glutenin dough was not only stable, but it was statistically similar to the β -sheet content of the gluten.

Apparently, adding the HMW glutenin to zein increased the stability of the β -sheet content of zein and maintained the β -sheet content during relaxation while only causing a slight increase in the α -helix content. Zein mixed with HMW glutenin and starch, incubated for 24 hours, mixed at 35°C, and relaxed at 25°C, had β -sheet structure similar to that of gluten maintained at <28°C. However, HMW glutenin as a co-protein for zein would not be acceptable in gluten-free products. Thus, Meija et al. (2012) determined casein to be an alternative co-protein to HMW glutenin because it is comparable to HMW glutenin in stabilizing β -sheet structures even at usage levels of 3% of the total protein content.

Finally, zein production can be costly as the solvents can be expensive and the process itself energy-intensive as it includes evaporation and distillation to remove the solvent (Shukla and Cheryan, 2001). An affordable idea includes extracting zein from whole grain corn versus from corn gluten meal (Shukla and Cheryan, 2001). Using whole ground corn could be advantageous to ethanol producers as well as they would have the distillation equipment and materials needed for recovery of alcohol. The cost of purified zein is \$10-40 per kg depending on the grade and purity (Anderson and Lamsal, 2011b), making zein too expensive for gluten-free products as other gluten alternatives, such as hydrocolloids, are significantly less expensive ranging from \$4.5 to \$12.50 per kg (De Guzman, 2008). With time, more uses for zein will be identified and this could drive production and decrease cost. However, increasing demand alone may not be enough to make it as affordable as other, less expensive alternatives.

Zein-based foods are believed to be the ideal choice for high quality, gluten-free products. As Durham (2010) stated, zein could potentially be an intermediate step toward achieving the “Holy Grail of gluten-free breads”. Research to manipulate zein, produce zein more efficiently, as well as to transform its behavior is exciting and has major implications for other non-gluten cereal proteins in other food applications through modification to impart different functional properties.

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