Cinnamon Hydrolysis

Enzymatic and Acid Treatments for Viscosity Reduction

Master's Thesis

Submitted to the Faculty of the Graduate School of The University of Minnesota By

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Dedication

I would like to dedicate this work to anyone who is passionate and hardworking enough to pursue their true dreams and not willing to settle for anything less.

Abstract

Cinnamon is a desirable spice in a variety of food products for flavor enhancement; however, it is a problematic ingredient in food processing because viscosity is increased due to large polysaccharides that give cinnamon its unique functionality. To help mitigate viscosity issues that arise during manufacturing food products, such as cereal, different treatment methods to hydrolyze the polysaccharides in cinnamon were conducted. Different treatment methods utilizing a variety of enzymes and acid resulted in viscosity reduction by rapid-visco analyzer (RVA) analysis followed by high pressure liquid chromatography size exclusion chromatography (HPLC-SEC), soluble and insoluble dietary fiber analysis and light microscopy verification methods. A cellulase, xylanase, pectinase in combination and a β-Glucanase were used in the study. Hydrochloric acid treatment resulted in 88% viscosity reduction, β-Glucanase resulted in a 50% viscosity reduction, cellulase, xylanase, and pectinase combined resulted in an 11% viscosity reduction.

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1. Review of Literature

1.1 Introduction and Objectives

Cinnamon is used throughout the world as a spice for its desirable flavor. The ground spice is used for flavoring baked products such as cakes, biscuits, puddings, chewing gum, desserts and cereals. Even though cinnamon is a desirable flavor in food products it may become problematic during food processing applications. There is a physical aspect to cinnamon that hasn't been explored to the fullest. Cinnamon is a particle in a food matrix that impacts physical behavior of that food matrix such as viscosity. This impact is important to know as food companies want to utilize spices for flavor in a variety of food products as described earlier.

Consumers today want their food to taste delicious while also having a "clean label." A clean label is not clearly defined, but it is a label consisting of ingredients which can be found in a pantry or grocery store avoiding chemicals or flavorings (Clean Label Conference, 2015). Today's market is full of health - conscience consumers that are no longer satisfied with artificial ingredients such as colors or flavors and prefer the raw ingredients (Clean Label Conference, 2015).

It is challenging to add cinnamon in high quantities to meet these demands due to processing issues that arise especially in cereal products. Cereal manufacturing consists of two main components: the base which is the puff or flake, and the slurry (semisolid mixture) which is the sugar coating on the outside of the base (Fast et al., 2000).

Cinnamon can be added in the slurry coating or base formulation; however, it is most commonly added in the slurry. Slurry processing requires high shear and elevated temperatures reaching 109°C (228°F) (Fast et al., 2000). At these conditions, cinnamon increases viscosity and the slurry is not able to be pumped or dispersed easily (U.S. Patent App. No. GMI7872US01, 2016). Due to this phenomenon, the processing areas of concern for viscosity during cereal manufacturing are mainly at the heat exchanger, spray nozzle, and enrober for a slurry application (Fast et al., 2000).

The specific objectives of this research were:

1. To determine viscosity contributing carbohydrates in cinnamon.
2. To discover a way to counteract negative impacts cinnamon has on viscosity to allow for ease of use during cereal processing.

Hypotheses include:

1. Carbohydrates with degree of polymerization (DP) over 200 such as cellulose, hemicelluloses, specifically arabinoxylan, β-glucan and pectin are the main cause of viscosity in cinnamon.
2. Enzymatic and acid hydrolysis will reduce viscosity of cinnamon.

1.2 Cinnamon Ingredient Functionality

1.2.1 Cinnamon sources and uses

Cinnamon is an evergreen tree 10-15 m tall of which the bark is commonly used as the spice. The leaves are 7-18 cm long and ovate-oblong in shape. It also consists of flowers

and berries; however, these parts of the plant are unused for industrial purposes except for reproduction. The bark oil, bark oleoresin and leaf oil are important value-added products from cinnamon. The ground spice is used for flavoring baked products such as cakes, biscuits, puddings, desserts and cereals. The bark oil and leaf oil is used in the manufacture of perfumes, soaps, toothpaste and as a flavoring agent for liquors. Bark oil is also used in the pharmaceutical industries (Leela, 2008).

The genus *Cinnamomum* consists of over 250 varied species of trees and shrubs throughout the world, which are all referred to as cinnamon but may have different characteristics (Leela, 2008). The cinnamon considered the "true cinnamon" and the highest quality cinnamon is *C. verum* which is native to Sri Lanka and South India. Other more common cinnamon species, *C. cassia, C. Loureiroi* and *C. burmannii,* are all referred to as cassia (Wang et al., 2013).

Cinnamon not only gives a desirable flavor impact but has many medicinal and pharmacological uses as well. It possesses various biological activities such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory and antiallergenic capabilities (Leela, 2008). The essential oil has been used as a preservative in food due to the antioxidant property of cinnamon, which prevents lipid oxidation. Phenolic compounds such as hydroxy cinnamaldehyde and hydroxycinnamic acid act as scavengers of peroxide radicals and prevent oxidative damage (Wu et al., 1994). The main flavor component, cinnamaldehyde, is an antibacterial that also shows insecticidal activity (Leela, 2008). Components of cinnamon such as the oil and extracts show antimicrobial

activity against several bacteria and fungi (Leela, 2008). For these reasons, cinnamon is a very versatile and desirable ingredient.

1.2.1.1 Industrial applications

Cinnamon may be used in cereal applications for flavor purposes. The cereal production consists of many processes but for the purposes of this research the focus is on the sugar coating application process because this is generally where cinnamon is added in a cereal formula. The major objective when formulating a cereal slurry coating is to combine a formula with an application method that produces a hard, transparent glaze or candy coating onto the cereal base (Fast et al., 1990). This involves very little added water but requires a very hot (even molten) spray solution (Massmann et al., 1954; Vollink, 1959). A common method of preparing a sugar spray solution is to make up an aqueous solution of sugar and pump it through a heat exchanger reaching 109°C (228°F) to drive off water concentrating it, then it is pumped to the spray nozzle where it is dispersed onto the cereal base which is being tumbled in a large drum or otherwise known as an enrober (Fast et al., 2000). It is important for product quality that only a thin layer of solution is evenly dispersed onto the base without being entirely absorbed by it resulting in a glassy coating. To accomplish this coating, a balance is required when formulating a cereal slurry. The more dilute the solution or the lower concentration of solids, the better the dispersion, the lower the viscosity, but higher risk of penetrating the base product collapsing the foamed puff therefore increasing base density (Fast et al., 2000). The more concentrated the solution or higher concentration of solids reduces the risk of penetration and minimizes the need for post drying, but results in a higher viscosity making even

dispersion of the protective coating more difficult, and can result in a hygroscopic product causing shelf life issues due to staling (Fast et al., 2000). Formulating a slurry where neither detrimental scenario takes place is the balance threshold and would result in a successful cereal product. The addition of cinnamon would ultimately result in more solids, making this balance very challenging. Adding or increasing the amount of cinnamon beyond the balance threshold can cause production difficulties, such as the clogging of machines or pipes, as well as build up on or fouling of a dryer making it infeasible to include significant amounts of cinnamon in a cereal slurry (U.S. Patent App. No. GMI7872US01, 2016). The addition of cinnamon to a slurry formula not only results in more solids causing an increase in viscosity but its composition has a profound impact on functionality as well such as the flow behavior.

1.2.2 Composition of cinnamon

Cinnamon is a crop and thus the composition of cinnamon varies depending on many factors such as the geographical origin, climate conditions, age of the tree, the time of harvest and storage conditions. These conditions affect components of the dried inner bark of cinnamon which is then processed and distributed. Cinnamon may be ground or kept in quills (a roll of bark after drying), may or may not be sterilized, then packaged and distributed. The general composition of the dried inner bark consists of volatile oil, fixed oil, tannin, resin, proteins, cellulose, pentosans, mucilage, starch, calcium oxalate and mineral elements (Leela, 2008). Nutritionally, cinnamon is mostly carbohydrates

(Tainter, D.R., 1993), see Table 1. The composition, including the carbohydrate components, may vary depending on the crop variability and the cinnamon species.

Composition	USDA	ASTA
Water (g)	9.52	10
Food energy (Kcal)	261	355
Proteins (g)	3.89	4.5
Fat (g)	3.18	2.2
Carbohydrates (g)	79.85	79.8
Ash (g)	3.55	3.5
Calcium (g)	1.23	1.6
Phosphorus (mg)	61	50
Sodium (mg)	26	10
Potassium (mg)	500	400
Iron (mg)	38.07	4.1
Thiamine (mg)	0.077	0.14
Riboflavin (mg)	0.14	0.21
Niacin (mg)	1.3	1.9
Ascorbic acid (mg)	28.46	40
Vitamin A activity (RE)	26	26

Table 1: Nutritional composition of Cinnamomum verum and Cinnamomum Cassia combined per 100 grams (Tainter, D.R., 1993). United States Department of Agriculture (USDA). American Seed Trade Association (ASTA).

There are several types of carbohydrates in the classes of monosaccharides, oligosaccharides, and polysaccharides. The characterization and quantification of each type of carbohydrate in cinnamon may vary depending on the cinnamon source. It is possible that the viscosity issues during cereal processing are most likely due to the larger molecular weight hydrocolloid polymers such as cellulose, β-glucan, arabinoxylan and pectin; which are classified as polysaccharides. Furthermore, it is most likely a combination of these different polysaccharides which make cinnamon viscous.

1.2.2.1 Polysaccharides that contribute to viscosity in cinnamon

Cellulose is the main contributing polysaccharide present in the cell-wall matrix consisting of more than 50% of the total carbohydrates (Coffey et al., 1995). Cellulose is formed from linear chains of β-(1→4)-linked D-glucopyranosyl units seen in Figure 1, ultimately linked glucose units, or referred to as β-(1→4)-glucan, where the degree of polymerization (DP) (The number of monomeric units in a macromolecule, an oligomer molecule, a block, or a chain shape) is usually 8,000-10,000 monomers, which produces chains of 4,000-6,000nm in length, with a molecular weight of well over one million (Coffey et al., 1995).

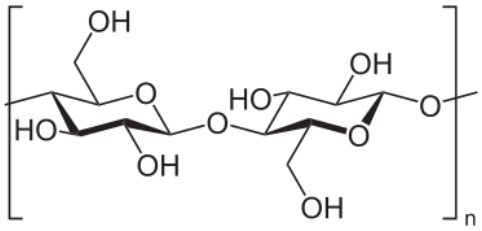


Figure 1: Cellulose, a linear polymer of D-glucose units (two are shown above) linked by β-(1→4)-linked D-glucopyranosyl bonds.

Individual glucan chains aggregate to form fibrillar structures, clearly visible by light microscopy, which are referred to as cellulose microfibrils. This association of glucan chains is stabilized by extensive inter-chain hydrogen bonding. Although, generally weak form of interaction, the sheer number of noncovalent bonds formed produce a three-dimensional molecular structure, hydrophobic in nature, which demonstrates a resistance to chemical or enzymatic attack far greater than that shown by the individual glucan chains. However, the degree of order of the microfibrils is not uniform. Regions in which the antiparallel glucan chains are precisely aligned and hydrogen bonding is

maximal (crystalline region) are found interspersed with regions in which the degree of order is far lower (amorphous region) seen in Figure 2.

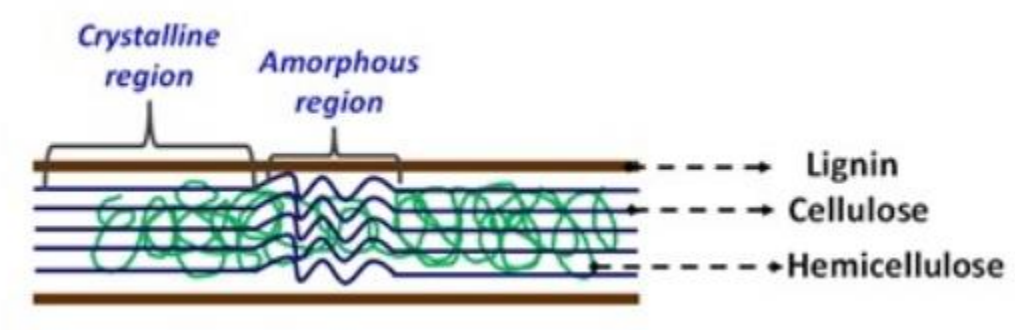


Figure 2: Plant cell wall depiction showing the crystalline region and amorphous region in cellulose.

Amorphous regions have a higher solubility compared to the crystalline regions and are more vulnerable to hydrolysis methods such as acid or enzymatic (Coffey et al., 1995). A characteristic of cinnamon is that it is partially soluble and insoluble, like any plant with high cellulose content.

Hemicellulose, unlike cellulose, has a random, branched, and amorphous structure with little strength. Hemicellulose is composed of several heteropolymers (matrix polysaccharides) including xylan, glucuronoxylan, arabinoxylan, glucomannan and xyloglucan which produces a (DP) usually 100 - 400 monomers.

Arabinoxylans can be found in the primary and secondary cell walls of plants including woods and cereal grains such as wheat, oat, barley, rice and rye and is characterized as a heteroglycan because it consists of two pentose sugars, arabinose and xylose (McCartney et al., 2005). Cinnamon is essentially a wood which may have arabinoxylans present. Arabinoxylans are mixed linkage (1→3) (1→4)-B-D-glucans that consist of a linear

chain backbone of β-D-xylopyranosyl residues linked through (1→4) glycosidic linkages (Foschia et al., 2013). Hemicellulose, unlike cellulose, has a random, amorphous structure with little strength. Arabinoxylans form highly viscous aqueous solutions and generally have a strong water-holding capacity (Foschia et al., 2013). Thus, in bread making and cereal extrusion, arabinoxylans are detrimental due to high viscosity impact, negative impact on loaf volume and crumb structure or puffing in a cereal product (Trogh et al., 2007). If hemicelluloses such as arabinoxylans are present in cinnamon, this may be one reason why viscosity is an issue during processing.

β-glucans form a natural component of the cell walls of bacteria, fungi, yeast, and cereals such as oat and barley. Each type of β-glucan comprises a different molecular backbone, level of branching and molecular weight which effects its solubility and physiological. The most common forms of β-glucans are those comprising D-glucose units with β-1,3 links. Yeast and fungal β-glucans contain 1-6 side branches, vary in size and shape and are insoluble. Oat β-glucans contain both β-1,3 and β-1,4 backbone bonds. Such (1→4, 1→3)-β-glucans are often called mixed-linkage β-glucans which produces a soluble (DP) of 5 - 28 monomers (Foschia et al., 2013). About 70% are linked (1→4) and about 30% (1→3). Such (1→4, 1→3)-β-glucans are often called mixed-linkage β-glucans seen in Figure 3.

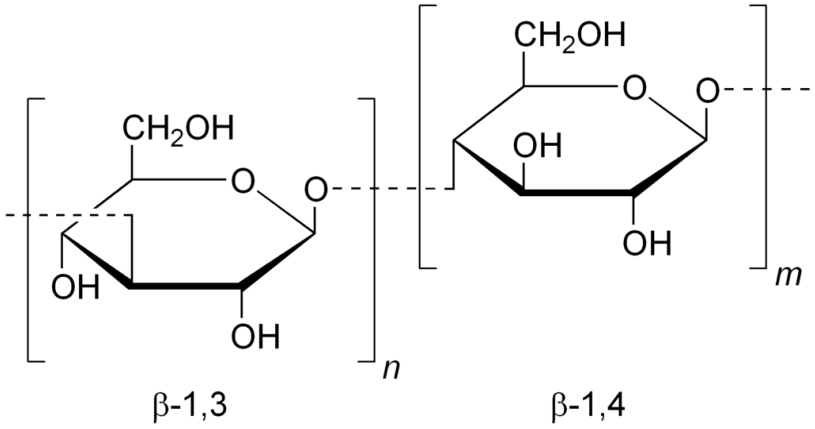


Figure 3: Chemical structure of (1→4, 1→3)-β-glucans, often called mixed-linkage β-glucans.

When taken orally in foods, these mixed linkage β-glucans reduce postprandial serum glucose levels and the insulin response, meaning they moderate the glycemic response (Fennema, 1996). However, β-glucans comprising D-glucose units with β-1,3 links vary with respect to molecular mass, solubility, viscosity, branching structure, and gelation properties, causing diverse physiological effects.

Pectin substances are a group of polysaccharides, ultimately made up of galactose units, from the primary cell walls and intercellular regions of higher plants. They are deposited mainly in the early stages of growth when the area of the wall is increasing (Voragen et al., 1995). Pectin is a linear backbone of α-(1→4)-linked D-galacturonic acid units (smooth region) with branched sections (hairy region) consisting of constituent sugars: D-galactose, L-arabinose, D-xylose as seen in Figure 4.

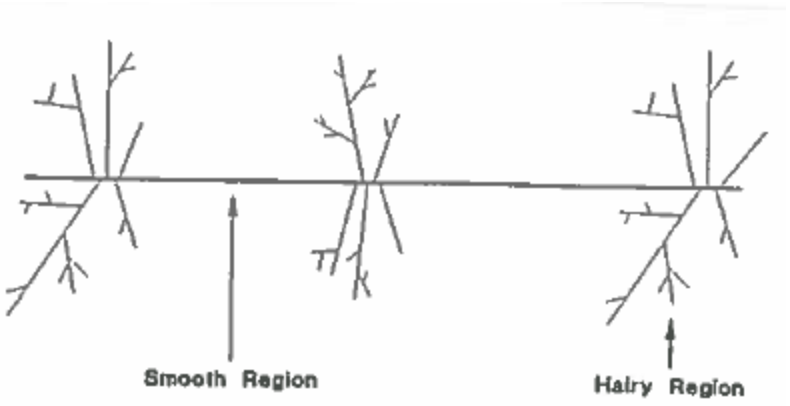


Figure 4: Schematic structure of pectin molecules (Voragen et al., 1995).

It is important to note that the hairy regions will vary in composition and will contain a variety of different combinations of 1,4 or 1,3,4-α-GalA, methyl ester at C6, 3O-acetylated GalA, 1,2 or 1,2,4-α-Rha, 1-β-Xyl, 1 or 1,3 or 1,3,6-β-Gal (Voragen et al.,1995). Pectin is not a homopolysaccharide which is why it is difficult to specify a molecular weight for it. Studies have shown that blocks of the smooth region range from 25, 40-60, 72-100 monomers with alternating hairy regions which would add to the total weight (Zobel, F.H., Stephen, A.M., 1995).

Starch which is similar in composition to cellulose, has an α linkage instead of a β linkage of (1→4)-linked D-glucopyranosyl units giving it a helical shape and consists of shorter chain lengths (DP 10-100, average 25) (Zobel, F.H., Stephen, A.M., 1995). Due to the relatively small molecular size compared to cellulose it may not be a substantial contributor to viscosity because it is not enhancing the entangled network greatly.

Lignin is a three-dimensional polymer comprised of phenyl propane units such as syringaldehyde and vanillin and these are linked through aliphatic three-carbon side

chains (Fennema, 1996). Lignification of cell walls, notably those of xylem and scherenchymatoous tissues, confers considerable rigidity and toughness to the wall. Pectin occupies the central location in the middle lamella, located between the primary cell walls of adjacent cells. Hydrolysis of pectin by enzymes can reduce intercellular adhesion and thus soften the tissue (Fennema, 1996). Therefore, focusing on pectin hydrolysis will ultimately degrade the lignin network. An example of a lignin network can be seen in Figure 5. However, a three-dimensional depiction of how the polysaccharides discussed in this section come together to create a cell wall can be seen in Figure 11.

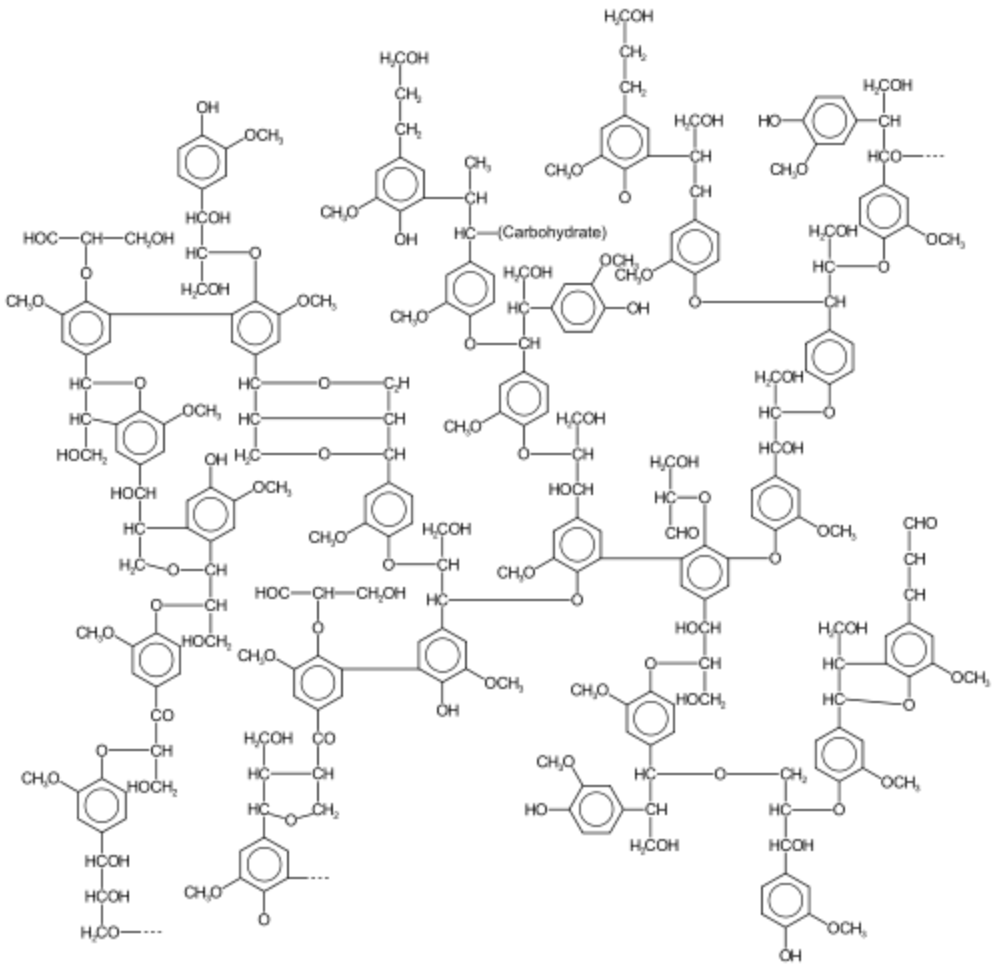


Figure 5: A molecular structure of a lignin network.

1.2.2.2 Dietary Fiber in Cinnamon

Dietary fiber consists of plant cell walls. Difference in the cell wall structure relate to differences in the type and proportion of the matrix polymers present (Chesson, 1995). Plant glucans (polysaccharide of D-glucose monomers), of which both cellulose and starch are specific forms, are among the few homopolysaccharides found within the plant kingdom. The cell walls of plants vary in composition depending on the cell type from which they derive and the phylogenic origin of the plant. It is an impossibly long and

arduous process to fractionate this heterogeneous material and to analyze the separated fractions, so that in practice an overall composition can be determined (Chesson, 1995). However, it is important to recognize that this heterogeneity exists.

Other than fractionation, discussed in section 1.6.4, cinnamon carbohydrate polymers may be characterized by separating them into soluble and insoluble dietary fiber. Dietary fiber is a nutritional term that is defined by the method used to determine chemical and physical properties (Fennema, 1996). Per the CODEX Alimentarius Commission, dietary fiber is defined as non-digestible polysaccharides of DP=3 or higher utilizing the CODEX method. Soluble dietary fiber (i.e. oligosaccharides, pectins, β-glucans) can help to lower blood cholesterol and regulate blood glucose levels whereas insoluble dietary fibers (i.e. cellulose, hemicellulose, and lignin) promotes the movement of material through the digestive system supporting the growth of intestinal microflora (Foschia et al., 2013). Solubility is a crucial factor to the viscosity of cinnamon which is discussed in further detail in section 1.3.1.

1.2.3 Physical behavior of cinnamon

1.2.3.1 Viscosity

Cereal processing often involves shear mixing in a slurry kettle and more importantly at the heat exchanger or condenser. Pumping the material through a series of pipes is also necessary. Processing problems arise when cinnamon is added to the cereal slurry formulation because cinnamon increases viscosity (U.S. Patent App. No. GMI7872US01, 2016). However, cinnamon has unique characteristics to its viscosity. Viscosity describes the physical property of a liquid to resist shear induced flow (Schramm, 1992).

Viscous flow is caused by linear chains slipping past one another and is the mechanism responsible for viscous flow in pipes and elongation flow under stress for example pulling of Silly Putty ® (Sperling, 2006, 1992). Cinnamon shows characteristics of viscous flow or mucilaginous (mucilage is a thick, gluey substance produced by plants) behavior during shear processing most likely due to the polysaccharides present such as cellulose which is a long linear polymer.

Several types of liquid materials may have differing flow behavior and can be classified. They can be classified into two main categories, Non-Newtonian liquids and Newtonian liquids. There are three types of Non-Newtonian flow behavior; pseudoplastic, dilatant and pseudoplastic with a yield point otherwise known as a plastic liquid, which can be seen in Figure 6. It is observed that cinnamon in solution has the characteristics of a non-Newtonian liquid. More specifically it may be observed that cinnamon in solution is a dilatant liquid where the viscosity increases whenever shear rate increases. However, the viscosity may become so high that the processing equipment will cease or it will slip and not mix the material behaving as a pseudoplastic material in solution meaning it becomes less viscous with higher shear. However, this is most likely due to the slippage from viscous flow that is occurring. The observation of pseudoplastic vs. dilatant liquid behavior is dependent on if the polymer network in cinnamon, also taking into consideration the other ingredients in the slurry solution, can align or not. However, neither scenario is ideal for production.

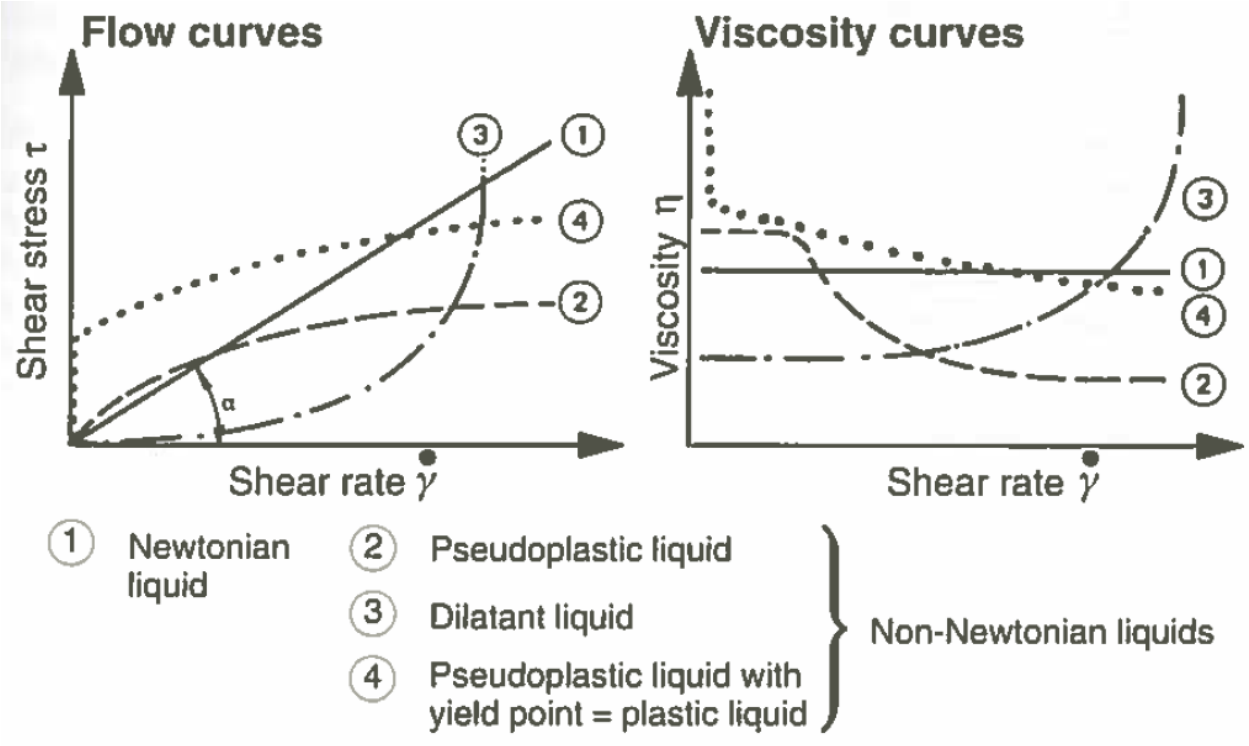


Figure 6: Various types of flow behavior (Schramm, 1992).

1.2.3.2 Creep Compliance

Creep is defined as something moving slowly. Creep experiments are when a constant stress is applied to a sample and the dimensions of how the sample reacts to that stress are recorded as a function of time (Sperling, 2006). These experiments can be generalized to include shear motion or compression for example. Stress can be applied by compressing a sample and holding constant. The sample reacts in one of two outcomes, the first, not being to withstand the stress and fractures or, the second, it slowly rearranges or moves itself, hence creep, to withstand and accept that stress. The storage modulus (E) of a material is a measure of its stiffness and usually reported in stress relaxation experiments whereas compliance (*J)* is its softness or malleability and

reported as the compliance (Sperling, 2006). Creep compliance (*J)* is the measure of how well a material comes to equilibrium when a force is applied (Sperling, 2006). Mathematically it can be represented by $J = \gamma/\tau$, where J = creep compliance, γ = equilibrium deformation and τ = stress (Sperling, 2006). Eventually, γ and τ will become equal at equilibrium resulting in $1 = J$.

Sperling explains that the molecular causes of stress relaxation and creep vary and can be grouped into five categories. The first being chain scission, caused by oxidative degradation and hydrolysis. The second, bond interchange, where chain portions change partners causing a release of stress (i.e. polyesters and polyiloxanes). The third, viscous flow, caused by linear chains slipping past one another. The fourth, thirion relaxation, is a reversible relaxation of the physical crosslinks or trapped entanglements in elastomeric networks. Lastly, the fifth being molecular relaxation, where the chains relax near the glass transition temperature (Tg) which is the temperature where a polymer transitions from a hard, glassy material into a soft, rubbery material (Sperling, 2006). It is possible that cinnamon may fall in any of these categories dependent upon the conditions. For example, cinnamon could be in the second, bond interchange, or fourth, thirion relaxation, categories when imbedded in a glass at ambient conditions; however, if the temperature reaches the Tg, then it may also be in the fifth, molecular relaxation. When cinnamon is in a water solution being sheared, it is observed in the third category, viscous flow, discussed in section 1.2.3.1. Ultimately, when enzyme or acid treatment takes place it is expected to be in the first, chain scission. Cinnamon can be in any one of these categories due to its unique polysaccharides discussed in section 1.2.2.1 and the

molecular network they make discussed in section 1.3.3. To understand how cinnamon creep compliance testing was conducted see section 2.1.

1.3 Primary factors impacting viscosity of cinnamon

1.3.1 Solubility

Polysaccharides such as hydrocolloids are used primarily to thicken and/or gel aqueous solutions to control the flow properties of that liquid or semisolid mixture of which only a small amount is needed to do so (0.25-0.50%) (Fennema, 1996).

Substances that solubilize, impact the viscosity whereas substances that do not solubilize, suspended solids, do not impact the viscosity nearly as much. Depending on particle size and concentration, they could impact flow but not to nearly the level of soluble polymers. Cinnamon has always been thought of as a suspended solid, but due to the potentially high cellulose content, it may also be considered a hydrocolloid due to the partial solubility from the amorphous regions and the partial insolubility from the crystalline regions. If, for example, 50% of cinnamon is cellulose and 50% of the cellulose is amorphous regions then 25% of cinnamon could be solubilized into solution which would not only impact the viscosity but also calculations that are critical when formulating such as solids to aqueous solution ratios.

1.3.2 Molecular size and shape

Another component of polysaccharide solubility is the size of the carbohydrate. One way to characterize the size of a carbohydrate is the degree of polymerization (DP). The DP is

the number of monosaccharide units in a polysaccharide and will vary among polysaccharides (Fennema, 1996). Few polysaccharides have a DP less than 100 monomers (mers); most are in the range of 200-3000 mers and some larger ones range from 7000-15000 mers such as cellulose (Fennema, 1996). The larger the molecule the more mass it will have, therefore more energy would need to be exerted resulting in a higher viscosity. However, the molecules can become so large that they are not able to absorb into solution; hence, suspended solids and therefore no longer impacting viscosity nearly as much as polymers in solution.

Not only is the size of the polysaccharide a contributing factor to viscosity but the shape is as well. The polymeric structure and its components are key factors that give a polymer complex its shape. The physical-chemical nature of the substance is determined by the molecules in that substance and is the primary influence on viscosity (Schramm, 1994). Polysaccharides may be linear, ribbon-like structures (cellulose) seen in Figure 7 or branched structures (hemicellulose) seen in Figure 8.

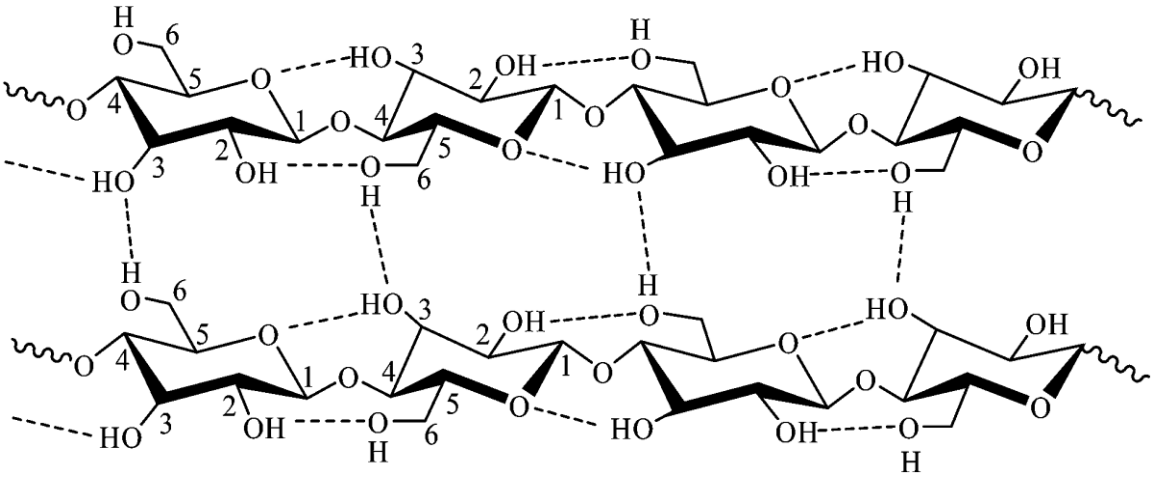


Figure 7: A molecular structure of two cellulose chains packing together.

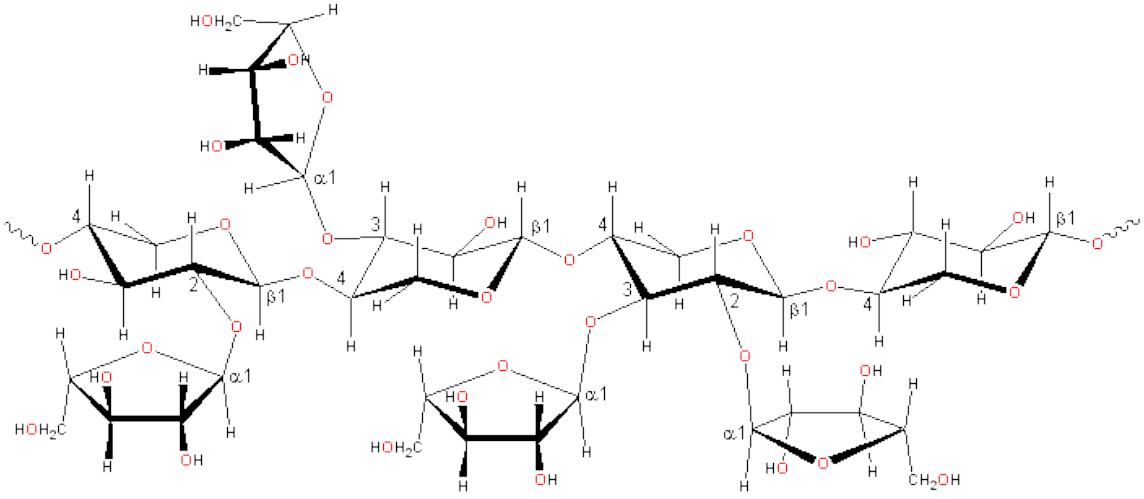


Figure 8: A molecular structure of hemicellulose, specifically arabinoxylan.

Linear polymers in solution move freely, sweeping out a large space until they collide with each other getting entangled, creating friction, consuming energy, and therefore producing viscosity (Fennema, 1996). If there are linear polymer chains containing only one type of ionic charge, usually at the ends, they will constantly arrange per the repulsion therefore producing solutions of very high viscosity (Fennema, 1996).

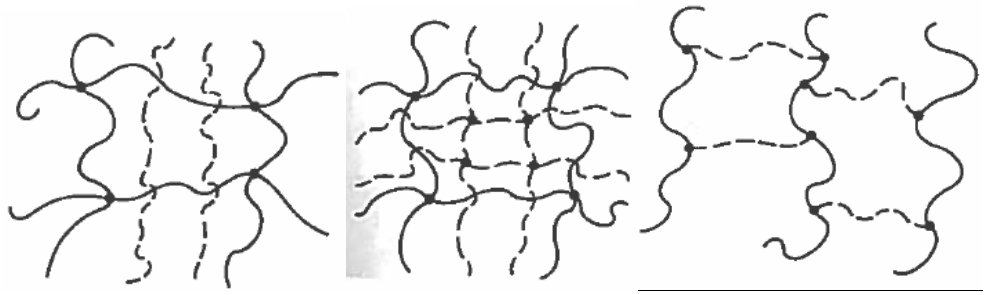
Branched polysaccharides, such as hemicelluloses, are not able to pack closely together to form strong hydrogen bonds therefore cannot form as ridged of structures like cellulose. Branched structures such as pectin and hemicelluloses are more vulnerable to cleavage because they have more potential cleavage sites throughout the molecule compared to large linear structures like cellulose where cleavage sites are only on the exterior of the packed chains or amorphous regions. According to Fennema, branched polymers are not able to have the same degrees of freedom in movement compared to a linear polymer thus not collide as frequently and will have lower viscosity (Fennema, 1996). Linear chains and branched molecules have differing degrees of freedom in movement due to their shape.

Viscosity depends both on the molecular weight and the extension and rigidity, that is, the shape and flexibility, of the solvated polymer chain (Fennema, 1996). Based on viscosity observations, cinnamon behaves as if it contains both linear polymer chains, and branched polymers.

1.3.3 Molecular network

Cinnamon is composed of many polysaccharides as discussed in section 1.2.2.1, rather than only one type. The polymer network of the carbohydrates will affect the viscosity of the cinnamon in solution due to entanglement of the polymers. There are many formations and cross linkages that could occur with two polymer types as seen in Figure 9, where three basic modes of linking two or more polymers are identified. The first, (a), is a semi-interpenetrating polymer network constituted by an entangled combination of two polymers, one of which is cross-linked, that are bonded to each other. The second,

(b), is an interpenetrating polymer network is entangled combination of two cross-linked polymers that are not bonded to each other. The third, (c), is a conterminously linked polymer, constituted by having the polymer II species linked, at both ends, onto polymer I. The total product is a network composed of two different polymers (Sperling, 1992).



(a) (b) (c)

Figure 9: Three basic modes of linking two or more polymers are identified. The total product is a network composed of two different polymers (Sperling, 1992).

On a larger scale, it might look something like Figure 10.

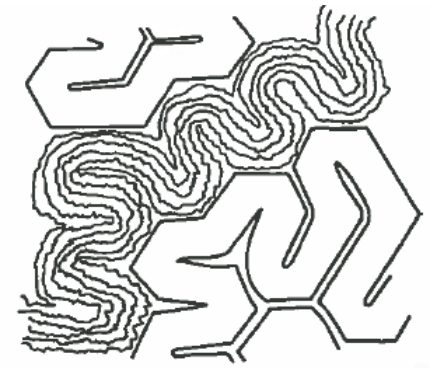


Figure 10: Pechhold's meander model of amorphous state polymers (Pechhold, 1973).

If the matrix of cinnamon consists of more than two polymer types, it would be more complex resulting in more entanglement and higher viscosity. An even larger scale example of how these polymers might pack together and interact can be seen in Figure 11.

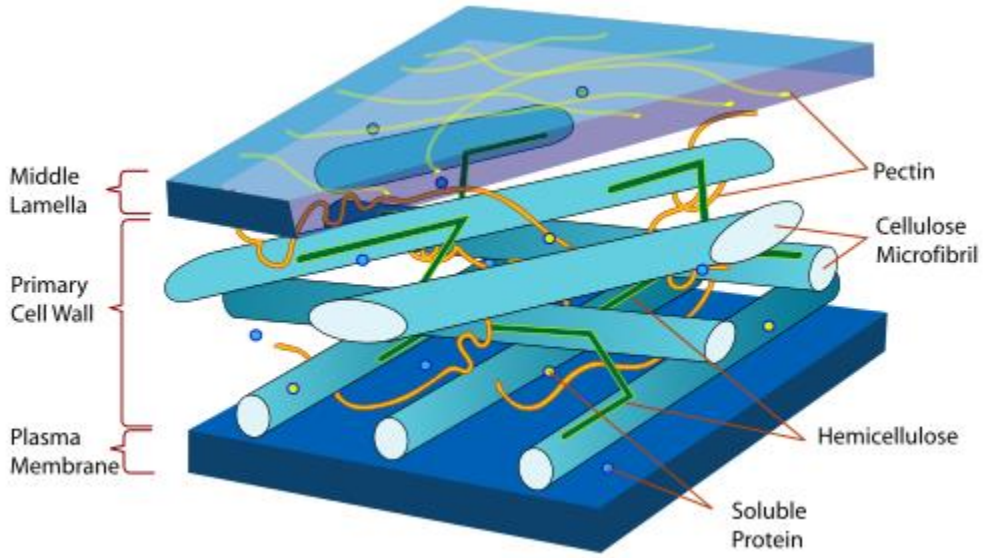


Figure 11: A three-dimensional depiction of a plant cell wall section (Wpclipart.com, 2017).

If the polymer network can be altered in a way to reduce viscosity, it could be a solution for processing issues that arise when using this problematic ingredient.

1.4 Enzymatic hydrolysis to reduce viscosity

1.4.1 Enzyme sources and uses

Enzymes are proteins synthesized by biological cells with powerful catalytic activity. They are involved in chemical reactions related to metabolism and exist in all organisms. Therefore, enzymes-catalyzed reaction also proceeds in many foods and thus enhance or deteriorate food quality. Examples of this phenomenon are the ripening of fruits and

vegetables, the aging of meat and dairy products, and the processing steps involved in the making of dough from wheat or rye flours and the production of alcoholic beverages by fermentation technology (Belitz et al., 2004).

Enzymes are globular proteins with greatly differing particle sizes. The enzyme structure in determined by its amino acid sequences and by its conformation, both secondary and tertiary, derived from this sequence. Larger enzyme molecules often consist of two or more peptide chains arranged into a specified quaternary structure. Some enzymes are complexes consisting of a protein moiety bound firmly to a non-protein component what is known as a substrate, which is involved in catalysis. The activities of other enzymes require the presence of a co-substrate which is reversibly bound to the protein moiety. The three-dimensional shape of the enzyme molecule and other components is what gives it the specificity and its effective role as a catalyst (Belitz et al., 2004).

The protein nature of the enzyme restricts its activity to a relatively narrow pH range and sensitivity to heat treatment which leads to loss of activity by denaturation. Enzyme inactivation or changes in the distribution patterns of enzymes in subcellular particles of a tissue can occur during storage or thermal treatment of food (Belitz et al., 2004). Since such changes are readily detected by analytical means, such as Polymerase Chain Reaction (PCR), they often serve as indicators for revealing such treatment of food. Examples are the detection of pasteurization of milk, beer, or honey, and differentiation between fresh and deep-frozen meat or fish (Belitz et al., 2004). Enzymes are very sensitive to their perspective environmental conditions and their function depends on the appropriate conditions.

1.4.2 Enzyme functionality

The catalytic activity of enzymes is exhibited only under specific conditions, such as pH, ionic strength, buffer type, presence of cofactors which activate the reaction and suitable temperature. Enzymes are substantially better catalysts than are protons or other ionic species used in non-enzymatic reactions (Belitz et al., 2004). Enzymes invariably surpass all chemical catalysts in relation to substrate and reaction specificities (Belitz et al., 2004). Theories have been developed to explain the exceptional efficiency of enzyme activity. They are based on findings which provide only indirect insight into enzyme catalysis. An enzyme molecule, glucose oxidase for example, is larger in size compared to the substrate, glucose in this example, by several orders of magnitude, 10^3 in this instance. This strongly suggests that in catalysis only a small locus of an active site has direct contact with the substrate. Specific parts of the protein structure participate in the catalytic process from the substrate binding to the product release from the so-called active site. The parts are amino acid residues which bind substrate and, if required, cofactors and assist in conversion of substrate to product. The lock and key hypothesis, and the induced fit model are two examples of mechanisms hypothesized for specificity of enzyme catalytic reactions.

Even though there is not a direct answer into enzyme catalysis mechanisms, the individual or combined effects which regulate the rates of enzyme-catalyzed reactions can be calculated. The reaction rate is dependent on the concentrations of the components involved in the reaction, mainly the substrate and the enzyme.

The reaction can be influenced by the presence of activators (cofactors) which enhance the reaction rate and inhibitors which decrease the reaction rate. Metal ions such as magnesium, calcium, zinc, iron, and copper are examples of activators where they are effective as cofactors with enzymes. These activators influence the substrate binding and participate in the catalytic reactions in the form of a *Lewis* acid or play the role of an electron carrier. On the other hand, inhibitors randomly react with the enzyme slowing or potentially terminating the reaction. Phenolic constituents in food, contaminated food that contain pesticides, heavy metal ions or other chemicals from a polluted environment can become inhibitors under some circumstances based on the conditions of the reaction (Belitz et al., 2004).

Other factors influencing the reaction due to the enzyme charge, are the pH, the ionic strength of the reaction medium, and the dielectric constant of the solvent, which is usually water. Each enzyme is catalytically active only in a narrow pH range and each has a pH optimum which is often between pH 5.5 and 7.5 (Belitz et al., 2004). The optimum pH is affected by the type and the ionic strength of the assay. The reasons for the sensitivity of the enzyme to changes in pH are mainly for two reasons. The first being sensitivity is associated with a change in protein structure leading to irreversible denaturation and the second that the catalytic activity depends on the quantity of electrostatic charges on the enzyme's active site generated by the prototropic groups of the enzyme (Belitz et al., 2004).

The thermal stability of enzymes is rather variable. Some enzymes lose their catalytic activity at lower temperatures (70°C), while others are capable of withstanding, at least

for a brief period of time, a stronger thermal treatment (140°C). Heat treatment may either accelerate desirable enzymatic reactions or inhibit undesirable changes by inactivation of enzymes. Temperature and time are two parameters responsible for the effects of a thermal treatment. Both should be selected carefully to ensure the desired reactions take place but the undesired reactions are kept to a minimum. Contrary to common chemical reaction, enzyme-catalyzed reactions as well as growth of microorganisms show a so-called temperature optimum, which is a temperature-dependent maximum resulting from the overlapping of two counter effects with significantly different activation energies: increase in reaction or growth rate, increase in inactivation or killing rate (Belitz et al., 2004). For example, starch hydrolysis by an α-amylase enzyme results in two different activation energies (E_a): E_a (hydrolysis) = 20 kJ/mol, and E_a (inactivation) = 295 kJ/mol. Because of the difference in activation energies, the rate of enzyme inactivation in substantially faster with increasing temperature than the rate of enzyme catalysis (Belitz et al., 2004). It is important to maintain an optimum temperature during an experiment for the hydrolysis to sufficiently effect changed attributes before increasing the temperature to inactivate the enzyme and stop the reaction entirely. An enzyme reaction is time and temperature dependent.

Since temperature is a crucial factor in enzyme activity, pressure ultimately is as well. The application of high pressure can inhibit the growth of microorganisms and the activity of enzymes. In contrast to thermal treatment, high pressure does not attack the primary structure of proteins at room temperature. Only H-bridges, ionic bonds, and hydrophobic interactions are disrupted (Belitz et al., 2004). The hydration of proteins is

also changed by high pressure because water molecules are pressed into cavities which can exist in the hydrophobic interior of proteins. In general, proteins are irreversibly denatured at room temperature by the application of pressure above 300 MPa while lower pressures cause only reversible changes in the protein structure (Belitz et al., 2004). In the case of enzymes, even slight changes in the steric arrangement and mobility of the amino acid residues which participate in catalysis can lead to loss of activity (Belitz et. al, 2004). Nevertheless, a relatively high pressure is often required to inhibit enzymes.

1.4.3 Enzymes to apply to cinnamon for viscosity reduction

Most of the enzyme properties are clearly and reliably revealed only with purified enzymes. Extraction of enzymes requires the disintegration and homogenization of biological tissue usually accompanied by a buffer which protects the enzymes from oxidation and traces of heavy metal ions. The crude enzyme isolate is then purified by a stepwise process such as fractional precipitation by column gel chromatography, then by ion exchange chromatography and further options may be various forms of preparative electrophoresis. Another more efficient method is affinity column chromatography where the enzyme is selectively and reversibly bound to the stationary phase through a substrate or an inhibitor thus, in contrast to the other proteins, its elution is delayed (Belitz et al., 2004). Each enzyme is unique based on its origin and specificity.

Enzymes can be classified based on their reaction specificity and are given an Enzyme commission (EC) number which is compiled of 4 numbers. All enzymes are classified into six major classes according to the nature of the chemical reaction catalyzed. Each class is then subdivided into subclasses which more specifically denote the type of

reaction. Then each subclass is divided into sub-subclasses. Lastly, they are given a serial number of the enzyme within the sub-subclasses which provide a systematic name or EC number resulting in 4 divided numbers. One application is for example Ascorbase which is classified as an oxidoreductase (1), donor naming: diphenols or ascorib acid (10), naming the acceptor: oxygen (3), serial number (3), resulting in E.C. 1.10.3.3. Since enzymes of different biological origin often differ in their properties, the source and, when known, the subcellular fraction used for isolation are specified in addition to the name of the enzyme preparation, (Belitz et al., 2004).

Enzymes of interest for applications on cinnamon include Endo -1, 3 (4)-β-D-Glucanases otherwise known as β-Glucanases, Pectinases, Cellulases and Xylanases due to the polysaccharides that contribute to viscosity of cinnamon as described in section 1.2.2.1. Most of the enzymes used in the food industry belong to the third class, hydrolases, of which these enzymes belong (Belitz et al., 2004). Hydrolysis may be described as transfer of the components of the substrate to water (Whitaker, 1994). An example of a hydrolysis reaction is represented in Figure 12.

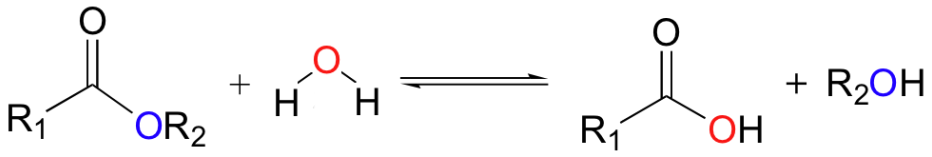


Figure 12: Generic mechanism of a hydrolysis reaction.

These enzymes of interest for cinnamon have an E.C. number starting with 3.2.1 which the 3 means they are hydrolases and the 2.1 means they act on glycosyl bonds (Whitaker, 1994). The glycoside bond is represented by the central oxygen atom, which holds the two monosaccharide units together as seen in Figure 13.

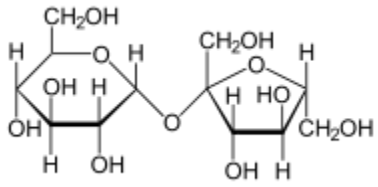


Figure 13: Sucrose molecular structure. The glycoside bond is represented by the central oxygen atom, which holds the two monosaccharide units together.

Sucrose is an example of a disaccharide meaning two monosaccharides, but there may be many disaccharide units linked together by glycoside bonds to form a chain, a polysaccharide, such as cellulose or form in more of a 3-dimensional network such as pectin. Polysaccharides such as β-Glucan, Pectin, Cellulose and Xylanase all have links of glycoside bonds, thus hydrolase enzymes in the endoglucanase enzyme subclass (endoglucanases cleave the middle of the polymer chain or network instead of the ends), will theoretically be the most effective in cleavage of the polysaccharides present in cinnamon which in turn will reduce entanglement resulting in an overall reduced viscosity. However, the sub-subclasses, biological origin, and the subcellular fraction used for isolation may all contribute to varying specificity and effectiveness even though they belong to the same class (hydrolases) and subclass (endoglucanases).

A study was conducted by David Ibarra, et al. (2010), using hardwood (eucalyptus) and softwood (mixture of Norway spruce and Scots pine) dissolving pulps which were

enzymatically treated with endoglucanases, and cellulases to enhance the accessibility and reactivity for viscous production. The effects of the enzymatic treatments were quantified by reactivity, viscosity measurements, and molecular weight distribution. The result was up to a 50% viscosity reduction and a 100% increase in cellulose reactivity. The weight-average degree of polymerization (DPw) was reduced as well proving the enzymes are cleaving the polymers. If cellulases and endoglucanases can be successful in viscosity reduction for hardwoods and softwoods such as Eucalyptus, Norway spruce and Scots pine, perhaps they will be successful for cinnamon since cinnamon is ground bark from a tree.

Enzymes have varying outcomes depending on the enzyme selection even if it is the same class. For example, in the study described, three different endoglucanase cellulases were used for enzymatic treatment but all had varying degrees of success. It was found that one enzyme of this class did not have nearly as much success as the other two. These varying results of success may be due to differing sub-subclasses, biological origin, and/or the subcellular fraction used for isolation of the enzymes. It will be a challenge selecting the appropriate enzymes, especially specific sub-subclasses for cinnamon viscosity reduction since the polymers have not been characterized yet and one can only act based on theoretically assumption. However, the results as to if the enzymes are successful in viscosity reduction, or not, give clues as to the structure of polymers that make up cinnamon. Enzymes are very selective and if known what works to break up the polymers in cinnamon it is an indication as to what polymers are in cinnamon.

1.5 Other methods to reduce viscosity

1.5.1 Acid hydrolysis

Another method to break down carbohydrate polymers is acid hydrolysis. A study conducted by Yao-Bing Huang and Yao Fu in 2013, used acid hydrolysis of different wood types investigating an effort to find a sustainable energy source. The result was lignocellulose was broken down into cellulose polymers of which were further broken down into glucose or oligosaccharides successfully. However, the mineral acids used, hydrochloric acid (HCl) and sulfuric acid (H_2SO_4) suffer from problems of product separation, reactor corrosion, poor catalyst recyclability and the need for treatment of waste effluent (Huang et al., 2013). The use of heterogeneous solid acids was also explored, but with very little glucose outcome (Huang et al., 2013). It is not a strategic polymeric cleavage method, but random. It is a very likely method to react and reduce viscosity but that would also mean it would react with everything including crucial flavor and aroma aromatics such as cinnamaldehyde and antioxidants in cinnamon. Acid hydrolysis of cinnamon may be similar to this study since cinnamon may also be considered a wood and may be the most likely to be successful in polymer cleavage. For this reason, acid hydrolysis would be a good model to use as a control, but may have impact to flavor and color resulting in undesirable attributes in food.

1.5.2 Irradiation treatment

Macromolecule polysaccharides such as starch, pectin and cellulose have shown to be degraded by ionizing radiation, otherwise known as irradiation (Heide et al., 1990). In a study where 10 different lots of cinnamon were subjected to 10 kGy of radiation the

viscosities were dramatically decreased by roughly 75% or more. However; this method could also degrade essential oils or antioxidants present in cinnamon and may cause off flavors and aromas. Also, consumer perception of irradiation is a controversial topic and consumers still are uncertain of the technology (Satin, 1996). This treatment method is not widely accepted as a clean label process (Clean Label Conference, 2015).

1.6 Cinnamon Analysis Methods

1.6.1 Light microscopy

One of the tools essential for studying microstructures is the bright-field or compound microscope. A compound microscope consists of a series of lenses and has widespread application. Bright-field microscopy is the simplest of all the optical microscopy illumination techniques. Sample illumination is transmitted (i.e., illuminated from below and observed from above) white light and contrast in the sample is caused by absorbance of some of the transmitted light in dense areas of the sample. Certain changes of the bright field system have been developed to increase the effectiveness of investigations of microstructures such as phase-contrast and dark-field instruments (G. Wistreich, 2003). In phase-contrast microscopy, the contrast between a transparent specimen and the surrounding background is increased while the transparency of the specimen remains unchanged (G. Wistreich, 2003). In dark-field microscopy, the image is formed from diffracted, reflected light, and specimen contrast against a dark background is thereby increased (G. Wistreich, 2003). The result is a brilliant image against a dark background in which minute structures usually too small to be observed directly are visible (G. Wistreich, 2003).

The resolving power of a microscope is inversely related to the wavelength of light used; that is, the shorter the wavelength, the greater the resolving power. Electron microscopy uses a beam of electrons used as the source of illumination and is focused by electromagnetic lenses instead of optical lenses. This electron beam has a considerably shorter wavelength than ordinary light and far exceeds the resolving power compared to a compound microscope if needed. Light microscopy which uses visible or ultraviolet light has a resolution of 100-300 µm depending on the method used (i.e. dark-field, phase contrast, bright-field) where electron microscopes are 0.2-10.0 µm (G. Wistreich, 2003). This amount of resolution may not be needed if observations can be made with a compound microscope.

1.6.2 Rapid visco analyzer (RVA)

There are many types of analytical ways to measure viscosity. The main one is a rotational rheometer/viscometer which have varying features. The principle of rotational rheometers with coaxial cylinder-, cone and plate and parallel plate sensor systems, allows the design of excellent and versatile absolute rheometers (G. Schramm, 1994). The temperature can also be controlled using a rotational rheometer. There are two basic methods one of which controls the stress input and determines the resulting shear rate and the other controls the shear rate input and determines the resulting shear stress (G. Schramm, 1994). Then there are two basic measuring systems one called Searle and the other Couette. Searle means that both the drive on the rotor and the torque detector act on the same rotor axis. The Couette design means that the drive acts on the outer cup while the viscosity-related torque is measure on the shaft on the inner cylinder. Couette

type measuring systems maintain laminar or constant flow, even when low viscosity liquids are tested at high shear rates. Searle type rheometers run into problems with low viscosity liquids at high shear rates since under those conditions the laminar flow may cause turbulence resulting in erroneous results (G. Schramm, 1994). Cinnamon in solution is a highly viscous substance where maintaining constant flow will most likely not be an issue.

The best option for this study to see the cinnamon degradation comparable to processing conditions is utilizing a Searle measuring system and a constant rate viscometer. This will allow viscosity comparisons to be made over time based on the constant rate being applied to the substances in the coaxial cylinder, or cup. The main apparatus is the inner cylinder, which may be a paddle, cone or rotor, is driven by a motor. Its speed is controlled for constant or programmed rotor speeds while the other cylinder, or cup, is held at rest. The cup is jacketed which is connected to an external thermal liquid temperature bath and circulator for an accurate temperature control of the sample. The driven inner cylinder forces liquid in the gaps to flow. The resistance of the liquid being sheared between two stationary and rotating boundaries of the sensor system results in a viscosity related torque working on the inner cylinder which counteracts the torque provided by the drive motor (G. Schramm, 1994). A torque detector, normally a spring that twists as a result of the torque applied, is placed between the drive motor and the shaft of the inner cylinder. The twist angle (0.5°) of the torque spring is a direct measure of the viscosity of the sample (G. Schramm, 1994).

1.6.3 Dietary soluble and insoluble fiber approach

Gravimetric methods are the methods of choice for determination of dietary fibers (Belitz et al., 2004). The sample is defatted, then the digestible components (1, 4-α-glucans, proteins) are enzymatically hydrolyzed (heat-stable α-amylase, glucoamylase, protease). The sample then goes through centrifugation where the insoluble fibers remain in the residue and the water-soluble fibers in the supernatant are isolated by precipitation with ethanol, ultrafiltration or dialysis. The protein and mineral matter remaining with the soluble and insoluble dietary fibers is deducted with the help of correction factors (Belitz et al., 2004).

1.6.4 High Pressure Liquid Chromatography – Size Exclusion Chromatography (HPLC-SEC)

Skoog explains that chromatography encompasses a diverse and important group of methods that allow the separation, identification, and determination of closely related components of complex mixtures; many of these separations are impossible by other means (Skoog et al., 2007). In all chromatographic separations, the sample is dissolved in a mobile phase, which may be a gas, a liquid, or a supercritical fluid. Skoog goes on to say that this mobile phase is then forced through an immiscible stationary phase, which is fixed in place in a column or on components of the sample distribute themselves between the mobile and stationary phases to varying degrees (Skoog et al., 2007). Those components strongly retained by the stationary phase move only slowly with the flow of mobile phase. In contrast, components that are weakly held by the stationary phase travel rapidly. As a consequence of these differences in migration rates, otherwise known as

elution times, sample components separate into discrete bands, or zones, that can be analyzed qualitatively and quantitatively (Skoog et al., 2007).

Liquid chromatography (LC) and gas chromatography (GC) are separation techniques where molecules elute off columns based on size. A detector that responds to solute concentration is placed at the end of the column and the signal is plotted as a function of time. A series of peaks is obtained resulting in a plot called a chromatogram. The positions of peaks on the time axis can be used to identify the components of the sample. The areas under the peaks provide a quantitative measure of the amount of each component. However, it is important to note that, although chromatograms may not lead to positive identification of species present in a sample, they often provide sure evidence of the absence of certain compounds. Thus, if the sample does not produce a peak at the same retention time as a standard run under identical conditions, it can be assumed that the compound in question is either absent or is present at a concentration level below the detection limit of the procedure (Skoog et al., 2007). For the purposes of this study it is more important to show differences in samples vs. determining what the components are for proof that cinnamon degradation took place. This may include but not be limited to peak areas and peak placement at certain times. There may be peaks that appear, disappear, or increase area, decrease area depending on if degradation took place.

1.7 Conclusion

To solve for viscosity issues that arise during processing with cinnamon as a component due to large polymers entangling with one another, acid hydrolysis will be a model;

however, enzyme hydrolysis is the best approach for practical applications in food systems. Enzyme hydrolysis is the best approach to solving viscosity issues that arise when using cinnamon while pleasing health conscience consumers at the same time.

From all the soluble and insoluble polysaccharides that may be present in cinnamon, focusing on hydrolysis of cellulose, arabinoxylan, β-glucan and pectin is the best chance at a viscosity reduction based on section 1.2.2.1. Therefore, enzymatic treatment utilizing a cellulase, xylanase, β-glucanase and pectinase is appropriate.

Viscosity reduction using Rapid Visco Analyzer (RVA) methods and molecular weight distribution by high pressure liquid chromatography (HPLC) as well as soluble and insoluble dietary fiber methods will be used to quantify success. Microscopy data will verify success and be qualitative information.

2. Materials and Methods

2.1 Creep Compliance Sample Preparation and Testing Procedure

The cinnamon used throughout the study was a ground, Korintjie cinnamon which had been cleaned by steam treatment. This cinnamon was used to make sample slurries for the texture analysis test. The formulas can be seen in Table 2 where there is a control sample which has no cinnamon, and then 2%, 5%, and 7% cinnamon based on percent solids of cinnamon in the formula. The slurries were boiled on a stovetop to a glassy state, control was boiled to 154 °C (310°F) and the cinnamon samples to 129˚C (265˚F). The liquid was poured into molds to harden and form a candy or confectionary phase where they were stored under desiccant for a minimum of 48 hours resulting in an approximate 3% moisture measured by Karl Fischer titration. These candies were then subjected to a texture analysis.

Sample	Water (%)	Sugar (%)	Canola Oil High Oleic (%)	Corn Syrup 36DE (%)	Cinnamon (%)	% solids of cinnamon in formula
Control (no cinnamon)	17.26	66.13	3.23	13.38	0	0
2% Cinnamon	16.61	64.68	3.16	13.09	2.46	2.05
5% Cinnamon	15.69	62.71	3.07	12.69	5.86	5.04
7% Cinnamon	14.99	61.66	2.99	12.36	8.00	7.07

Table 2: Slurry formulas utilizing various levels of cinnamon for texture analysis testing

A creep compliance test using texture analysis was conducted to understand how cinnamon is impacting visco-elastic properties in a slurry matrix. A TA.XT Plus equipped with a 30kg load cell utilizing Exponent software (Texture Technologies, Hamilton, MA) was used. A creep compliance test using Exponent software was used with modifications to create the method. The samples were held in a candy press while a 5-mm cylinder stainless steel probe software (Texture Technologies, Hamilton, MA), came down at a rate of 0.1mm/sec until desired depth of 1mm (0.175mm for control) was reached. Adjustments had to be made to the depth and trigger force applied to the control sample vs. the cinnamon samples otherwise all the control samples would fracture and creep compliance data could not be collected. Three candy pucks, measured three times in different spots on the candy puck for a total of nine trials per sample type were measured.

2.2 Viscosity Testing Procedure

Viscosity of cinnamon samples were measured using a Rapid Visco Analyzer (RVA 4500, Perten Instruments, Springfield, IL.) Calibration and sample viscosity measurements utilized an uncoated aluminum RVA can (NCI Packaging Ltd. Upper Hutt, New Zealand) and double skirted plastic paddle (PPC Moulding Services, Villawood NSW, Australia). A new can and paddle were used for each sample and calibration took place before each sample measurement. Samples were run in triplicate. The solution contents were added to the aluminum can and inserted into the RVA. The paddle speed

for the test was 960 rpm for 10 seconds to mix the sample, then 160 rpm for the remainder of the test. The temperature was held at 50˚C for 10 minutes, then ramped to 82˚C and held for the remaining 16 minutes. The total time for the RVA test was 26 minutes. After which the viscosity versus temperature profile was analyzed utilizing Thermocline for Windows (TCW) software (Perten Instruments, Hägersten, Sweden).

2.3 Methods of Sample Preparation

2.3.1 Sample preparation utilizing control cinnamon

The cinnamon used throughout the study was a ground, Korintjie cinnamon which had been cleaned by heated steam and packed (McCormick & Company, Sparks, MD). The control sample was prepared by adding 6.25 grams of cinnamon to an uncoated, aluminum RVA can (NCI Packaging, Upper Hutt, New Zealand). Then 18.75 grams of deionized (DI) water was added to the can and stirred using the plastic double skirted paddle (PPC Moulding Services, Villawood NSW, Australia) by hand until the cinnamon was incorporated into solution. This resulted in 25 total grams in the can where cinnamon was 25% of the solution and DI water is 75%. The can and paddle were inserted into the RVA for testing. This was repeated in triplicate. The samples were refrigerated at 4.4°C and kept for further analysis.

2.3.2 Sample preparation of treated cinnamon utilizing combination of cellulase, xylanase, pectinase enzymes provided by Sigma Aldrich

The sample was prepared by adding 18.44 grams of DI water to a 50-mL plastic centrifuge tube with a screw cap (VWR International, Radnor, PA) and placed in a water bath held at 50˚C. Using a temperature probe, once the temperature of the DI

water reached 50˚C the enzymes were added to the centrifuge tube. 0.103 grams of cellulase from *Aspergillus niger*, EC#3.2.1.4, powder (Sigma Aldrich, St. Louis, MO) was added, 0.103 grams of xylanase from *Thermomyces lanuginosus*, EC#3.2.1.8, powder (Sigma Aldrich, St. Louis, MO) was added, and 0.103 grams of pectinase from *Aspergillus niger* EC#3.2.1.15 in an aqueous glycerol solution (Sigma Aldrich, St. Louis, MO) was added. The enzymes were incorporated by gently stirring the tube by hand for 5 seconds. All the enzymes had been stored under refrigeration at 4.4°C hours prior to use.

The cinnamon was measured (6.25 grams) and added to the RVA can. The enzyme solution was poured from the centrifuge tube into the RVA can containing the cinnamon. This resulted in 25 total grams in the can where cinnamon was 25.00% of the solution, and the enzymes were 1.24% and DI water is the remaining 73.76%. The RVA can and paddle were inserted into the RVA for testing. The sample was transferred to a 50ml centrifuge tube with a screw cap (VWR International, Radnor, PA) and refrigerated at 4.4°C for 48 hours and kept for further analysis. This process was repeated in triplicate.

2.3.3 Sample preparation of treated cinnamon utilizing β-glucanase enzyme provided by Novozymes

The samples were prepared by adding 6.25 grams of cinnamon to an RVA can. Then 18.44 grams of DI water was added to the can. After which, 0.31 grams of β-glucanase (endo-1,3 (4)-) from *Aspergillus aculeatus* EC#3.2.1.6 provided by Novozymes (Bagsvaerd, Denmark) was added to the RVA can. This resulted in 25 total grams in the can where cinnamon was 25.00% of solution, the enzyme is 1.24% and DI water is the

remaining 73.76%. The enzyme had been stored under refrigeration at 4.4°C prior to use. It was stirred using the paddle by hand until the cinnamon was incorporated into solution. Then the can and paddle were inserted into the RVA for testing. The sample was transferred to a 50ml centrifuge tube with a screw cap (VWR International, Radnor, PA) and refrigerated at 4.4°C for 48 hours and kept for further analysis. This process was repeated in triplicate.

2.3.4 Sample preparation of treated cinnamon utilizing acid

The sample was prepared by adding 6.25 grams of cinnamon to an RVA can. Then 18.44 grams of DI water was added to the can. After which, 0.5 grams of 10 N hydrochloric acid (J.T.Baker, Center Valley, PA) was added to the RVA can. It was stirred using the paddle by hand until the cinnamon was incorporated into solution. More acid was added to solution compared to the enzyme methods in hopes to push the boundaries of degredation of cinnamon in comparison. The pH was 1, determined by a pH test strip dipped into the solution. This resulted in 25.19 grams in the can, cinnamon being 24.82%, acid being 1.98% and DI water being the remaining 73.20%. The can and paddle were inserted into the RVA for testing. To neutralize the solution, 0.5 grams of 10 N sodium hydroxide (Sigma Aldrich, St.Louis, MO) was added. The neutral pH of 7 was determined by a pH test strip dipped into the solution. After which the solution resulted in 25.69 grams where the solution was 24.32% cinnamon, 1.95% acid, 1.95% base, 71.78% DI water. This process was repeated in triplicate. The samples were refrigerated at 4.4°C for 48 hours and kept for further analysis.

For simplicity purposes the enzymes used are given codes in Table 3 and the final compositions after sample preparations can be seen in Table 4.

Code	**EC #**	**Type**
C	3.2.1.4	Cellulase
X	3.2.1.8	endo-1,4-β-xylanase
P	3.2.1.15	Pectinase (endopolygalacturonase)
B	3.2.1.6	endo-1,3(4)-β-glucanase

Table 3: Enzymes used for treatment of cinnamon

Sample	**Cinnamon (g)**	**%**	**DI Water (g)**	**%**	**Enzyme (g)**	**%**	**Acid (g)**	**%**	**Base (g)**	**%**
Control	6.25	25.00	18.75	75.00	0	0	0	0	0	0
CXP	6.25	25.00	18.44	73.76	0.309*	1.24	0	0	0	0
B	6.25	25.00	18.44	73.76	0.31	1.24	0	0	0	0
Acid	6.25	24.82	18.44	73.2	0	0	0.5	1.98	0	0
Base addition	6.25	24.32	18.44	71.78	0	0	0.5	1.95	0.5	1.95

*= (0.103 of each)

Table 4: Composition of samples (control, CXP, B, acid with base addition) based on different preparation methods

2.4 Testing Procedures

2.4.1 Light Microscopy Analysis

The samples prepared as in sections 2.3.1, 2.3.2, 2.3.3, and 2.3.4 were further analyzed after the RVA procedure utilizing an Olympus AX70 light microscope with a 20X objective lens (Olympus Scientific Equipment Group, Center Valley, PA). The samples were rapidly frozen on a cold plate to prevent ice crystal damage and cut into 10 mm

diameter, 16-micron cross section disks utilizing a Leica CM1950 cryostatic microtome (Leica Biosystems Inc., Buffalo Grove, IL). The samples were transferred onto glass slides and a drop of mineral oil (EMD Millipore, Billerica, MA) was used as a mounting media. Each slide was imaged in transmitted and polarized light. Sample cross sections were stained with a drop of dilute 0.1 N iodine (Alfa Aesar, Wardhill, MA) 1:10 (v/v) in DI water and imaged using transmitted light. The iodine colored the starch blue-purple which allows for changes in the starch structure to be observed by light microscopically (Flint. O., 1994).

2.4.2 Soluble and Insoluble Fiber Analysis

This method measures the total dietary fiber as defined by the CODEX Alimentarius Commission, by Codex soluble and insoluble fiber analysis (AOAC method 2011.25). Dietary fiber is defined as non-digestible polysaccharides of DP=3 or higher for this method. This method followed the RVA procedure for all samples. Homogenous samples were digested at 37°C for 16 hours in 2-(N-Morpholino) ethane sulfonic acid hydrate (MES) (Sigma-Aldrich, St. Louis, MO), pH 6.1 buffer, with constant shaking utilizing a New Brunswick Scientific Innova 44 Incubator Shaker Series (Eppendorf International, Hamburg, Germany) in the presence of porcine pancreatic α-amylase E.C. 3.2.1.1 and amyloglucosidase E.C. 3.2.1.3 (Megazyme International, Bray, Co. Wicklow, Ireland) which digest non-dietary fiber polysaccharides. After briefly boiling the samples to inactivate the enzymes, cooled to room temperature and pH adjusted with Tris (hydroxymethyl) aminomethane (Sigma-Aldrich, St. Louis, MO), further enzymatic digestion with *Bacillus licheniformis* protease (Subtilisin A) E.C. 3.4.21.62 (Megazyme

International, Bray, Co. Wicklow, Ireland) was performed with stirring at 60ºC to degrade proteins.

To determine insoluble dietary fiber (IDF) and soluble dietary fiber (SDF), the enzyme digested sample solution was then centrifuged using an Avanti J-26XPI (Beckman-Coulter Inc., Chaska, MN) at 81,800 x g for 30 min at 15ºC in a 50-mL tube to separate the IDF (pellet) from the SDF (supernatant). The supernatant was aspirated off the top of the IDF pellet and transferred to a 500-mL centrifuge bottle where it was brought to a concentration of 85 % reagent alcohol (200 proof reagent alcohol ACS grade, 95% ethanol: 5% isopropanol) (Pharmco-AAPER, Shelbyville, KY) and chilled for at least 3 hours at -20ºC to precipitate soluble dietary fiber. After the 3-hour precipitation of the soluble fiber, the mixture was centrifuged at 13,700 x g for 30 min at 4ºC. The supernatant containing low-molecular-weight resistant oligosaccharides was removed from the SDF pellet by aspiration. The SDF pellet was de-fatted by washing with 2:1 (v/v) acetone/methanol solution. The IDF pellet was de-fatted by washing with a 2:1 (v/v) acetone/methanol solution with stirring at 60ºC. The de-fatted IDF was centrifuged using an Avanti J-26XPI (Beckman-Coulter Inc., Brea, CA) 81,800 x g for 30 min at 25ºC outfitted with an inert nitrogen atmosphere to minimize flammability. The organic solvent supernatant containing the fat was aspirated off the SDF and IDF pellets and the remaining SDF and IDF were placed in separate flasks and inserted in a VirTis Freezemobile Shell Bath Freezer (SP Scientific, Warminster, PA) where the flasks containing the samples were in a rotational bath containing 200 proof ethanol held at -80°C until the samples were frozen. The frozen samples were dried using a VirTis Sentry 2.0 freezemobile 35EL Freezer (SP Scientific, Warminster, PA) for 16 hours to a

moisture of 0%. The freeze-dried SDF and IDF were separately homogenized and separately transferred to caped storage tubes. One aliquot of each was assayed for total protein by Dumas combustion and the remaining was assayed for total ash. The protein, ash and blank control results are subtracted from the dried IDF weight to give the IDF in the original sample. The protein, ash, and blank control results from the SDF sample are subtracted from the dried IDF weight to give the SDF in the original sample. The IDF and the SDF values can be summed to give a calculated total dietary fiber value.

2.4.3 High pressure liquid chromatography (HPLC) system using size exclusion chromatography (SEC) Method

The molecular weight distribution was determined utilizing a high-pressure liquid chromatography (HPLC) system using size exclusion chromatography (SEC) or to be referred to as SEC-HPLC. SEC-HPLC was performed on a Waters e2796 AllianceBio chromatography system equipped with a Bischoff RI detector. The samples were analyzed after the RVA procedure. Samples were mixed with a minimum volume of DI water for 10 minutes at room temperature with a stir bar and magnetic stirrer. The sample mixture was transferred to a centrifuge tube, and spun on a Beckman Coulter (Brea, CA) centrifuge at 5,300 RPM for 20 minutes at 18°C. An aliquot of supernatant was removed and diluted 1:2 with DI water then filtered through a 0.22 µm 13 mm syringe filter membrane into HPLC vials for analysis.

The molecular weight determination was performed on a Shodex OHpak guard column with a Shodex SB-806m HQ and Waters Ultrahydrogel Linear column in series at 60°C with DI water at a flow rate of 0.5 ml/min. The percent insoluble fraction was

determined by SEC-HPLC on the Shodex-Waters columns based on the response of the insoluble fraction to a glucose standard. The calibration of the Shodex-Waters Columns can be seen in Figure 14.

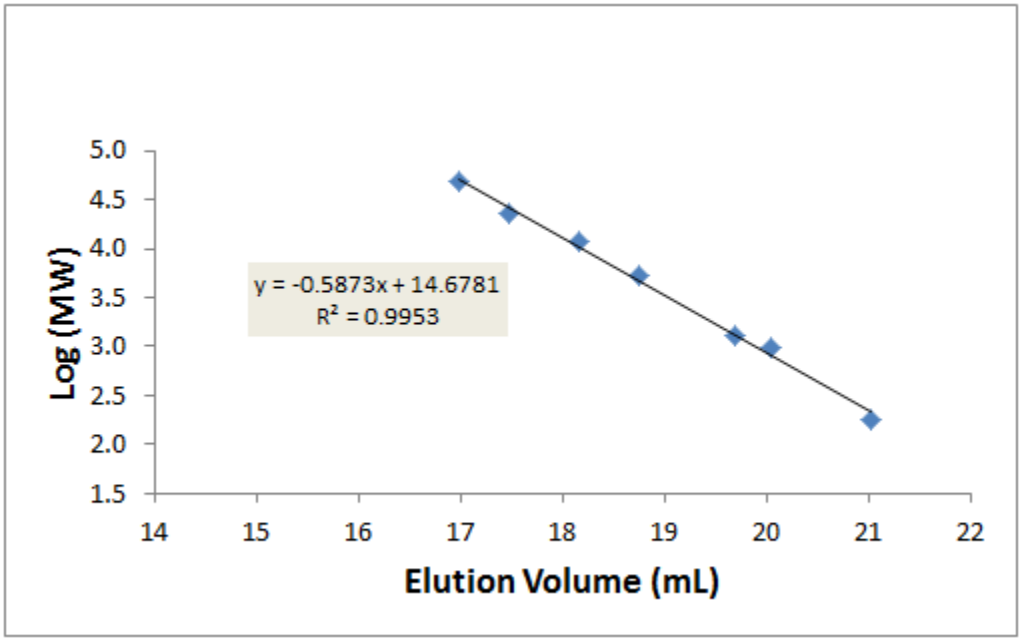


Figure 14: SEC-HPLC Calibration of standards of the Shodex-Waters Columns (SB806m HQ and Ultrahydrogel).

The saccharide and dextran standards were purchased from Sigma- Aldrich (St. Louis, MO) at >99.5% purity. DI water was generated by a Purelab Ultra filtration unit as needed. Samples and standards were weighed on a Mettler Toledo balance, and dispensed into class A volumetric flasks and diluted to volume with DI water where applicable.

3. Results and Discussion

3.1 Creep compliance of cinnamon

Creep compliance is the measure of how well a material comes to equilibrium when a force is applied as discussed earlier in section 1.2.3.2. The relaxation rates of the candies consisting of different percentages of cinnamon can be seen in Figure 15 and the exact values can be seen in Table 5.

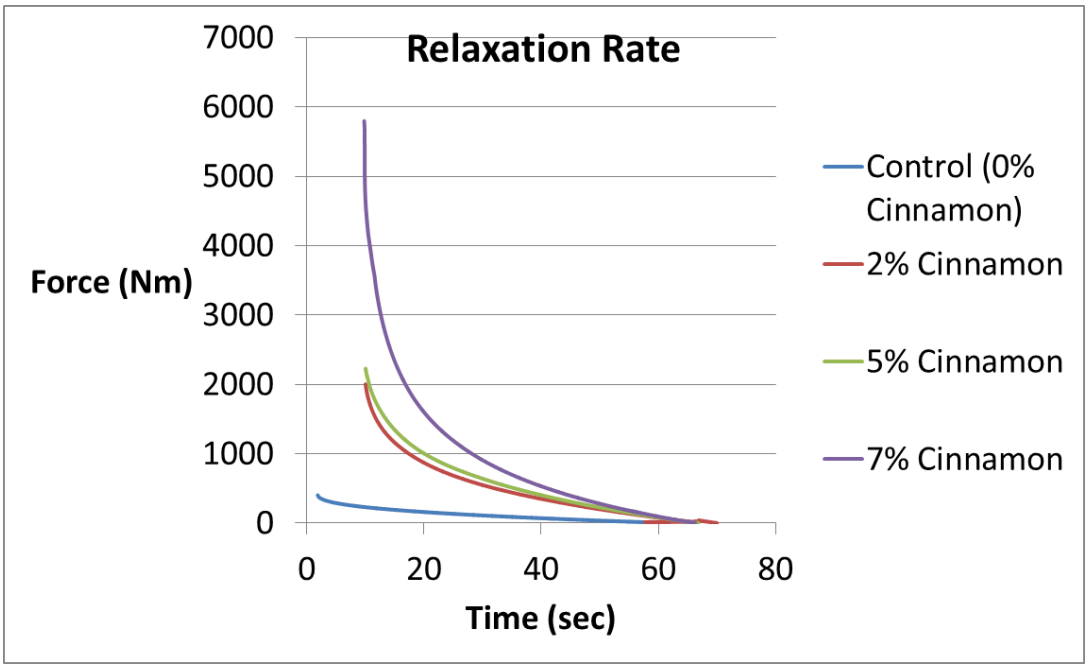


Figure 15: Relaxation rate of candies made with varying percentages of cinnamon (U.S. Patent App. No. GMI7872US01, 2016).

Sample	Depth (mm)	Trigger force (Nm)	Number of fractures (N=9)	Average area (g*sec)	Maximum force (Nm)	Moisture (N=1)
Control (No Cinnamon)	0.175	14.7	0	7,185 ± 1,624	403.59 ± 101.48	4.33%
2% Cinnamon	1	29.4	1	48,094 ± 18,800	2004.05 ± 752.60	2.50%
5% Cinnamon	1	29.4	0	54,546 ± 26,241	2227.62 ± 1043.64	3.43%
7% Cinnamon	1	29.4	1	106,723 ± 37,993	5796.55 ± 3823.31	2.75%

Table 5: Texture Analyzer results of candies made with varying percentages of cinnamon

The results show that the addition of cinnamon increased the toughness of the candies (U.S. Patent App. No. GMI7872US01, 2016). It is also apparent that the toughness of the candy samples corresponds to the amount of cinnamon addition to the candy pucks meaning the more cinnamon the tougher it is.

The results imply that cinnamon incorporated into the glass candies are changing the viscoelastic properties making them strong, yet flexible. The maximum on the graph is the storage modulus meaning the initial force it can withstand and implies how brittle or stiff a material is. The greater the maximum the stiffer the material. The slope is the compliance or the creep recovery meaning how soft or malleable the material is. Cinnamon incorporated into a glass can withstand the initial force meaning it is very stiff, then it can relax and come to equilibrium with that force meaning it is malleable. This demonstrates that it is stiff yet amorphous material resulting in a high creep compliance. The combination of stiff yet malleable characteristics is described as tough; strong yet flexible.

The control samples with 0% cinnamon had a low creep compliance because it is not able to relax against the force being applied nearly as well as the sample having 7% cinnamon which had the greatest creep compliance. The control sample also was not able to withstand the same force as the samples with cinnamon in them meaning the cinnamon adds strength to the slurry. Refer to the appendix for further information on this subject matter.

This physical behavior pertaining to creep compliance for cinnamon may be due to the complex network of polymers present in cinnamon discussed in further detail in section 1.3.3. It is possible that the polymer network may be rearranging to absorb the force being applied by the probe giving cinnamon its flexible yet strong characteristic. For example, bond interchange is going on constantly in polysiloxanes with or without stress; however, in the presence of stress the statistical rearrangements tend to reform the chains so that the stress is reduced (Sperling, 2006, 1992).

3.2 Microscopy Comparisons

Microscopy methods are used to see possible differences to insoluble tissue that other analytical methods are not able to capture. The insoluble tissues did not show a change from any of the treatment methods and look similar to control. Birefringent, fibrous structures remain unchanged in the transmitted light microscopy cross section in oil which can be seen in Figures 16, 17, 18, 19 and polarized light microscopy cross section in oil methods which can be seen in Figures 20, 21, 22, 23.

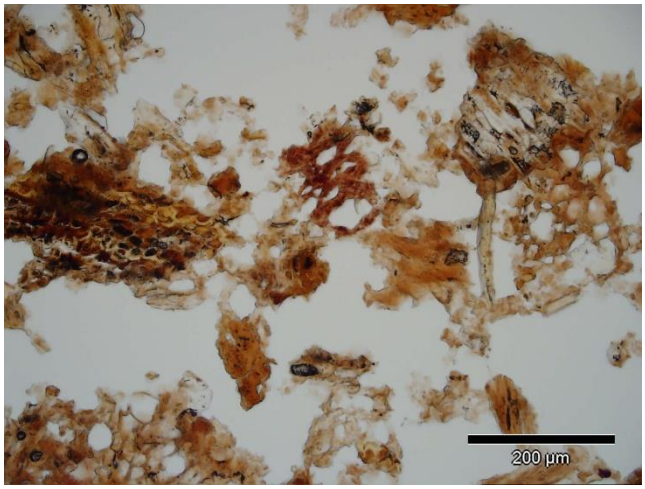


Figure 16: Cinnamon Control Trial 3 sample cross section in oil utilizing transmitted light microscopy.

Figure 17: Cinnamon with CXP enzyme treatment trial 3 sample cross section in oil utilizing transmitted light microscopy.

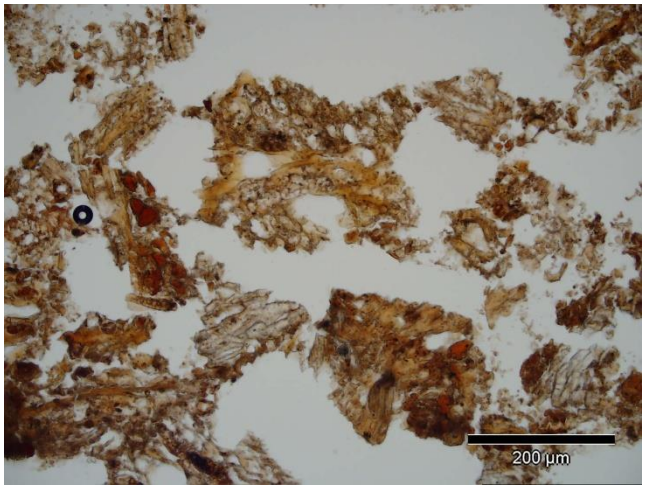


Figure 18: Cinnamon with B enzyme treatment trial 3 sample cross section in oil utilizing transmitted light microscopy.

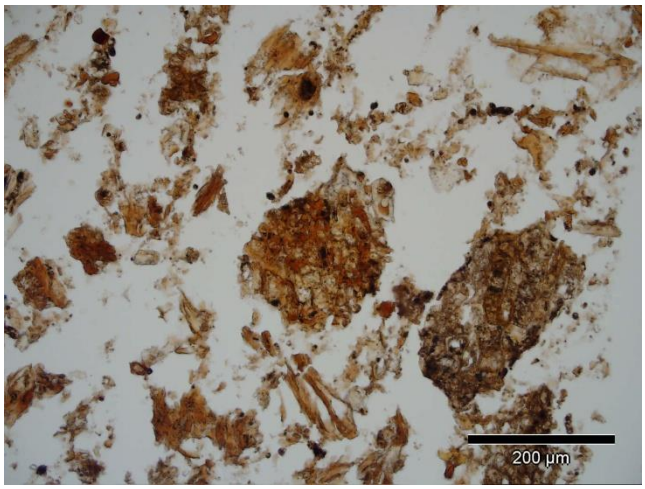


Figure 19: Cinnamon with acid treatment trial 3 sample cross section in oil utilizing transmitted light microscopy.

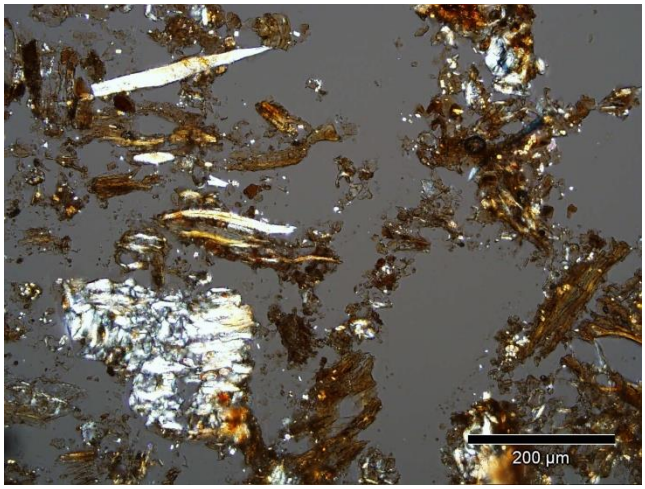


Figure 20: Cinnamon control trial 3 sample cross section in oil utilizing polarized light microscopy.

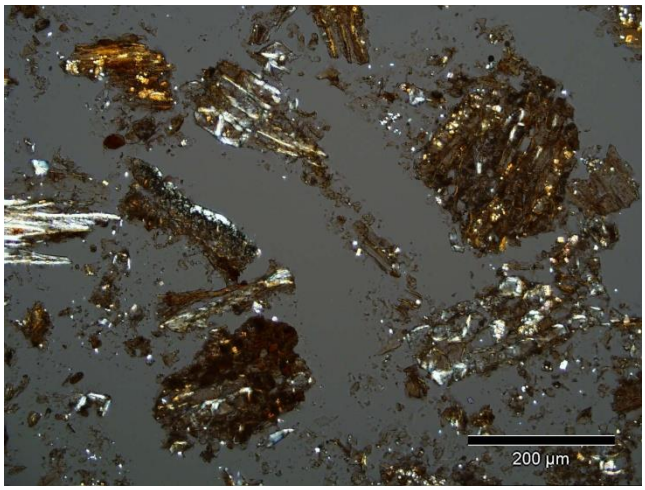


Figure 21: Cinnamon with CXP enzyme treatment trial 3 sample cross section in oil utilizing polarized light microscopy.

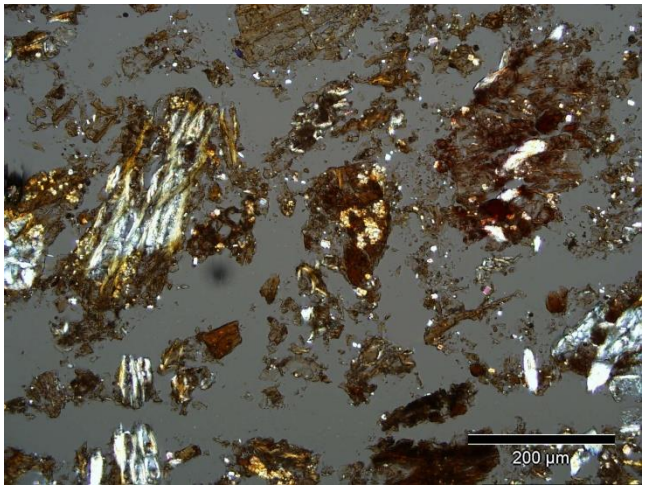


Figure 22: Cinnamon with B enzyme treatment trial 3 sample cross section in oil utilizing polarized light microscopy.

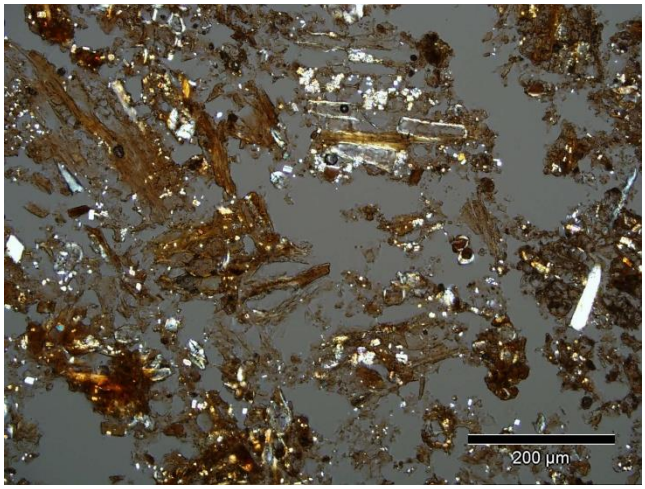


Figure 23: Cinnamon with acid treatment trial 3 sample cross section in oil utilizing polarized light microscopy.

The light microscopy starch stained with aqueous dilute 0.1 N iodine method showed that the starch was partially digested in the acid treatment and was sticking to the other components which can be seen in Figure 27. This digestion was represented by a purple haze seen in the acid treatment sample, Figure 27, but the enzyme treatments as well as control did not show change to the starch which are represented by purple spheres seen in

Figures 24, 25, and 26. This may be a possible reason why the viscosity in the acid treatment was the lowest further discussed in section 3.3. The total insoluble tissues did not change by the treatment methods implying that the insoluble tissues did not break down into soluble ones; however, changes to the soluble components would not be able to be observed light microscopically.

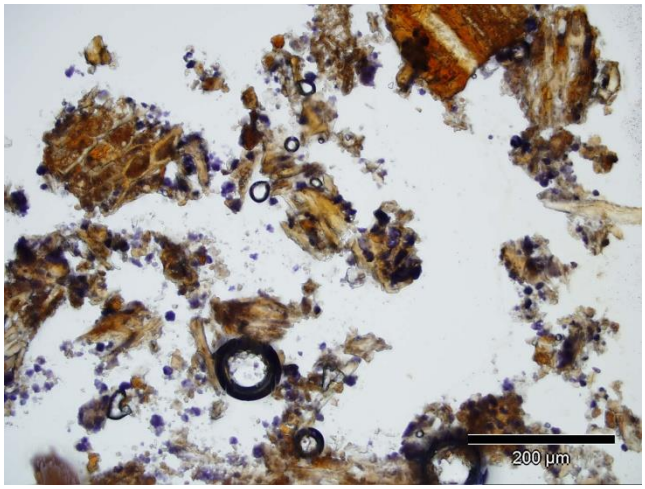


Figure 24: Cinnamon control trial 3 sample cross section in oil starch stained with aqueous dilute 0.1 N iodine utilizing transmitted light microscopy.

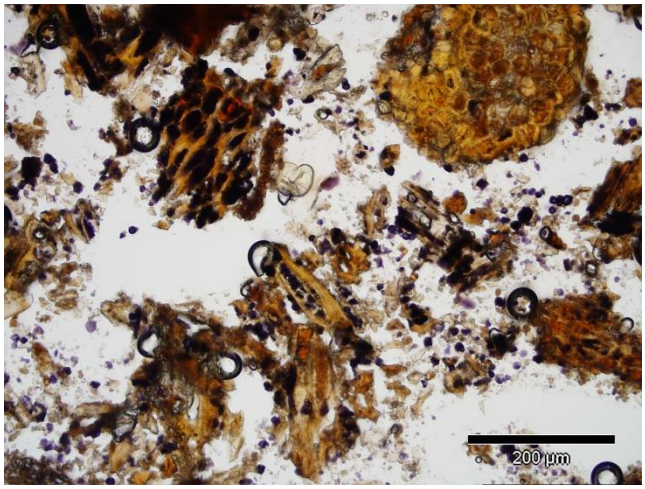


Figure 25: Cinnamon with CXP enzyme treatment trial 3 sample cross section in oil starch stained with aqueous dilute 0.1 N iodine utilizing transmitted light microscopy.

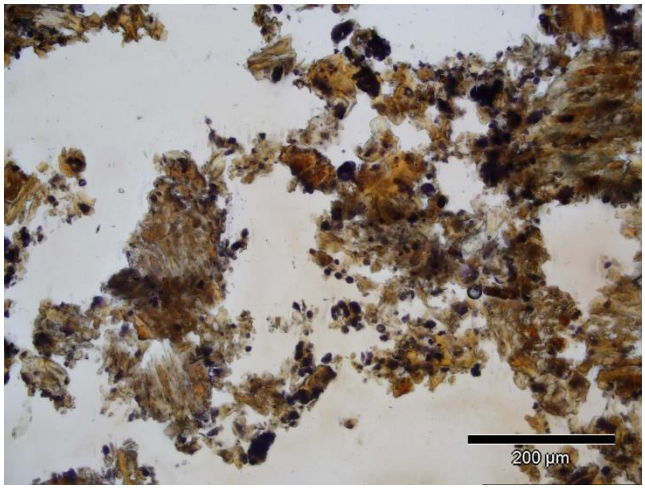


Figure 26: Cinnamon with B enzyme treatment trial 3 sample cross section in oil starch stained with aqueous dilute 0.1 N iodine utilizing transmitted light microscopy.

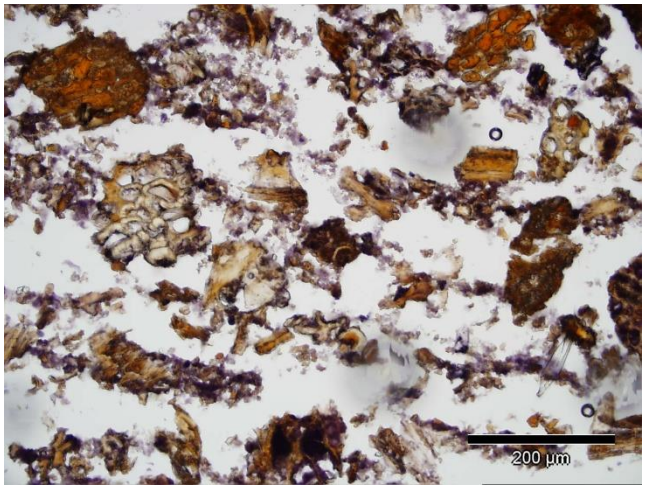


Figure 27: Cinnamon with acid treatment trial 3 sample cross section in oil starch stained with aqueous dilute 0.1 N iodine utilizing transmitted light microscopy.

3.3 Effect of hydrolysis methods on viscosity

When comparing the different hydrolysis methods, the acid hydrolysis reduced the end viscosity the greatest by 88%, the B enzyme reduced the end viscosity by 51% and the

CXP combination reduced the end viscosity by 11% (Table 6). A graph of viscosity vs. the temperature profile for the different treatment methods can be seen in Figure 28.

Sample	Trial	Ending Viscosity (cps)	STDEV
Control	1	3542	
	2	3289	
	3	3900	
	Average	3577	307
CXP	1	3195	
	2	3983	
	3	2332	
	Average	3170	826
B	1	1279	
	2	1677	
	3	2353	
	Average	1770	543
Acid	1	409	
	2	366	
	3	480	
	Average	418	58

Table 6: Ending viscosity measurements of cinnamon in solution treated with enzymes CXP, B and acid using a RVA method

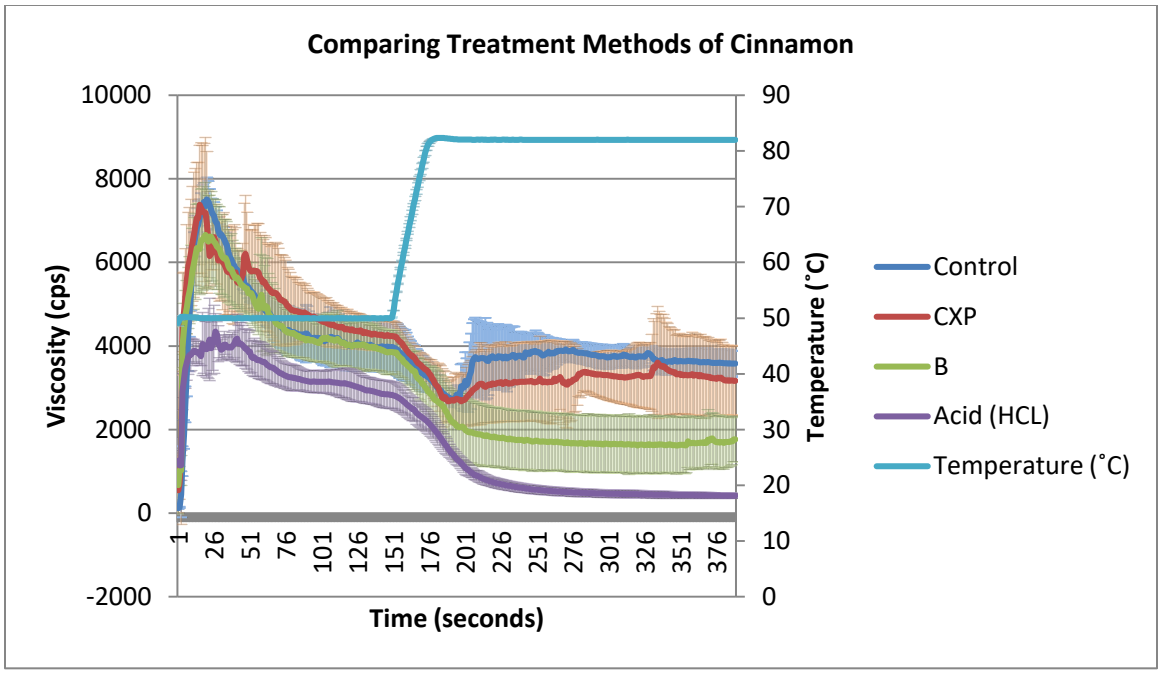


Figure 28: Viscosity of cinnamon in solution treated with enzymes CXP, B and acid using a RVA method.

This data confirms the hypothesis that enzymatic and acid treatment with cinnamon can reduce the viscosity in solution with water. The β-glucanase enzyme supplied by Novozymes also has cellulase, xylanase and pectinase activity and it had a much greater impact on viscosity reduction compared to the Sigma-Aldrich cellulase, xylanase, and pectinase enzymes. The hydrocolloids are being cleaved thus the polymer networks is being broken down by the acid and enzyme methods allowing for viscosity reduction and ease of shear processing.

The differences may be due to the addition of the β-glucanase enzyme or it may be due to the differing suppliers and their proprietary specifications on process and concentrations. The temperature, time, pH and concentration parameters may need to be further optimized. Since the carbohydrates in cinnamon have not been characterized or

quantified, it was challenging to pick enzymes that would be the most effective. However, the β-glucanase enzyme supplied by Novozymes resulted in the greatest viscosity reduction for enzyme treatment. This is a starting point for understanding what makes up cinnamon. If there is evidence that the enzymes are having an effect, then that means these molecules must be components in cinnamon.

Acid treatment had the greatest impact on reducing viscosity, but it also had the greatest undesirable outcomes such as cinnamon color change, undesirable odors and potential off-flavors. The acid treatment may have also degraded the desirable qualities of cinnamon such as antioxidants and the crucial flavor and aroma compounds present. It is a harsh process that would result in chemical waste and may damage processing equipment. Enzyme treatment does not have the undesirable aspects that acid treatment appears to have and is therefore the more amiable approach to solving viscosity issues that arise when using cinnamon during cereal processing.

3.4 Effect on dietary fiber from hydrolysis methods

The acid and enzymatic treatment methods on cinnamon showed no distinguishable differences compared to control from the Codex soluble and insoluble fiber analysis seen in Table 7.

Sample	SEC-HPLC % Soluble Fiber	Codex % Soluble Fiber	Codex % Insoluble Fiber
Control	1.28 ± 0.31	1.03 ± 0.21	15.70 ± 0.17
B	1.08 ± 0.13	1.27 ± 0.59	15.33 ± 0.59
CXP	1.10 ± 0.13	1.60 ± 0.10	15.57 ± 0.81
Acid (HCl)	4.32 ± 0.36	1.53 ± 0.47	15.73 ± 0.42

Table 7: Soluble and insoluble dietary fiber percent of cinnamon after enzymatic or acid hydrolysis based on the Codex fiber assay and SEC-HPLC

It was hypothesized that the treatment methods would break down the insoluble fiber into soluble fiber, but this analysis shows that did not happen based on the Codex fiber assay. However, this method is designed to determine soluble vs. insoluble fiber and does not account for change in molecular weight within fiber classifications which means it cannot determine if the insoluble fibers are breaking down into smaller insoluble fibers or if the soluble fibers are breaking down into shorter soluble fibers.

The HPLC-SEC was not able to detect the insoluble portion, but did show the acid hydrolyzed samples had 4 times the amount of soluble fiber compared to control. This may be an indication that the acid is able to digest the insoluble fibers into soluble ones which the Codex method did not detect. The acid treatment resulted in free sugars appearing to be glucose and possibly arabinose which indicates the extent of hydrolysis. seen in Figure 29.

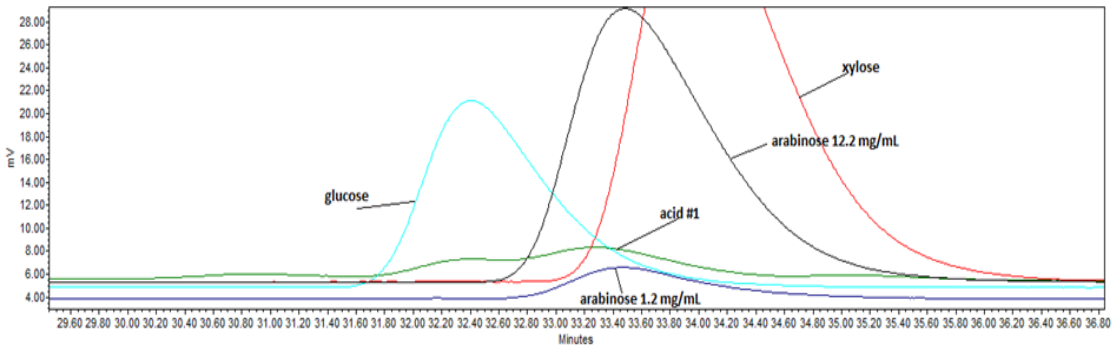


Figure 29: HPLC-SEC chromatogram of cinnamon hydrolyzed with acid, trial 1 sample (acid #1), glucose, arabinose, and xylose standards.

It is peculiar to not find xylose or a confirmed arabinose present in the enzymatically treated cinnamon samples because cellulase and xylanase were expected to generate arabinose and xylose. Even though arabinose and xylose are not present for the enzymatically treated samples, glucose is present which indicates hydrolysis took place seen in Figure 30. Discontinuing the reaction results in a desired molecular weight distribution where sugars are not substantiated therefore not negatively impacting a nutritional label or ingredient statement.

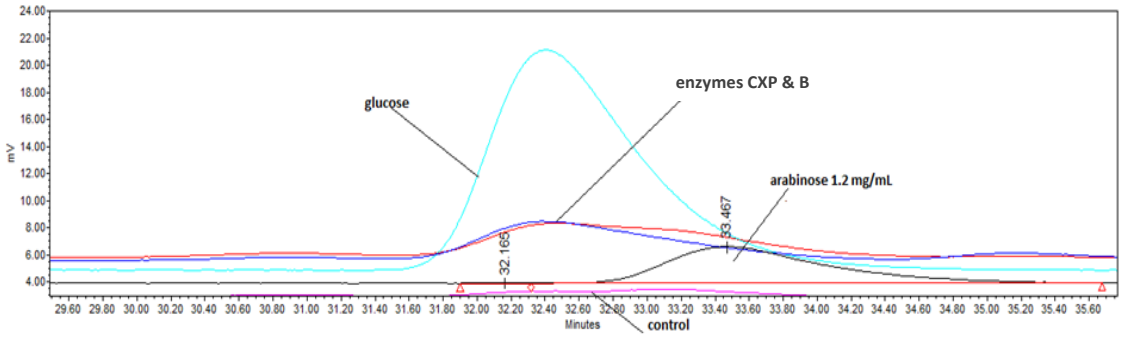


Figure 30: HPLC-SEC chromatogram of cinnamon treated with cellulase, xylanase, pectinase (CXP) enzymes and *β-glucanase (B) enzyme*, 1 trial for each sample, against glucose and arabinose standards.

There is not a substantial amount of sugars being generated from the treatment methods; however, the change in viscosity suggests a compositional change is occurring. One

possibility is that there may be inhibitors present that may be discontinuing the reaction. Inhibitors such as sulfide, carbon monoxide, thiol groups, heavy metal ions may be forming covalent bonds with the reactive groups of the enzymes (Whitaker, 1994). A more likely scenario is that the enzymes are cleaving but not the point of substantial sugar generation possibly due to the shape or size of the carbohydrates present in cinnamon.

The polymer network of the carbohydrates will affect the viscosity of the cinnamon in solution. Cinnamon has more than two polymer types; it may have many different polymer types that make the matrix even more complex and entangled as discussed in section 1.2.2.1. One possible solution is that the enzymes are cleaving polymers to break up the entangled polymer network to form smaller blocks of the matrix also combined with smaller polymer chains that can break from the block and move freely. The matrix can spread out in solution therefore not entangle as much with one another reducing the viscosity when stirred by the RVA paddle.

3.5 Molecular weight distribution impact from hydrolysis methods

All the treatments resulted in breaking down the molecules into smaller ones compared to control. Control has an average of 52.75% of the molecules in the >48.6k Da (Dalton (Da) is a unified atomic mass unit equal to 1.660 539 040(20) x 10^{-27}kg) range which is the largest molecular weight of the distribution. The acid treatment had the greatest impact on the amount of molecular weight distribution which can be seen in Table 8.

Sample	%>48.6k Da	%>48-40k Da	% 30-20k Da	%19-10k Da	%9.2-6k Da	%2.6-0.2k Da	% 180 Da
Control 1	59.95	2.92	0	0	0	0	37.13
Control 2	48.32	3.79	0	0	0	0	47.88
Control 3	49.98	3.33	0	0	0	0	46.68
B 1	30.21	8.27	0	2.15	0	0	59.38
B 2	31.19	7.42	0	2.15	0	0	59.25
B 3	32.00	7.90	0	2.18	0	0	57.92
CXP 1	22.26	0	0	2.24	0	8.70	66.80
CXP 2	26.33	0	0	4.33	0	7.55	61.80
CXP 3	27.34	0	0	6.32	0	8.74	57.60
Acid 1	10.83	0	47.27	6.58	5.94	6.04	23.39
Acid 2	10.14	0	45.87	3.12	7.08	6.27	24.53
Acid 3	8.09	0	44.36	5.27	8.77	8.03	25.48

Table 8: Molecular weight distribution of cinnamon after cellulase, xylanase, pectinase (CXP) or β-glucanase (B) enzymatic or acid treatment

An average of only 9.77% of the molecules were in the >48.6k Da range after acid treatment. The larger molecules have been broken down into smaller ones and acid treatment was the only treatment that resulted in molecules in the 9.2-6k Da and 30-20k Da ranges and the largest percentage of the distribution was in the 30-20k Da range. It is remarkable that the acid treatment did not break down the molecules to have more in the range of 180 Da. Perhaps the molecules starting in the 180 Da range were further broken down into even smaller molecules such as sugars which can be seen in Figure 29. The acid treatment had the smallest percentage of larger molecules therefore less entanglement, which correlates with the viscosity results.

The β-glucanase enzyme resulted in the molecules being broken down into the 48-40k Da range, doubling that of control. All the treatment methods had molecules in the 19-10k Da range, but the β-glucanase enzyme had the least amount in that range compared to acid and cellulase, xylanase, pectinase enzyme combination treatments. Most of the

molecules are in the 180 Da range, an average of 58.85%. This means that the larger molecules in the >48.6k Da have been broken down into the 180 Da range, to almost the opposite of control. This also correlates with the viscosity reduction results.

The cellulase, xylanase, pectinase enzyme combination had a small percentage of molecular distribution in the 19-10k Da and 2.6-0.2k Da ranges equaling an average of 12.63% combined. Most of the molecules were in the 180 Da range, an average of 62.07%, leaving an average of 25.31% in the larger molecular range of >48.6k Da. This treatment broke down slightly more of the larger molecules into smaller ones compared to the β-glucanase enzyme treatment; however, the viscosity results show that β-glucanase enzyme impacted viscosity reduction more than the cellulase, xylanase, pectinase enzyme combination as seen in Figure 31.

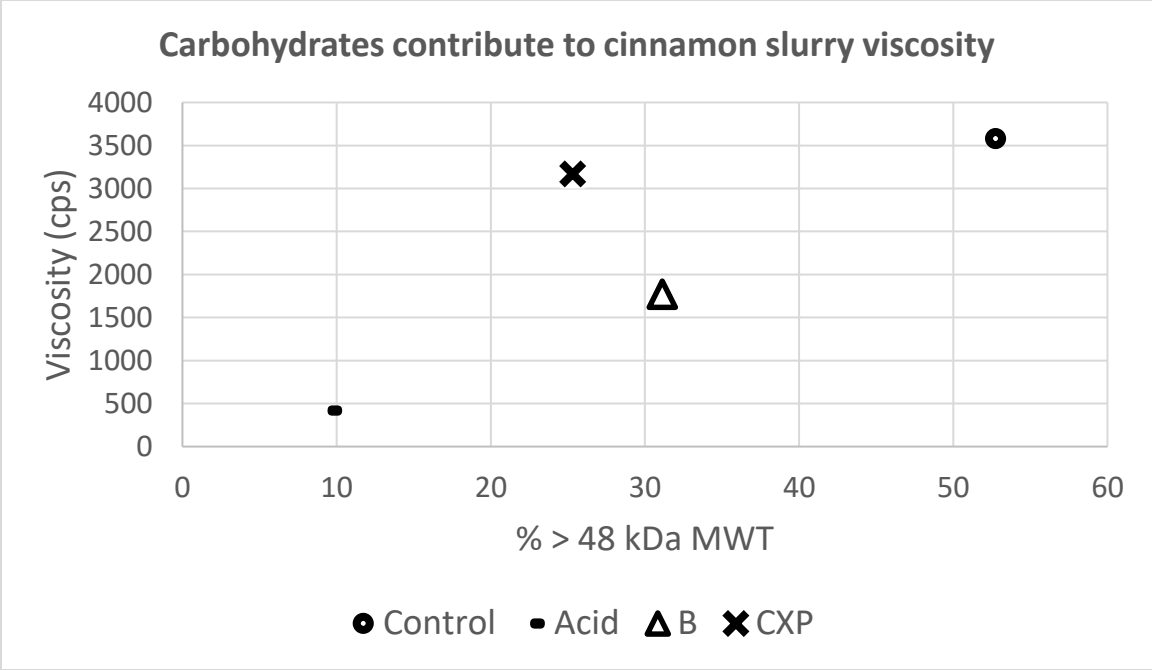


Figure 31: Viscosity of cinnamon vs. molecular weight distribution of % > 48 kDa after cellulase, xylanase, pectinase enzyme combination (CXP), β-glucanase (B) or acid treatment.

It is interesting to find that the cellulase, xylanase, pectinase enzyme combination broke down more of the larger molecules in the % >48.6k Da compared to β-glucanase enzyme but had a higher viscosity. Perhaps the β-glucanase enzyme treatment was breaking up more of the crucial molecules that are entangling, impacting viscosity, therefore resulting in a molecular distribution that interacts more appropriately for viscosity reduction in cinnamon.

4. Conclusions, Implications, and Recommendations

To reduce molecular weight and therefore reduce shear rate issues that arise during processing, acid and enzyme hydrolysis has demonstrated to be effective. However, enzyme hydrolysis was the best approach for practical applications in food systems. Enzyme hydrolysis, specifically the β-glucanase was the best approach to solving

viscosity issues that arose when using cinnamon while pleasing health conscience consumers.

Enzymatic treatment utilizing a cellulase, xylanase, pectinase and β-glucanase were successful. This is also an indication as to what key polymers exist in cinnamon, i.e. the polysaccharides. The exact concentration and characterization of these polymers warrant further study. Acid treatment was the most successful in hydrolysis and therefore viscosity reduction, which also degraded the starch as seen in the microscopy data. A recommendation would be to explore amylase enzyme treatment on cinnamon as well as in combination with the β-glucanase, cellulase, xylanase and pectinase to determine if starch or other polysaccharides are crucial components to viscosity in cinnamon. It is worth exploring other polysaccharide structures that may be present such as lignin and mucilage in further investigation, but how to quantify larger carbohydrate structures will be needed. Modifications to dietary fiber assays, HPLC-SEC or more exploratory technologies such as electrophoresis should be investigated as quantification methods.

It is recommended that cinnamon be characterized. This could be accomplished by a solvent retention capacity test before and after treatment methods which could then be compared to solubility characteristics. Since cinnamon is a crop from different geographic sources and growth conditions different results may be expected. Testing multiple cinnamon species is recommended for comparative purposes. This would be helpful for understanding the capability of the starting ingredient and the treated ingredient, if needed, vs. the capability of the processing equipment. Selecting cinnamon species based on process ability may reduce the need for ingredient treatment.

If hydrolysis can reduce viscosity of cinnamon, perhaps it can reduce the viscosity for other comparative ingredients such as cocoa or any cellulose based spice/herb or ingredient for that matter. Spices and herbs carry many positive flavor and health related attributes but are not used in abundance potentially due to processing difficulties that arise. The flavor components are extracted, and the waste is simply viewed as that, waste. However, theoretically the waste stream could be modified and used as a beneficial ingredient. The so called "waste" after flavor extraction could potentially be used as a bulking agent in sugar reduction efforts. It could be used as natural colors, antioxidant enrichment, thickening agents or dietary fiber addition. It is important to remember that the physical component of an ingredient is as or even more important than the flavor and that both can be modified if discovered how to do so.

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6. Appendix

Patent Application Reference# GMI7872US

FOOD PRODUCT AND METHOD OF MAKING THE SAME

BACKGROUND OF THE INVENTION

[0001] The present invention pertains to food products, such as ready-to-eat (RTE) cereals, and, more particularly, to making food products with certain natural ingredients, such as cocoa and/or cinnamon, while also taking measures to enable the effective use of such ingredients.

[0002] When producing food products, it is important that the component ingredients, as well as any ingredient mixtures, are not so viscous or sticky as to cause production difficulties. Certainly, it is important that the ingredients and mixtures thereof are not so viscous as to clog the machines (*e.g.*, extruders) and pipes through which they pass as such clogs can lead to the shutdown of production lines, thereby increasing costs and potentially reducing the amount of product that can be produced. Also, when intermediate products are placed on a dryer belt, for example, the stickiness of these products can lead to build up or fouling. Unfortunately, some otherwise desirable ingredients, and mixtures containing these ingredients, are sufficiently viscous or sticky so as to cause difficulties during production. For instance, both cocoa and cinnamon increase viscosity and stickiness when used in food products. As a result, in certain food products, it has generally been infeasible to include significant amounts of cocoa, cinnamon or both natural ingredients.

[0003] These production issues have also made it difficult to remove or reduce the amount of other ingredients. For example, in order to meet health-related metrics, the amount of sugar used in many food products has been reduced, which has had the effect of making processing the overall products more difficult as the sugar acts as a lubricant during processing. Also, certain artificial ingredients, such as artificial food coloring, are commonly employed when producing a wide range of food products. Increasingly, there has been a desire to reduce the use of such ingredients. However, when it is desired to remove artificial coloring for example, it is

not as simple as replacing the artificial coloring with a non-artificial (*i.e.*, natural) ingredient when making a food product having the color expected by the consumer, particularly if the same productions lines are to be used with the new formulation. Instead, the use of natural coloring ingredient can lead to a host of problems. For instance, natural coloring ingredients such as cocoa and cinnamon alter the viscosity and stickiness of the formulation so as to cause difficulties during production.

[0004] Due to various constraints, it is not always feasible to reduce the viscosity of ingredients by conventional means, *e.g.*, by simply adding additional water. Accordingly, and in view of the above, it would be desirable to provide further ways of reducing the viscosity and/or stickiness of certain ingredients, thereby enabling the addition or increased use of these ingredients or the reduction of removal of other ingredients with current production equipment.

SUMMARY OF THE INVENTION

[0005] The present invention is directed to dough based food products, such as cereal products, as well as a method of making the food products. More specifically, the invention is concerned with making food products where one or more ingredients are pre-treated with one or more enzymes in order to control the viscosity or stickiness of the overall formulation. Although various ingredients can be utilized, certain embodiments of the invention employ cocoa and/or cinnamon. The cocoa and/or cinnamon are treated or combined with multiple enzymes to form a mixture that is later added to the other ingredients of the food product.

[0006] In particular, a dough based food product is produced by mixing a plurality of ingredients to form a cereal dough. The plurality of ingredients includes at least water, syrup and grains or grain flour. An additional ingredient is mixed with at least one enzyme to form a mixture, and the mixture is combined with the cereal dough or coated on cereal pieces formed from the cereal dough. In one embodiment, the mixture is combined with the cereal dough and then the cereal dough is formed into the food product. In another embodiment, the cereal dough is formed into pieces and then the mixture is sprayed onto the cereal pieces as a coating slurry.

[0007] In a preferred embodiment, the additional ingredient is at least one of cocoa and cinnamon, and the at least one enzyme is one or more of cellulase, xylanase, beta-glucanase,

pectinase, protease and lipase. Mixing the cocoa and/or cinnamon with the at least one enzyme reduces the viscosity of the mixture by reducing a carbon chain length of or hydrolyzing the cocoa and/or cinnamon. The at least one enzyme is deactivated prior to the mixture being combined with the cereal dough, during further processing of the cereal dough or prior to being coated on the plurality of cereal pieces.

[0008] Additional objects, features and advantages of the invention will become more readily apparent from the following detailed description of preferred embodiments thereof when taken in conjunction with the drawings wherein like reference numerals refer to common parts in the several views.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] Figure 1 is a schematic illustration of a portion of a process for producing a ready-to-eat (RTE) cereal product in accordance with the invention;

[0010] Figure 2 is a graph of relaxation rate over time for several candy samples;

[0011] Figure 3 is a graph of slurry viscosity versus temperature for treated and untreated cocoa slurries;

[0012] Figure 4 is a graph of slurry viscosity versus temperature for treated and untreated cinnamon slurries; and

[0013] Figure 5 is a flowchart of a process employed in connection with producing the RTE cereal product in accordance with the invention.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0014] A wide variety of food and snack products are prepared from cooked cereal doughs, including ready-to-eat (RTE) or breakfast cereals. Generally, in the preparation of cooked cereal dough, cereal or farinaceous ingredients, such as various cereal flours, are first admixed with other dry ingredients, such as salt, minerals, starch and sugars, to form a dry blend of ingredients. This dry blend is then further blended with various liquid ingredients (including water), heated and worked to gelatinize or cook the starch fraction of the cereal ingredients and

other starchy materials. A wide variety of blending, cooking and working apparatuses and techniques are known in the art. More recently, the preparation of cooked cereal dough using a cooker extruder, especially a twin-screw extruder, has become commonplace. The cooked cereal doughs so prepared can be processed to form finished products of various sizes, textures and shapes. Typically, a post-cooking cereal dough formation step involves forming suitably sized and shaped individual pieces, such as shreds, flakes, biscuits or puffs. Thereafter, the finished dried cereal base pieces can have a topical coating applied thereto in order to provide desired taste and texture attributes. For instance, in the preparation of a breakfast cereal, the topical coating can include a sugar coating.

[0015] Of particular interest to the present invention is the production of RTE cereal products. With initial reference to Figure 1, a portion of a production arrangement for preparing RTE cereal in accordance with the present invention is shown. Cereal ingredients 100-102, as well as a natural food coloring ingredient 103, are supplied to a cooker extruder 105 to form a cooked cereal dough. Although not shown, cooker extruder 105 can be defined by an Archimedes-type single- or twin-screw extruder that is driven by a motor 110. Motor 110 is linked through a communication line 115 to a controller (or control system) 120. Cereal ingredients 100-102 can take the form of a wide range of cereal components suitable for cooking and extrusion. By way of example, cereal ingredient 100 is a dry, processed starchy cereal ingredient, cereal ingredient 101 is a syrup and cereal ingredient 102 is water.

[0016] For the sake of completeness, it should be noted that cereal ingredient 100 can take many forms known in the art, such as being formed with appropriately sized particles of whole grains supplied by any of the major cereal grains including wheat, corn (maize), oats, barley, rye, rice and mixtures thereof. In one form, cereal ingredient 100 has only fine particles so as to constitute a cereal flour. Cereal ingredient 100 can comprise about 40 to 99% (dry basis) of the cooked cereal dough. In terms of organoleptic attributes and reductions in RTE cereal piece frangibility, better results are obtained when cereal ingredient 100 comprises about 75 to 99% of the cooked cereal dough. Cereal ingredient 100 can also include various minor ingredients or additives such as sugar(s), salt and mineral salts (*e.g.*, trisodium phosphate) and starches that can be conveniently pre-blended with the other portions of cereal ingredient 100. Cereal ingredient 101 can be constituted by various liquid ingredients, such as corn (maize) or malt syrups or the like. The amount of moisture from cereal ingredients 101 and 102 will depend on various factors including the particular cereal ingredients, cooking temperature and working

techniques employed. The moisture content of the cooked cereal dough typically ranges from about 20 to 30% prior to the cooked cereal dough exiting cooker extruder 105.

[0017] If desired, the cereal dough can additionally comprise, by dry weight, about 0.1 to 20%, particularly about 0.5 to 5%, sugar(s) or, synonymously herein, nutritive carbohydrate sweetening agents. Such materials are also well known in the RTE cereal art. One sugar component useful herein is sucrose. However, the sugar component can alternatively comprise fructose, maltose, dextrose, honey, fruit juice solids, brown sugar and the like. In addition to providing desirable sweetness, the sugar component also beneficially affects the cereal color and texture. Conveniently, the sweeteners can be added in with cereal ingredient 100 or cereal ingredient 101. If desired, the cereal dough composition can additionally include a variety of materials designed to improve the aesthetic, organoleptic or nutritional qualities of the cereal. These adjuvant materials can include vitamin or mineral fortification, colors, flavors, high potency sweeteners and mixtures thereof. The precise concentration of such ingredients can certainly vary. Generally, however, each of these ingredients can comprise about 0.01 to 2% dry weight of the cereal composition. One especially useful material is salt (*i.e.*, table salt or common salt).

[0018] Important with respect to the present invention is the addition of natural coloring ingredient 103. By way of particular examples, the invention will be described in connection with the use of cocoa or cinnamon as natural food coloring ingredient 103 since cocoa and cinnamon are two non-artificial ingredients that can be advantageously used to impart color to a food product. Unfortunately, adding either cocoa or cinnamon to a cereal slurry will significantly increase the overall viscosity and stickiness of the cereal formulation. This problem is illustrated by Figure 2 which shows the results of a texture analysis. Specifically, samples having different formulations of sugar, water, canola oil, corn syrup and cocoa or cinnamon were prepared. The ingredients were boiled on a stovetop, and the liquid was poured into molds to harden and form a candy or confectionary phase. These candies were then subjected to a texture analysis. As can be seen in Figure 3, the addition of cocoa or cinnamon increased the toughness of the candies (*i.e.*, made the candies less brittle). This indicates that the addition of cocoa or cinnamon will increase the viscosity and stickiness of a cereal slurry. As a result, adding or increasing the amount of these ingredients can cause production difficulties, such as the clogging of machines or pipes, specifically cooker extruder 105, as well as build up on or fouling of a dryer belt.

[0019] To address these concerns, the present invention involves treating natural coloring ingredient 103 with enzymes to reduce the viscosity of ingredient 103, thereby reducing the viscosity of slurries containing ingredient 103 without negatively affecting the flavor of the resulting RTE cereal products. Without being bound to any particular theory, the enzymes function to reduce carbon chain lengths and cause hydrolysis of the natural coloring ingredient 103. In connection with both cocoa and cinnamon, the treatment includes the use of particular enzyme combinations. That is, different enzymes and combinations of enzymes were found to be significantly more effective for treating cocoa and cinnamon, and not all enzymes and enzyme combinations can be used to effectively treat these ingredients. Also, even among the enzymes and enzyme combinations that are effective, the effectiveness varies. In terms of the specific enzymes, the following enzyme combination was found to be particularly beneficial in connection with cocoa: beta-glucanase, cellulase, xylanase, pectinase, and protease (including at least neutral and alkaline protease). For cinnamon, the following enzyme combination was found to be particularly beneficial: beta-glucanase, cellulase, xylanase, and pectinase. To test the effectiveness of these treatments, cocoa and cinnamon were treated with enzymes and incorporated into cereal slurries. Figure 3 is a graph of slurry viscosity versus temperature for treated and untreated cocoa slurries, while Figure 4 is a graph of slurry viscosity versus temperature for treated and untreated cinnamon slurries. As can be in these figures, the slurries containing treated cocoa or cinnamon are less viscous than the slurries containing untreated cocoa or cinnamon over much of the temperature range shown. The viscosity difference is particularly significant at the lower end of the temperature range. These results are reproduced in part in Table 1, which provides the viscosities of the slurries at 220 °F.

[0020] Table 1:

Variable	Viscosity at 220 °F (Pa.s)	Viscosity Reduction (%)
Red Dutched Control	0.351	0
1E Treated Cocoa	0.321	3
2E Treated Cocoa	0.255	9.6
2% Cinnamon Control	0.085	0

2% Treated Cinnamon	0.078	0.7
3% Cinnamon Control	0.151	0
3% Treated Cinnamon	0.156	-0.5

[0021] In a particular formulation of the cocoa enzyme combination, the beta-glucanase, cellulase, xylanase and pectinase makes up 50% of the combination while the protease makes up the remaining 50%. The cellulase and xylanase have been determined to be the most important portions of the cocoa and cinnamon enzyme combinations in reducing the carbon chain length or hydrolyzing, with the xylanase acting as a catalyst for the cellulase. When cocoas having higher fat contents are used (*e.g.*, 20-22% fat), it was found that the addition of lipase to the enzyme combination is further beneficial in connection with reducing the carbon chain length or hydrolyzing. Although specific combination of enzymes are set forth, it should be recognized that good results can still be obtained when fewer than all of the listed enzymes are used.

[0022] Figure 5 is a flowchart illustrating a portion of a process for producing RTE cereal products in accordance with the present invention. At step 500, a plurality of ingredients is mixed together in cooker extruder 105 to form a cereal dough, the plurality of ingredients including at least water, a syrup and grains or grain flour. At step 505, cocoa or cinnamon is mixed with at least one enzyme in order to reduce a carbon chain length of or hydrolyze the cocoa or cinnamon. In one exemplary enzymatic treatment process, water is heated to 50 °C, and the chosen enzymes are added to the water. Preferably, the enzymes are added in an amount equal to about 5% of the weight of the cocoa or cinnamon to be added (*i.e.*, 12.5 g of enzymes per 250 g of cocoa or cinnamon), and 8 parts water is used to 2.5 parts cocoa or cinnamon (*i.e.*, 800 g of water per 250 g of cocoa or cinnamon). Once the enzymes are mixed with the water, the cocoa or cinnamon is gradually added until all of the cocoa or cinnamon is in solution. The mixture is then maintained at 50 °C for 10 minutes. Afterwards, the mixture is heated to 82 °C and held for 20 minutes to deactivate the enzymes. At this point, the mixture can be placed in a container and refrigerated or frozen until use when it will be pumped to cooker extruder 105 as coloring ingredient 103. Alternatively, the mixture is not stored in a refrigerator or freezer after it is made but is instead pumped directly to cooker extruder 105. In either case, coloring ingredient 103, *i.e.*, the enzymatically treated cocoa or cinnamon, is added to the cereal dough in step 510. Then, at step 515, the cereal dough is extruded. In an alternative embodiment, at step 520, the

enzymatically treated cocoa or cinnamon is sprayed, such as from a slurry tank (not shown), onto the cereal dough as a coating or part of a coating after the cereal dough is extruded or otherwise formed in step 515. Typically, the coating is a semisolid mixture that acts as a sugar coating for the cereal dough, which is generally formed into shreds, flakes, biscuits or puffs at this stage. Although there are many potential ingredients, the coating can include water, sugar, syrups and oil. While the embodiment described above involves deactivating the enzymes before the mixture is added to the cereal dough, it is also possible to add coloring ingredient 103 with the enzymes earlier in the cooking process, such that the enzymes are actually deactivated during the cooking of the cereal dough or heating of the coating slurry. In addition, while step 500 is shown taking place prior to step 505, these steps can take place simultaneously or in reverse order.

[0023] Although the present invention has been described in connection with RTE cereal products, it should be recognized that enzymatically treated cocoa and cinnamon are useful in other food products. For example, in cinnamon rolls, cinnamon inhibits yeast from forming bubbles due to the increased viscosity of the wet product, which meant that, previously, only small amounts of cinnamon could be added. If the cinnamon is treated, greater amounts can be added. In addition, it should also be understood that other ingredients can potentially be treated with appropriate enzymes to reduce their viscosities. Furthermore, while portions of the above discussion have focused on adding additional cocoa or cinnamon to a food product to impart color, these ingredients can also be added to a colored food product simply to impart cocoa or cinnamon flavor. For food products that already contain chocolate or cinnamon, the amount of these ingredients can be increased. Otherwise, chocolate and/or cinnamon can potentially be added to a new or existing formulation. In addition, the amount of cocoa or cinnamon can be maintained while the amount of sugar is reduced, with the treatment of the cocoa or cinnamon mitigating the increased viscosity caused by the sugar reduction.

[0024] Based on the above, it should be apparent that either adding or increasing the amount of cocoa or cinnamon in an operational product stream can cause production difficulties. In fact, even maintaining existing amounts of cocoa and cinnamon can be difficult if the amounts of one or more other ingredients are modified, *e.g.*, if the amount of sugar is reduced. Although it is possible to replace or upgrade existing equipment or rework cereal formulations, such options may be impractical, expensive and/or otherwise undesirable. However, the present invention provides a way to reduce the viscosity and stickiness of food product ingredients and ingredient mixtures, thereby preventing clogging or fouling of machinery and pipes during production of the

food product. Accordingly, the enzymatic treatment described above alleviates the problems caused by the use of relatively more viscous ingredients and allows food products to be produced with natural ingredients, reduced sugar content, increased amounts of cocoa or cinnamon or the use of both cocoa and cinnamon, for example. Although described with reference to preferred embodiments, it should be readily understood that various changes or modifications could be made to the invention without departing from the spirit thereof.

CLAIMS

1. A method of producing a ready-to-eat cereal product, the method comprising:

mixing a plurality of ingredients to form a cereal dough, the plurality of ingredients including at least water, syrup and grains or grain flour;

mixing an additional ingredient with at least one enzyme to form a mixture; and

combining the mixture with the cereal dough or with cereal pieces formed from the cereal dough.

2. The method of claim 1, wherein mixing the additional ingredient with the at least one enzyme includes mixing at least one of cocoa and cinnamon with the at least one enzyme.

3. The method of claim 2, wherein mixing the at least one of cocoa and cinnamon with the at least one enzyme includes mixing the at least one of cocoa and cinnamon with cellulase and xylanase.

4. The method of claim 3, wherein mixing the at least one of cocoa and cinnamon with the cellulase and xylanase includes mixing cocoa with cellulase, xylanase, beta-glucanase, pectinase and protease.

5. The method of claim 4, wherein mixing the cocoa with the cellulase, xylanase, beta-glucanase, pectinase and protease includes mixing cocoa with cellulase, xylanase, beta-glucanase, pectinase, protease and lipase.

6. The method of claim 3, wherein mixing the at least one of cocoa and cinnamon with the cellulase and xylanase includes mixing cinnamon with cellulase, xylanase, beta-glucanase and pectinase.

7. The method of claim 2, wherein mixing the at least one of cocoa and cinnamon with the at least one enzyme includes deactivating the at least one enzyme.

8. The method of claim 2, wherein:

mixing the plurality of ingredients includes mixing the plurality of ingredients in a cooker extruder; and

combining the mixture includes combining the mixture with the cereal dough by adding the mixture to the cooker extruder.

9. The method of claim 2, wherein combining the mixture includes applying the mixture as a coating onto the plurality of cereal pieces formed from the cereal dough.

10. The method of claim 2, wherein mixing the at least one of cocoa and cinnamon with the at least one enzyme to form the mixture includes reducing the viscosity of the mixture.

11. The method of claim 10, wherein reducing the viscosity of the mixture includes at least one of reducing a carbon chain length of and hydrolyzing the at least one of the cocoa and cinnamon.

12. The method of claim 11, further comprising: deactivating the at least one enzyme after reducing the viscosity of the mixture.

13. A method of producing a food product, the method comprising:

mixing at least one of cocoa and cinnamon with at least one enzyme to form a mixture;

deactivating the at least one enzyme;

combining an ingredient with the mixture after the at least one enzyme is deactivated; and

forming the food product.

14. The method of claim 13, wherein mixing the at least one of cocoa and cinnamon with the at least one enzyme includes mixing the at least one of cocoa and cinnamon with cellulase and xylanase.

15. The method of claim 14, wherein mixing the at least one of cocoa and cinnamon with the cellulase and xylanase includes mixing cocoa with cellulase, xylanase, beta-glucanase, pectinase and protease.

16. The method of claim 15, wherein mixing the cocoa with the cellulase, xylanase, beta-glucanase, pectinase and protease includes mixing cocoa with cellulase, xylanase, beta-glucanase, pectinase, protease and lipase.

17. The method of claim 14, wherein mixing the at least one of cocoa or cinnamon with the cellulase and xylanase includes mixing cinnamon with cellulase, xylanase, beta-glucanase and pectinase.

18. The method of claim 13, further comprising:

adding the mixture to a cooker extruder, wherein combining the ingredient with the mixture includes adding the ingredient to the cooker extruder.

19. The method of claim 13, wherein combining the ingredient with the mixture includes applying the mixture onto the ingredient as a coating.

20. The method of claim 13, wherein mixing the at least one of cocoa and cinnamon with the at least one enzyme to form the mixture includes reducing the viscosity of the mixture.

21. The method of claim 20, wherein reducing the viscosity of the mixture includes reducing a carbon chain length of or hydrolyzing the cocoa or cinnamon.

22. A dough based food product comprising:

water;

syrup;

grains or grain flour;

at least one of cocoa and cinnamon; and

at least one deactivated enzyme selected from the group consisting of cellulase, beta-glucanase, xylanase, pectinase and protease.

23. The dough based food product of claim 22, wherein the at least one deactivated enzyme constitutes cellulase and xylanase.

24. The dough based food product of claim 23, wherein the at least one of cocoa and cinnamon constitutes cocoa, and the at least one deactivated enzyme constitutes cellulase, xylanase, beta-glucanase, pectinase and protease.

25. The dough based food product of claim 24, wherein the at least one deactivated enzyme further includes lipase.

26. The dough based food product of claim 23, wherein the at least one of cocoa and cinnamon constitutes cinnamon, and the at least one deactivated enzyme constitutes cellulase, xylanase, beta-glucanase and pectinase.

27. The dough based food product of claim 22, wherein the at least one deactivated enzyme is deactivated prior to being combined with the water, syrup, and grains or grain flour.

28. The dough based food product of claim 22, wherein the at least one of cocoa and cinnamon and the at least one deactivated enzyme form a coating provided on the dough based food product.

29. The dough based food product of clam 22, wherein the food product is cereal dough or a plurality of cereal pieces made from cereal dough.

ABSTRACT

A dough based food product is produced by mixing a plurality of ingredients to form a cereal dough. The plurality of ingredients includes at least water, syrup and grains or grain flour. An additional ingredient is mixed with at least one enzyme to form a mixture, and the mixture is combined with the cereal dough. In one embodiment, the cereal dough is extruded or otherwise formed into the food product after the mixture is combined with the cereal dough. In another embodiment, the mixture is coated onto individual food products, such as cereal pieces, formed from the cereal dough. Preferably, the additional ingredient is cocoa and/or cinnamon, and the at least one enzyme is one or more of cellulase, xylanase, beta-glucanase, pectinase and protease, with the at least one enzyme being deactivated during making of the food product.

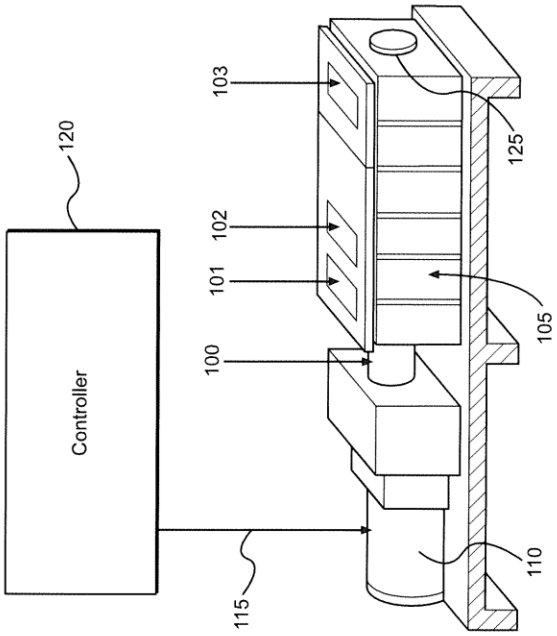

FIG. 1

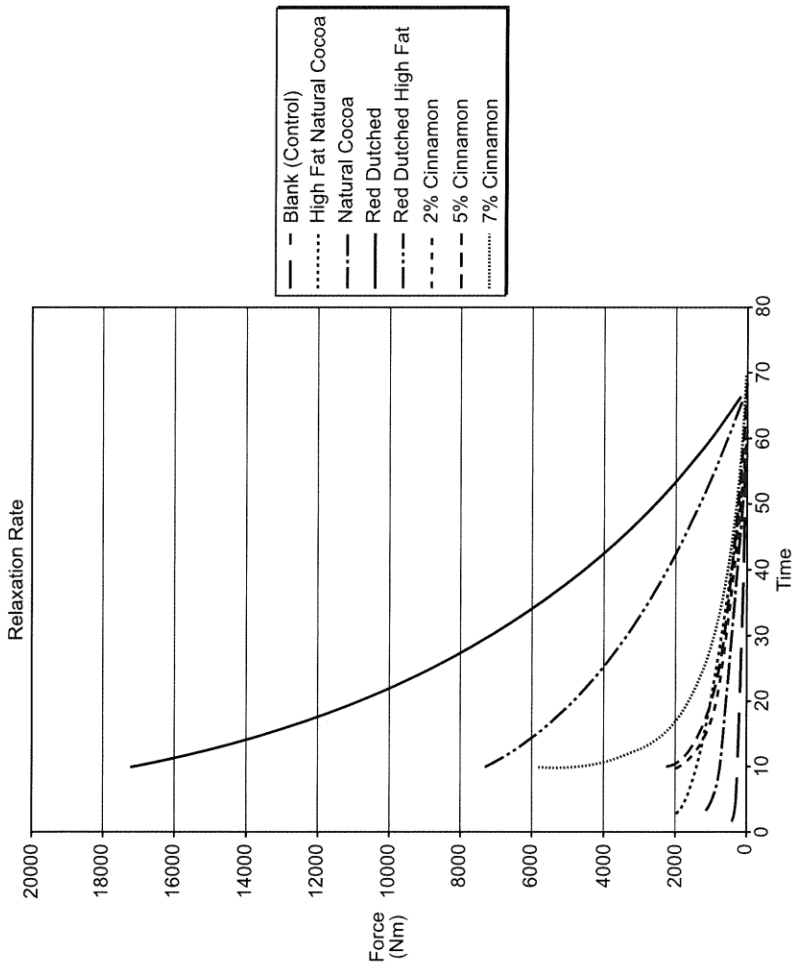
Blank (Control)
High Fat Natural Cocoa
Natural Cocoa
Red Dutched
Red Dutched High Fat
2% Cinnamon
5% Cinnamon
7% Cinnamon
Relaxation Rate
Force (Nm)
Time
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
0
10
20
30
40
50
60
70
80

FIG. 2

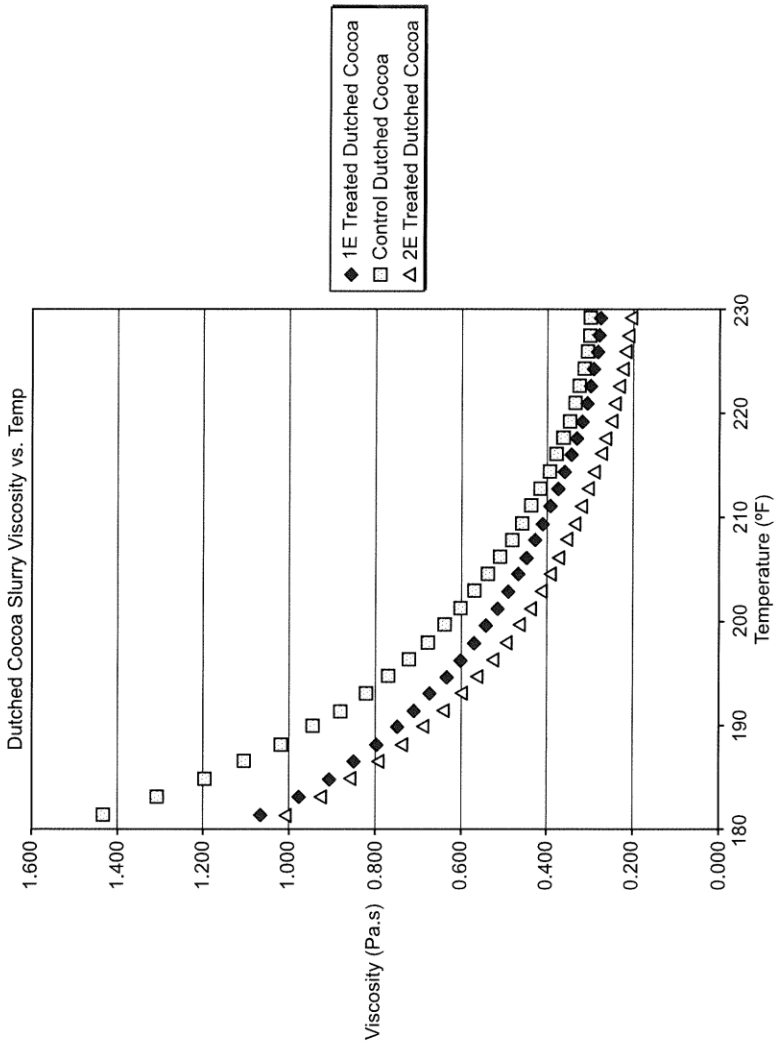
Dutched Cocoa Slurry Viscosity vs. Temp
1E Treated Dutched Cocoa
Control Dutched Cocoa
2E Treated Dutched Cocoa
Viscosity (Pa.s)
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Temperature (°F)
180
190
200
210
220
230

FIG. 3

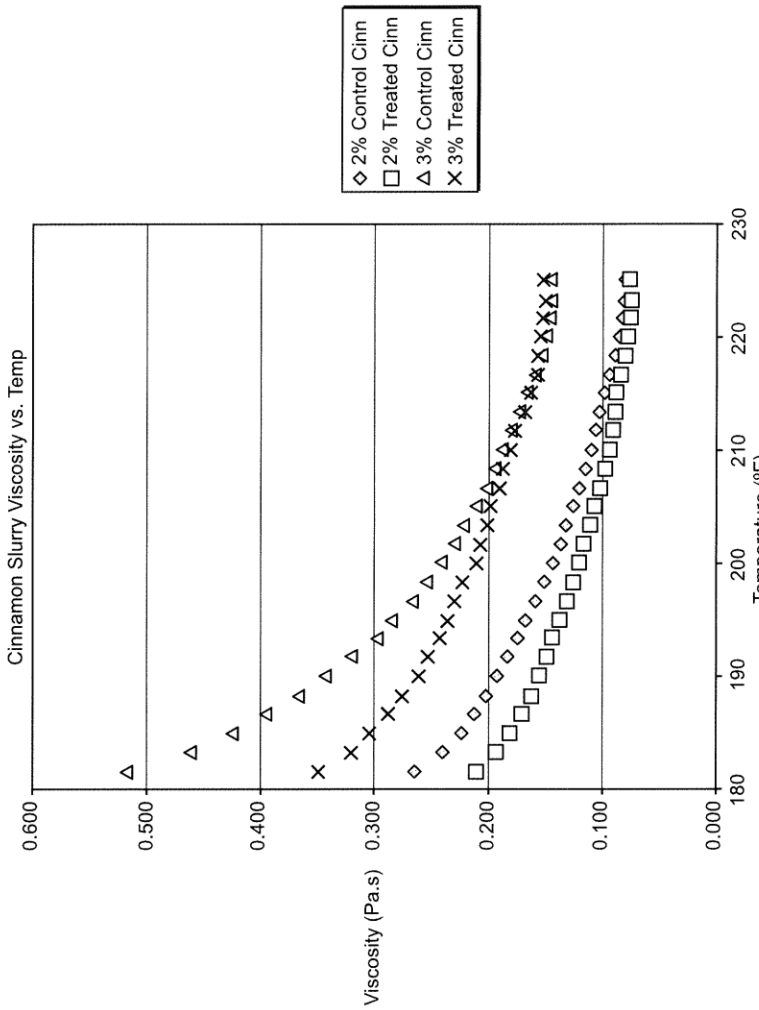
Cinnamon Slurry Viscosity vs. Temp
◇ 2% Control Cinn
□ 2% Treated Cinn
△ 3% Control Cinn
× 3% Treated Cinn
Viscosity (Pa.s)
0.600
0.500
0.400
0.300
0.200
0.100
0.000
Temperature (°F)
180
190
200
210
220
230

FIG. 4

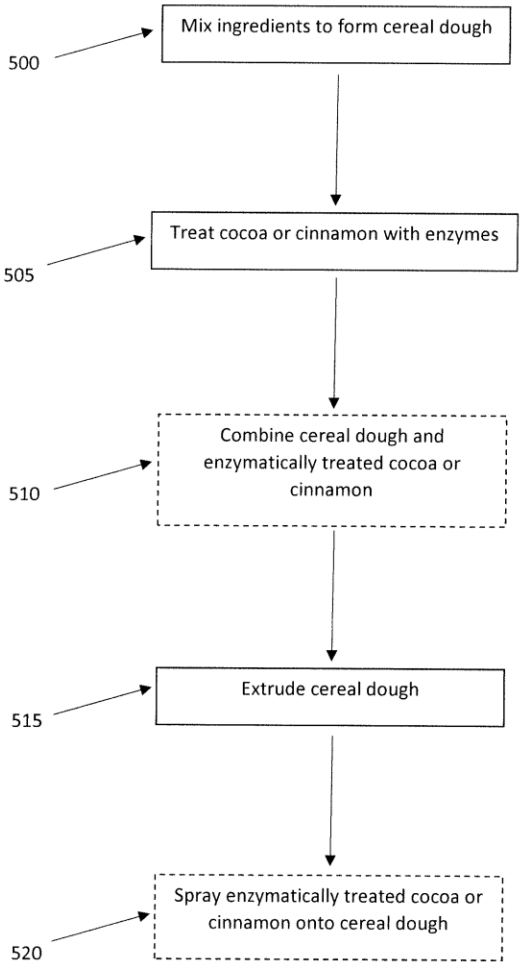
Mix ingredients to form cereal dough
500
Treat cocoa or cinnamon with enzymes
505
Combine cereal dough and enzymatically treated cocoa or cinnamon
510
Extrude cereal dough
515
Spray enzymatically treated cocoa or cinnamon onto cereal dough
520

FIG. 5