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THE EFFECT OF DI-POTASSIUM PHOSPHATE AND SODIUM CHLORIDE ON THE PASTING PROPERTIES OF CEREAL FLOURS

by
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Title: The Effect of Di-potassium Phosphate and Sodium Chloride on the Pasting Properties of Cereal Flours

Phosphorus is the second most abundant mineral in the body making up 1% of the total human body weight. In addition to its main function in bone and teeth formation, phosphorous is an essential element used by proteins for cellular and tissue renewal, regeneration and repair. It assists the body in producing adenosine triphosphate (ATP) which contributes to the control of food intake. Preload phosphorus intake was reported to decrease subsequent food intake.

It is well documented that cereal flours and starches contain traces of phosphorus; however, the form of this phosphorus in these staple foods renders it inaccessible or non-bioavailable in the human body. Accordingly, in this study we have explored the effect of added phosphorus in the form of di-potassium phosphate on the pasting properties of wheat, corn, and rice flours. For this purpose, di-potassium phosphate was added in different percentages (0.25, 0.5, 1, and 2%) to the various cereal flours. In addition, sodium chloride was added in different percentages (1 and 2%) to explore the synergistic effect of the salts on the pasting properties. Consequently, the pasting properties of these flours were assessed using a starch pasting cell attached to a TA Instruments DHR3 rheometer following AACC procedures for flours. The obtained results show that although the pasting properties of the flours followed a trend when the salts were added, these observed changes were not significant at 95% confidence interval when compared to samples with no salts added. It is worthy to note that the identified trends were dependent on the concentrations of both di-potassium phosphate and sodium chloride.

Keywords: Phosphorus, wheat, corn, rice, pasting properties, gelitinization

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CHAPTER 1
INTRODUCTION

Phosphorus is an essential mineral, it is required by proteins for cellular and tissue renewal, regeneration, and repair, and is necessary for bone and teeth formation. Phosphorus is needed in Hepatic ATP synthesis as well, the level of which control food intake. Moreover, it has been proven that the increase in phosphorus intake in one meal, fuels the production of Hepatic ATP, which will result in an earlier satiation in the next meal.

Obesity and overweight are spreading rapidly and are affecting all segments of society, low and high income societies, and especially developing countries. The rise in obesity has been associated with the urbanization and westernization of diets that are characterized by the consumption of saturated fats, carbohydrates, and refined cereals. This western diet is a high energy diet and a low cost one. On the other hand, healthier diets are nutrient rich diets, but unfortunately they are high-cost diets. Thus, low-cost diet, or energy dense diets, and low-nutrients diets, hence low in phosphorus are behind the rise in obesity. Cereal products and starches make up to 90% of our energy intake, and the most consumed are wheat, corn and rice. They contain traces of phosphorus, but in a form that is not bioavailable. In addition most of the phosphorus is lost during processing and refining. In this study, phosphorus in the form of dipotassium phosphate (K_2HPO_4), as well as sodium chloride ($NaCl$), was added to wheat, corn, and rice flours. In order to investigate the effect of enriching cereal flours with dipotassium phosphate on manufacturing processes of cereal products, the pasting properties and gelatinization of the abovementioned flours were tested.

A. Phosphorus

Phosphorus, the second most abundant mineral in the body, makes up 1% of the total human body weight (IoM, 1997). In addition to its main function in bone and teeth formation, phosphorous is needed by proteins for cellular and tissue renewal, regeneration, and repair (IoM, 1997); moreover it is involved in the structure of cell membranes as phospholipids and in DNA and RNA coding . Phosphorus, in its inorganic form (P_i) assists the mineralization of bone and the intermediary metabolism of body tissue. Furthermore, it assists the body in the production of adenosine triphosphate (ATP) the levels of which are shown to contribute to the control of food intake (IoM, 1997; Menon & Ix, 2013).

The Recommended Dietary Allowance (RDA) of phosphorus for healthy adults is 700 mg/day. Phosphorus is found in most food sources: Dairy products, meat, fish, and cereal foods. Dairy products contribute to 20-30% of adults' dietary intake of phosphorus, and another 20-30% comes from meat, fish, poultry, and legumes. These sources are high in protein making them high in organic phosphorus. However, plant seeds such as beans, peas, nuts, and cereals store their phosphorus in the form of phytic acid. Phytic acid cannot be metabolized by the human digestive system, thus rendering the phosphorus in plant seeds unavailable (IoM, 1997).

With the exception of the abovementioned foods, phosphorus is found in a readily hydrolysable from, organic phosphate esters. Nevertheless, in cereal grains and unleavened bread, phosphorus is stored as phytic acid, a form indigestible by the human intestine given that it lacks the enzyme Phytase which is for the hydrolysis and metabolism of phytic acids. Hence, phytate phosphorus is not bioavailable in any given human diet. On the other hand, yeasts are able to hydrolyze phytic acid, which renders the phosphorus available in leavened cereal based foods (Reddy, Sathe, & Salunkhe, 1982).

Phosphorus metabolism differs from that of other minerals. For instance, in the case of calcium the body is dependent on environmental surfeit to maintain its calcium homeostasis. However, since phosphorus is needed for the growth of organisms and the renewal and regeneration of tissue, the regulatory components responsible for its homeostasis are optimized. Moreover, phosphorus in its inorganic form is involved in glycogen synthesis and the storage of energy in general, and buffers the pH of the extracellular fluid (Penido & Alon, 2012).

1. *Phosphorus and Obesity*

There has been evidence that obesity and overweight are spreading rapidly and are affecting all segments of society, high and low-income countries. In fact, worldwide it has more than doubled in the past 35 years. In 2014, 1.9 billion (39%) adults aged 18 and older have been reported to be overweight and 600 million (13%) of them obese (WHO, 2016). This trend, although can be related to genetics, is also associated with the urbanization and westernization of diets. These diets are high in refined foods, sugars, refined cereals, saturated fats, and accompanied with a lifestyle characterized by lower activity levels (Popkin & Gordon-Larsen, 2004). The western diet is low phosphorus since none of the above mentioned foods contain a good amount of phosphorus, especially cereals which most of the phosphorus that they contain are phytic phosphorus that are not bioavailable and where 70% of the phosphorus is lost by refinement (Obeid, 2013). Today, a person consumes about 1.3 grams per day of phosphorus even though the upper limit of intake is 4 grams per day (Ervin, Wang, Wright, & Kennedy-Stephenson, 2004). The rise of obesity in the developing world and industrialized societies has been attributed to energy-dense foods such as snacks, added sugars, added fats, fast foods, beverages, and refined grains. Obesity and all degenerative diseases (such as type 2 diabetes, hypertension, and heart related diseases) follow a socioeconomic gradient where the highest rates are seen in poor countries and the developing world. This is explained by the fact that energy dense diets are low-cost diets, and healthy diets that are nutrient rich are high-cost diets that are unaffordable by the aforementioned communities (Drewnowski, 2009). Low-cost and low-nutrients diets which are usually low in phosphorus may be one of the factors contributing to the increase in obesity (Obeid, 2013). According to Obeid, Dimachkie, and Hlais (2010), increasing the phosphorus content of one meal can stimulate hepatic ATP synthesis of the following meal; this in turn contributes to satiation. In the postprandial state, insulin is produced and stimulates the uptake of phosphorus and several other metabolites by extrahepatic cells, which will decrease the availability of phosphorus for hepatic ATP production. Thus, Obeid et al. (2010) hypothesized that if we increase the phosphorus intake in a meal, the hepatic phosphorus status will improve resulting in an increase in hepatic ATP synthesis, which will cause an earlier satiation in the next meal. Therefore, increasing the uptake of phosphorus can be an effective factor in shaping a better diet for developing countries, hence contributing in the decrease of degenerative diseases especially obesity.

B. Cereals

Cereals are the most important staple food for humans. In fact, 90% of our energy intake comes from cereals. They are also considered as an important protein source and a subsistence crop, especially for poor people and the Third World. Cereals are by far the most important crop since it is easy to grow, harvest, store, process into food products, and a good product for commercial farmers. The major and mostly used are wheat corn and rice. 60% of the cultivated land in the world is used to grow cereals, and these three types of cereals are produced in the largest quantities. In fact, in 2010 wheat took up to 217 million ha of the cultivated area, corn 162 million ha, and rice 154 million ha. (David A. V. Dendy, 2001; Koehler & Wieser, 2013)

Globally, the consumption of cereal has remained stable over time and societies representing around 50% of energy intake. However, subtle changes in the share of dietary energy intake derived from cereals in developing countries where it has fallen from 60% to 54% in 10 years. According to FAO's projections, cereals will continue to represent a big part of the dietary energy intake globally, but will suffer a downward trend particularly wheat and rice (WHO/FAO, 2003).

Botanically, cereals are member of the grass family (Gramineae); they produce one-seeded fruits or a caryopsis which is also called a "kernel" or "grain". The anatomy of cereal grains basically consists of the outer layer which is the bran and encloses the starchy endosperm and the germ.

Chemically, cereals have moisture of 11-14% and are known for their high content of carbohydrates. Mainly, the available carbohydrates are in the form of starch in the endosperm and amount to 56-74%. Cereal grains contain a good amount of proteins, which compose 8-11% of the chemical groups available in cereal grains. 2-13% of the components are fibers and are located in the bran. Lipids are present in cereal grains but in low amounts (2-4%) except in oat they can reach 7%, along with minerals which compose only 1-3%. Furthermore, cereal grains have a very high content of B-vitamins. These chemical components and the differences in their amount and availability affect the quality of products made from cereal grains.

Starch, in the endosperm, is the major storage of carbohydrates in cereals (55-70%). It is a very important material in food, in particular bread, because it has unique properties that affect the texture of food. Starch is also considered a functional ingredient, it is added to several products such as sauces, low-fat products, and confectionery and its functionality differs between products. It is usually added to products to improve their texture: to fluids to improve their viscosity and stability and to semisolid products to improve their fat and water-holding properties. In fact, worldwide only 5% of starchy staple food comes from root crops (potato, cassava, and yams) the rest comes from cereal grains (Eliasson, 2004; Hermansson & Svegmark, 1996; Jan A. Delcour, 2010; Wischmann, Norsker, & Adler-Nissen, 2002).

C. Starches

Starch exists in the endosperm of the cereal grain in the form of granules to act as an energy reservoir for the seed to grow. It is basically composed of polymers of one monosaccharide (glucose) but has a complicated structure and function. Usually, food processes using cereals destroy the native granular structure of starch which is the process of gelatinization. The smallest granules are found in rice (around 5 μm in diameter). The granules of corn starch are irregular in shape and size varying between 5 and 20 μm. In wheat, granules exist in two types, A-granules and B-granules. A-granules are large, have a lenticular form and a diameter of around 14 μm. B-granules are small spherical structures with a diameter of around 4 μm. The ratio between A and B granules was shown to be important for baking processes, at about respectively 25% to 35% by weight. The granules in cereals have surfaces that are 90-95% composed of carbohydrates, but they also contain proteins and lipids (Buchanan, Gruissem, & Jones, 2015; David A. V. Dendy, 2001; Eliasson, 2004; Tomlinson & Denyer, 2003).

Starch or starch granules are 65-85% composed of amylopectin, one of the biggest molecules in nature. It can even reach 100% in some mutant plants called “waxy starch”. Amylopectin, a highly branched polysaccharide is composed of numerous short chains of α-(1,4)-linked D-glucose residues. The glucose residues are linked by glycosidic bonds, and the branching takes place at reducing ends by α-(1,6) bonds. Together the chains form a large macromolecule with many non-reducing ends, and a single glucose with a free reducing end group. Amylopectin is soluble in water, and is easily digested by amylase (Buchanan et al., 2015; David A. V. Dendy, 2001; Manners & Matheson, 1981)

Another major component of starch is a less branched polysaccharide called amylose, with a small number of glucan chains. Amylose usually makes up to 20-30% of starch by weight, depending on the botanical source. Amylose can differ in size depending on the source as well. It is also a polymer composed of α-(1,4)-linked D-glucose units. Since it has a highly tight structure, amylose is more resistant to digestion than amylopectin (an important form of resistant starch), more readily crystalized, insoluble in cold water, and can render the starch hard to be infiltrated by water.(S. J. Wang, Li, Copeland, Niu, & Wang, 2015) In fact, Corn contains 25% amylose; rice around 20%, and wheat between 20 and 30% (Hegenbart, 1996).

1. Amylose/Amylopectin Content

Amylose content is a factor of interest when using starches in food processing because amylose affects the texture and quality of the end product. Moreover, it has been shown that starches with high amylose content have a nutritional importance since it is digested slower and contributes to resistant starch. One important property of starch is its ability to absorb water which leads to gelatinization and loss of granular structure, and as mentioned before in contrast to amylopectin amylose tends to retard the process (Jane et al., 1999; Kim, Patel, & BeMiller, 2013; Lii, Tsai, & Tseng, 1996; Sajilata, Singhal, & Kulkarni, 2006). Thus, amylose and swelling power are negatively correlated. Blazek and Copeland (2008) investigated the influence of amylose content on pasting properties of wheat flour. This study showed that with the increase in total amylose content the peak (indicating lower swelling power) and final viscosities decreased as well as the breakdown. However, the longer the amylopectin chains the more they contribute to the gelling of starch and therefore to the increase in peak and final viscosity. Thus, the amylose-amylopectin ratio of starch influences the swelling and pasting properties of starch. Actually, El-Khayat, Samaan, and Brennan (2003), found negative linear relations between amylose content of durum wheat starch and peak viscosity and breakdown. Li, Shoemaker, Ma, Shen, and Zhong (2008) also found that for rice starch, the pasting onset and pasting properties correlated positively with amylose content.

D. Pasting Properties

Rheological properties are the study of how fluids materials react when subjected to stress. Fluids exist in many types, which can be separated into simple or structured fluids. Simple fluids are material formed of one phase such as water. Structured fluids are comprised of several phases such as solid particles dispersed in a liquid. Most foods are structured fluids, starch semisolid particles mixed with water; it has multiple phases and exhibit complex fluid behavior (Rao, 2014). The behavior of structured fluids is affected by many factors such as the viscosity of the liquid phase forming dispersion, particle size, shape, concentration, and the continuous phase. When structured fluids are subjected to stress, they do not exhibit a Newtonian flow, which means that the stress applied and the flow do not have a linear relationship (Rao, 2007a, 2007b; Sahin & Sumnu, 2006).

Mostly, starch testing consists of mixing the starch or flour with water while heating and cooling it in order to monitor the viscosity. This yields a characteristic curve called the pasting curve. The pasting properties, or the shape and size of the curve depend on the type of starch being tested (Ragaee & Abdel-Aal, 2006; N. Singh, Singh, Kaur, Singh Sodhi, & Singh Gill, 2003). Starch granules, when unmodified, are insoluble in water. When heated beyond a certain temperature, they start absorbing water and swell to many times their original size. This marks the start of an irreversible process called gelatinization. This process consists of crystalline melting, loss of birefringence, unwinding of double helices, breakage of hydrogen bonds, and the solubilization of starch in water (Ratnayake & Jackson, 2008; Sullivan & Johnson, 1964).

To understand the gelatinization process, a starch pasting cell can be used to test the viscosity of a sample so as to quantify the pasting properties. When the temperature rises beyond a certain critical temperature called the gelatinization temperature, starch granules start absorbing water and swelling which causes shear forces between the swollen granules. This yields the viscosity to increase. This temperature is called the pasting temperature (PT) and is the minimum temperature required for the specific type of starch being tested to cook. This gives an idea about the manufacturing processes of the starch, it indicates if other ingredients are soluble at this temperature and how much energy is needed, hence the cost (Bao, 2008; Brennan, Suter, Luethi, Matia-Merino, & Qvortrup, 2008; Krog, 1973).

When the starch starts cooking, the granules swell which causes the viscosity to increase rapidly over a range of temperatures. This range depends on the heterogeneity of the granules and is reflected by how steep the rise in viscosity is. The more heterogeneous they are, the less steep it is. The granules keep on swelling with the rise in temperature until they rupture. The Crystalline structure of amylose and amylopectin is lost, and the soluble amylose starts leaching out into the water and in some cases the amylopectin follows but at a lower rate (Olkku & Rha, 1978; Tester & Morrison, 1990).

Once gelatinization occurs, pasting process takes place. The swelling of granules and amylose/amylopectin leaching causes the viscosity to increase rapidly. At the equilibrium point of the polymer leaching and swelling the peak viscosity (PV) is attained. The rupture of granules follows and the alignment of the polymers, this causes the viscosity to decrease. Accordingly, two pasting properties can be extracted: the peak viscosity and peak time (Pt). Each starch has a different water-binding capacity where the peak viscosity gives a good indicator to how well the mixture can bind to the water. This reflects on the product's final quality and the load/torque required by the mixing cooker (Crosbie, 1991; Olkku & Rha, 1978).

The sample is subjected to a constant temperature and mechanical shear for a period of time. During this time, the granules are further disrupted and the polymers continue to leach out and align, which causes the viscosity to decrease till it reaches a minimum value. This viscosity is one of the pasting properties, known as holding strength, hot paste viscosity (HPV) or tough. For many processes, how the material withstands the shear stress and high temperature is important, and is shown by the rate and extent of the reduction in viscosity from peak till it reaches the trough. This breakdown (BDV) is affected by the holding temperature, the mechanical shear stressed applied, and the nature of the sample (Han & Hamaker, 2001).

After the holding period, the the mixture cools down causing the re-association of amylose and the formation of a gel. When the mixture cools, the viscosity increases and stabilizes at a final viscosity (FV). This pasting property is the most commonly used, it indicates how well this type of starch forms a viscous paste or gel after cooking and cooling (Alcazar-Alay & Meireles, 2015).

The Setback (SBV) is associated with the re-ordering of the starch molecules also known as retrogradation. The setback viscosity is measured by calculating the difference between the final viscosity and the trough usually, using the following equation:

$$SBV = FV - HPV \qquad (1)$$

However, in some cases such as rice, the final viscosity does not plateau thus it is measured as the difference between the final and peak viscosity. Setback can be very apparent and is associated to the texture of the final product and the tendency of amylose in a starch paste to retrograde. It is also correlated to syneresis, which is the expulsion of the liquid from the gel. A high setback indicates high synergistic effects during freezing and thawing cycles (S. J. Wang et al., 2015). Much attention is given to retrogradation since it has a strong effect on end products. To give a more detailed definition of retrogradation, after heating the starch granules in the presence of water, as mentioned before the crystalline structure is lost and amylose and amylopectin leach out, as the cooling process starts amylose and amylopectin chains re-associate into a new ordered structure. Here the paste viscosity increases with gel formation, exudation of water, and reforming of a crystalline structure different than that of the granules. Thus retrogradation results in the formation of a gel consisting of a three dimensional network. Starches with high amylose content form strong elastic gels, and waxy starches form soft gels (Bao, Shen, & Jin, 2007; Gudmundsson, 1994).

Retrogradation of starch (specifically amylopectin) is related to bread or starch based foods' staling, which makes its retardation and inhibition extremely relevant on an industrial level(Miles, Morris, Orford, & Ring, 1985). However, studies have shown that retrogradation can have a positive effect on human nutrition. Starch is digested enzymatically in the upper gut of the digestive system. When it is digested rapidly it causes the rapid release of glucose into the bloodstream which causes detrimental effects triggering the increase of risk for diabetes, cardiovascular disease, and cancer. Conversely, when starch is digested slowly or is undigested, such as resistant starch, the glucose is not rapidly released in the bloodstream. This leads to a better control of blood glucose, and has prebiotic effects by promoting the colonic microflora. The rapid retrogradation of amylose causes the digestibility of starch to decrease, as well as the retrogradation of amylopectin at a slower rate causes the gradual decrease in digestibility of starch. This is explained by the fact that retrogradation leads to the formation of crystallites and double helices that increase the resistance of starch to enzymatic hydrolysis (Annison & Topping, 1994; Chung, Liu, Lee, & Wei, 2011; Eerlingen, Jacobs, & Delcour, 1994; Fuentes-Zaragoza, Riquelme-Navarrete, Sánchez-Zapata, & Pérez-Álvarez, 2010; Oladele, 2016; S. Wang & Copeland, 2013). In addition, retrogradation is influenced by many factors such as water content, the type of starch, storage condition, and food components and additives such as salts (Ambigaipalan, Hoover, Donner, & Liu, 2013; S. J. Wang et al., 2015).

Starch pastes are shear-thinning, they exhibit a non-Newtonian viscosity. When the shear-thinning rate is increased the paste's viscosity decreases. They also are thixotropic which means that at constant shear rates the viscosity is reduced. Therefore, the measurement of pasting properties depends on several factors such as concentration, the starch's nature, heating and cooling rates, holding time and temperature.

1. *Factors Affecting Pasting Properties:*

The gelatinization and pasting properties of starch can be affected by many factors. The source of the starch, such as cereal grains, roots or tubers, waxy or non-waxy, amylose/amylopectin content, and size of granules plays a role in defining the pasting properties of the starch. In addition to the source, the conditions under which the starch is being tested affect the pasting curve. Cooling/ heating rates, shear rate, sample preparation can highly affect the gelatinization of starch. Moreover, starch modification by any chemical process such as hydrolysis or enzymatically alters the pasting properties of the starch dramatically in order to suit a particular application. Finally, starch pasting can be intensely modified by the interaction of the starch with its environment. (J. Singh, Kaur, & McCarthy, 2007)This means that the addition of any type of ingredient, whether sugars, lipids, emulsifiers, gums, proteins, or salts causes variations in observed pasting properties of starch. It is affected by any additional component affecting water activity (BeMiller, 2011; Jyothi, Sasikiran, Sajeev, Revamma, & Moorthy, 2005; Shi & BeMiller, 2002). For example gluten, a protein present in different types of flours can reach 12 to 14% in hard wheat flours. In fact, Chen, Deng, Wu, Tian, and Xie (2010) found that gluten influences the pasting properties of starch considerably, when an increase in the addition of gluten in wheat flour was found, peak viscosity, trough viscosity, final viscosity, setback, and peak time were shown to have significant downtrends. However, pasting temperature and pasting time were not affected by the addition of gluten(Lund & Lorenz, 1984; Rooney & Pflugfelder, 1986).

Although the effects of salts on gelatinization of starch have been studied extensively, there is scarce literature on the effect of the addition of K_2HPO_4 and its synergistic effect with NaCl. Moreover, considerable confusion is found between the literatures on how salts alter the pasting properties of starch.

Oosten (1990) examined the effect of sodium chloride on the initial gelatinization temperature of corn starch granules, with and without the addition of sodium hydroxide. His results showed that the initial gelatinization temperature increased in the presence of low concentrations of sodium hydroxide, but then the trend reversed, with sodium hydroxide destabilizing the granules as its concentration increased. His results showed that the initial gelatinization temperature increased in the presence of low concentrations of sodium hydroxide, but then the trend reversed, with sodium hydroxide destabilizing the granules as its concentration increased. Oosten (1982) examined the effect of sodium chloride on gelatinization temperature of corn starch, with and without the addition of sodium hydroxide. His results showed that the initial gelatinization temperature increased in the presence of low concentrations of sodium chloride, but to a certain extent. However, upon adding sodium hydroxide to the solution, the gelatinization temperature increased significantly.

Rice flour is a very popular traditional ingredient in China, used as is or to manufacture novel products such as breakfast cereals, puffed grains, and snacks. Phosphates are extremely used in the manufacture of rice products especially rice noodles and rice vermicelli which are widely consumed in China. Therefore, W.S. WU1 (2011) investigated the effect of phosphates (sodium phosphate dibasic, sodium tripolyphosphate, and sodium trimetaphosphate) on the pasting properties of rice flour from waxy and non-waxy varieties. The addition of phosphates to non-waxy rice flour significantly lowered the peak viscosity, trough viscosity, final viscosity, and the set back. Reduced final viscosity and lower setback shows that the addition of phosphates retards retrogradation and reduces synersis of non-waxy rice flour. In the case of waxy rice flour, the addition of phosphates significantly decreased pasting viscosities, and with the increase in the amount of phosphates added, peak viscosity and final viscosity decreased significantly. However, tough, breakdown, and setback were not dependent on the amount of phosphates added. The decrease in peak viscosity suggests that the starch granules did not swell as much as in the control before rupturing; this might be explained by the presence of the negatively charged phosphates. This study showed that the addition of phosphates significantly changes the pasting properties of rice flour, which is possibly due to the formation of a strong network between phosphates and starch.

Zaidul, Yamauchi, Kim, Hashimoto, and Noda (2007) studied the pasting properties of wheat flour mixed with high phosphorus (HPS), medium phosphorus (MPS), and low phosphorus (LPS) potato starches using a rapid visco analyzer (RVA). The peak viscosity was found to be highest in HPS-wheat mixture, followed by MPS-wheat and LPS-wheat, with significance in the difference between them. Since phosphorus is covalently bonded to amylopectin, high amounts of phosphorus indicate a high concentration of amylopectin and lower amylose content. The lower amounts of amylose may be behind the rise in peak viscosity in HPS-wheat mixture. Another reason is the higher swelling power of starch granules in potato starches. This means that with the increase of potato starch in the mixtures the peak viscosity increased. Peak time decreased with the increase in the amount of phosphorus present in mixture as well as with the increase in potato starch. At 50% potato starch, which means at a constant concentration of potato starch, peak time was shorter in HPS, followed by MPS, then LPS. With the increase of phosphorus and amount of potato starch the breakdown and trough viscosities increased. The final viscosity followed the same trend. Hence, with the increase in phosphorus content the peak viscosity, breakdown, and final viscosity increased significantly. The setback viscosity followed the reverse trend, and the differences between HPS-wheat and MPS-wheat were not significant at 10-30% potato starch. At 10-50% potato starch, the differences between HPS-wheat and LPS-wheat and between LPS-wheat and MPS-wheat in peak viscosities were significant.

Lu, Donner, Yada, and Liu (2012) investigated the synergistic effects of amylose and phosphorus on rheological, thermal and nutritional properties of potato starch and gel. In fact, results of this study showed that with the increase in phosphorus content the granular structure is less rigid, which makes it easier to rupture and to swell into larger granules causing a higher peak viscosity and breakdown. Nutting (1952) explained that this increase in peak viscosity can be related to the ionization of phosphate groups when the starch is pasted causing it to charge negatively; thus creating columbic repulsion and stabilizing the amylopectin in the water. The results of this study showed that a higher content of phosphorus produces a more rigid and well-formed gel, and enhances the retrogradation. Higher contents of phosphorus are usually related to high amount of amylopectin with long chains, which are responsible for the increase in retrogradation. In addition, since phosphates are present in relatively low amounts and since during cooling starch mobility is decreased, the negatively charged ions and their columbic repulsion do not affect the retrogradation. This in turn decreases the salvation of amylopectin and enhances its re-crystallization into a rigid gel. Moreover, other than the enhancement in re-crystallization during retrogradation, higher amounts of phosphorus decreases the digestibility of potato starch (increases the amount of resistant starch), because they do not gelatinize readily when cooked. This in turn, results in lower glycemic index, lower blood glucose, and hence lower insulin. S. J. Wang et al. (2015) stated that NaCl addition can change starch gelatinization and retrogradation greatly, it actually decreases retrogradation when stored between 4 and 25^{o}C, however when starch is stored at -20^{o}C the addition of 5% NaCl increased retrogradation. M. J. Beck, M.; Becker, T studied the effect of NaCl and several other salts on the retrogradation of maize starch. The authors observed that NaCl delays retrogradation by binding Na^+ ions to amylopectin causing a high rate of re-crystallization. Likewise, M. Beck, Jekle, and Becker (2012) investigated the impact of NaCl on wheat flour dough for yeast-leavened products also. NaCl decreased retrogradation by inclusion of Na^+ ions in starch molecules.

Alamri, Hussain, Mohamed, Al-Ruquie, and Qasem (2015) investigated the effect of urea and NaCl on the pasting properties of corn starch. The results showed that the peak viscosity, pasting temperature, increased significantly with the addition of NaCl. The authors stated that NaCl prevented amylose-amylose interaction.

E. Objective

Phosphorus not only is a major mineral for bone and teeth formation, a contributor in tissue and cell renewal, regeneration, and repair, but it also takes part in controlling food intake, hence obesity and degenerative diseases. Cereal flours and starches, which are major contributor to the energy intake of people especially in the poorer countries, usually contain traces of phosphorus. However, the form of phosphorus in these staple foods is not bioavailable for the human body. In this study, we investigated the effect of enriching wheat, rice, and corn flours with di-potassium phosphate (K_2HPO_4), a bioavailable source of phosphorus at 0.25, 0.5, 1, and 2 percent. In addition, we explored the synergistic effect of K_2HPO_4 on the pasting properties of flours when added with sodium chloride (NaCl) at 0, 1 and 2 percent.

CHAPTER 2
MATERIALS AND METHODS

A. Materials

Wheat, rice and corn flours were purchased from Bob's Red Mill Natural Foods (Milwaukie, OR, USA) and used without any further treatment.

NaCl and K_2HPO_4 were purchased from Sigma Aldrich (St. Louis, Missouri, USA).

B. Methods

1. Chemical proximate analysis

The moisture content of the raw flour samples was measured according to the approved AACC 44-40 method (2011). The total nitrogen content was evaluated using Kjeltec™ model 8400 (FOSS, Denmark); the protein content was then calculated adopting 6.25 as a conversion factor. The amounts of amylose were determined using the "Amylose/Amylopectin Assay Kit" purchased from Megazyme (Co. Wicklow, Ireland) following the methods developed by (Yun & Matheson, 1990). Starch samples were first completely dispersed by heating in dimethyl sulphoxide (DMSO). After that lipids were removed by precipitating the starch in ethanol and recovering the precipitated starch. Then, the precipitated sample was dissolved in an acetate/salt solution. In order to remove amylopectin; the sample was first precipitated by the addition of Con A (lectin concanavalin A) and then centrifuged. The amylose, in an aliquot of the supernatant, was enzymatically hydrolyzed to D-glucose, which was analyzed using glucose oxidase/peroxidase reagent. The total starch, in a separate aliquot of the acetate/salt solution, was similarly hydrolyzed to D-glucose and measured colorimetrically by glucose oxidase/peroxidase. The concentration of amylose in the starch sample was estimated as the ratio of GOPOD absorbance at 510 nm of the supernatant of the Con A precipitated sample to that of the total starch sample.

Amylose, % (w/w)

= Absorbance (Con A Supernatant)/ Absorbance (Total Starch Aliquot) x 66.8 (2)

All chemical analyses were run in triplicates.

2. *Pasting properties*

The pasting properties of the samples were measured using a Discovery Hybrid Rheometer DHR-3 (TA Instruments, New Castle, DE, USA) equipped with a Starch Pasting Cell (SPC). The pasting properties of the flours were determined at 0, 0.25, 0.5, 1, and 2% (w/w flour based) K_2HPO_4 as well as 0, 1, and 2% (w/w flour based) NaCl following the methodology described by AACC 76-21.01 (2000) while adjusting both powders and water to 14% moisture basis. The dispersions were first conditioned for 1 min at 50 °C while stirring at 16.755 $rad \cdot s^{-1}$ before being subjected to a flow temperature ramp at a rate of 6 $°C \cdot min^{-1}$ and stirring speed of 16.67 $rad \cdot s^{-1}$, then holding for 10 min at 95^oC. After that the dispersion were cooled to 50 °C at a flow rate of 6 $°C \cdot min^{-1}$ and a speed of 16.67 $rad \cdot s^{-1}$; and finally holding at 50 °C for 5 min while stirring. Viscosity-time and viscosity-temperature profiles of the suspensions at the various dipotassium phosphate and sodium chloride concentrations were recorded during heating and subsequent cooling. Consequently, the following parameters were obtained from the resulting curves: peak viscosity (PV), viscosity at trough (also known as minimum viscosity after peak, MV), final viscosity (FV), pasting temperature and peak time were obtained using the TRIOS Software v4.1 (TA Instruments, New Castle, DE, US) and OriginPro 9.1 (OriginLab Corporation, Northampton, MA, USA). In addition, the breakdown viscosity (BDV) and setback viscosity (SBV) were calculated using equation 1. All procedures were performed in at least triplicate.

3. Statistical analysis

All experiments were carried out in triplicates. The data presented corresponds to the means and standard deviations of each experiment. A one-way analysis of variance (ANOVA) and Tukey's test were used to establish the significance of differences among the means with 95% confidence level. The statistical analyses were performed using OriginPro 9.1 (2014) (OriginLab Corporation, Northampton, MA, USA).

CHAPTER 4
RESULTS AND DISCUSSION

A. Proximate Analysis

The proximate analysis results including amylose content of wheat, corn, and rice flours are summarized in table 1.

Table 1: Proximate Composition of Wheat, Corn, and Rice flours (mean ± standard deviation; N=3)

	Moisture (%)	Protein (%) (6.25xN)	Fat (%)	Ash (%)	Amylose content (%w/w)
Wheat flour	12.85 ± 0.06	13.30 ± 0.13	4.47 ± 0.35	0.55 ± 0.02	26.75 ± 3.2
Corn flour	11.06 ± 0.04	8.41 ± 0.06	3.30 ± 0.17	1.29 ± 0.02	27.59 ± 1.2
Rice flour	11.85 ±0.01	6.69 ± 0.11	2.51 ± 0.14	0.55 ± 0.07	21.17 ± 2.1

B. General Discussion

Starch pasting or gelatinization is water dependent(Rolee & Le Meste, 1997). Some literature suggests that adding a solute to water-starch suspension will decrease the amount of water available for gelatinization by competing with the starch. However, in the case of NaCl, the water available decreased with the increase in its concentration, but the effect on gelatinization did not conform to the theory (Eleni Chiotelli, Rolée, & Le Meste, 2000; D'appolonia, 1972; Evans & Haisman, 1982; Spies & Hoseney, 1982).

Water enhances the loss of the crystalline structure of starch. It acts as a plasticizer by reducing the glass transition temperature of the amorphous part. Thus, adding a solute to water can either enhance its plasticizing effect or can have an anti-plasticizing effect on starch. Moreover, water is a mixture of hydrogen bonded parts and free water molecules. Hence, high charge ions can interact with water molecules more readily, reducing the free water available for starch gelatinization. Moreover, the size of the ions also plays a role in the hydration of starch granules. At high concentrations of NaCl, the viscosity increases, while at high concentration of KCl the viscosity decreases. This is due to the fact that K^+ is smaller than Na^+. Nonetheless, starch gelatinization does not only depend on water-salt interactions, but is also affected by the interactions between salt and starch (E. Chiotelli, Pilosio, & Le Meste, 2002).

Starch is a weak acidic ion exchanger containing many OH-groups. When pure starch is suspended in water, alcohol molecules dissociate and increase the concentration of H^+ in the starch. This creates a gradient between the water phase and the starch which will cause the H^+ molecules to migrate from granules to the water until equilibrium is reached. Thus, at this stage, the starch is negatively charged and the water phase is positively charged. This difference in potential is called the Donnan potential. It comes from the dissociation of chemical groups into ions free to move around and one ionic group attached to a still structure, the starch network (Oosten, 1982). The occurrence of the Donnan potential tends to prevent anions from entering the starch particles, and consequently stabilizes the granules. However, because of its electronegative nature, starch attracts cations, destabilizing its structure. This repulsion or attraction is proportional to the charge of the ion in subject. Moreover, anions have been qualified as the active gelatinizing agent since they are responsible for the breaking of hydrogen bonds between starch molecules

(Oosten, 1990). So by changing the environment, by adding salt, the Donnan Potential will play a role in determining the quality of the end product.

Oosten (1982) observed that at low concentrations of electrolytes, the cations are attracted by the negative charge of the starch and anions are repelled more. However, when the concentration of electrolytes increases, (Oosten, 1982) claims that the Donnan potential is reduced and therefor the driving force of anions is higher than the electrolyte exclusion effect. Taking the example of NaCl in Oosten (1990)'s study, at low concentrations, Na^+ cations enter the starch granules by attraction and concentration gradient, and the Cl^- anions are repelled by the Donnan potential into the water. Upon increasing the concentration of NaCl, driving force of anions becomes higher than the protective Donnan potential. In addition, as mentioned before, the repulsion or attraction forces are proportional to the charge density, which means that polyvalent anions are excluded more efficiently than monovalent anions (Oosten, 1990). Oosten (1982) also explained that if the environment contains an agent to bind with the anions released more cations will penetrate the starch granules resulting in a higher Donnan potential making the starch granules more stabilized.

C. Pasting Properties

1. Peak Viscosity

- Rice Flour

The peak viscosities of rice flour at different concentrations of NaCl and K_2HPO_4 are shown Figure 1. When K_2HPO_4 was added to the starch-water mixture, each di-potassium phosphate was converted to its composing ions, two K^+ and one HPO_4^{2-}.

Figure 1 shows that, at 0% NaCl and low concentrations of K_2HPO_4 (0.25 and 0.5% w/w), the peak viscosity decreased. However, the peak viscosity increasesd when higher concentrations of K_2HPO_4 were added (1 and 2% w/w).

As explained earlier, at low concentrations of salt, the negative Donnan Potential repelled the anions (HPO_4^{2-}) protecting the starch from destabilization, and attracted the positive K^+ cations. This rendered the starch granules more stable and less susceptible to swell and absorb water. However, upon increasing the K_2HPO_4 concentration in water phase, the gradient in the concentration of K^+ decreased reducing the Donnan potential. Hence, at high concentrations of K_2HPO_4 the driving force of anions was more powerful than the repulsing force of the Donnan potential. In this case, at 1 and 2% K_2HPO_4, the HPO_4^{2-} entered the starch granules and induced gelatinization, increasing the peak viscosity since K^+ replaced H^+ ions; this made the starch molecules dissociate faster and more efficiently. Another reason behind the decrease in viscosity may have been the reduction in water available for starch. HPO_4^{2-}, being a divalent ion, bonded with water molecules readily reducing the free water available for the swelling of granules.

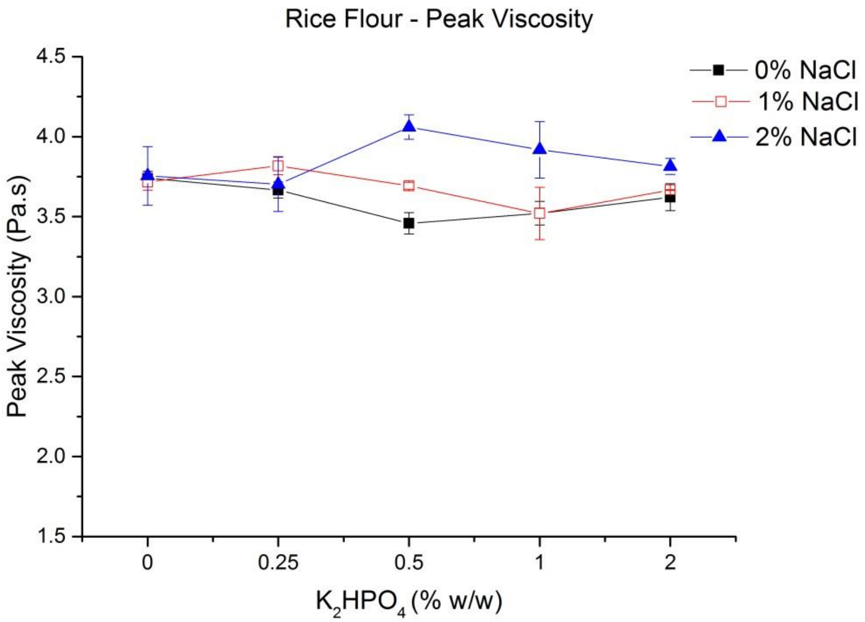

Figure 1: Peak Viscosity (mean N=3, ± standard deviation) of Rice flour as function of K_2HPO_4 and NaCl

As shown in Figure 1, the peak viscosity at 2% NaCl decreased when 0.25% of K_2HPO_4 was added due to the elevation in the Donnan potential. On the other hand, the peak viscosity at 0% NaCl increased due to the excess in cations. When HPO_4^{2-} were present in high amounts in water (1 and 2%), the phosphate anions bonded with the water available for the granules to absorb and swell, hence decreased the peak viscosity.

Comparing the three curves in figure 1, we can see that at 0% K_2HPO_4, the peak viscosity decreased slightly when 1% NaCl was added, and then increased slightly at 2% NaCl. This conforms to the Donnan potential theory. Then when K_2HPO_4 was further augmented, we can see that the peak viscosity does not follow a specific trend. This can be explained by the fact that Na^+ is smaller than K^+, which makes its effect more dominant since it can enter the starch molecules more easily. Another reason is the presence of a divalent anion that can direct the force of the electric field and affect more the gelatinization. W.S. WU1 (2011) observed a decrease in peak viscosity of waxy and non-waxy rice flour at 0.1, 0.2, and 0.5% (w/w dry basis) of phosphates addition. This correlated with our data since, as we concluded, at low concentrations of phosphates the peak viscosity decreased.

- Wheat Flour

Wheat flour contains amylopectin and amylose, but it also contains another macromolecule, gluten, which is mixture of proteins. In fact, considering the proximate analysis (Table 1), the wheat flour used in this study contained around 13.30% protein (hard wheat). Chen et al. (2010) showed in his study that gluten influenced considerably the pasting properties of wheat starch; gluten was observed to decrease the peak viscosity.

Looking at the trends in Figure 2 we can see that the addition of salts increased the peak viscosity of wheat. This can be related to the formation of bonds between the anions (especially the divalent HPO_4^{2-}) and the gluten matrix which inhibited the influence of gluten on pasting properties of starch, and thus increased the peak viscosity. Additionally, since anions of HPO_4^{2-} were combining with gluten the increase of the salt increased the gradient of cations between starch and water which subsequently increased the Donnan potential, the dissociation of starch, and therefore the peak viscosity. Likewise, Ragaee and Abdel-Aal (2006) evaluated the pasting properties of soft and hard wheat. Soft wheat, which has lower protein content, exhibited a higher peak viscosity than hard wheat. At 1 and 2% NaCl, A. Kaur (2011) observed an increase in peak viscosity after the addition of NaCl and different types of salts with the exception of substituting all NaCl with $CaCl_2$. In addition, it was shown that at lower levels of substitutions of NaCl with other salts the peak viscosity was highest. Zaidul et al. (2007) also reported an increase in peak viscosity when wheat flour was mixed with high phosphorus potato starch.

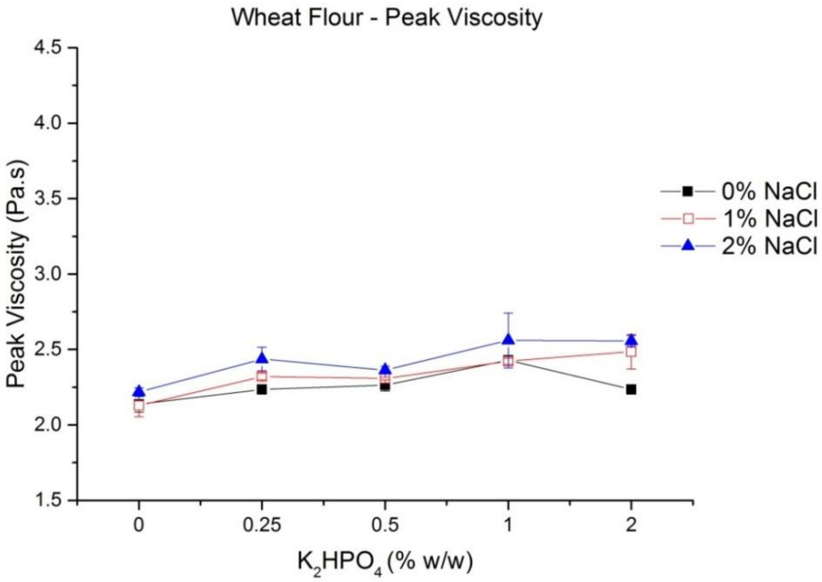

Figure 2: Peak Viscosity (mean N=3, ± standard deviation) of Wheat flour as a function of K_2HPO_4 and NaCl

- <u>Corn Flour</u>

The results of the peak viscosities of corn flour reported in figure 3 show that at 1% NaCl, and at a low concentration of 0.25% K_2HPO_4 the peak viscosity decreased. Then with the increase in K_2HPO_4, the peak viscosity was elevated.

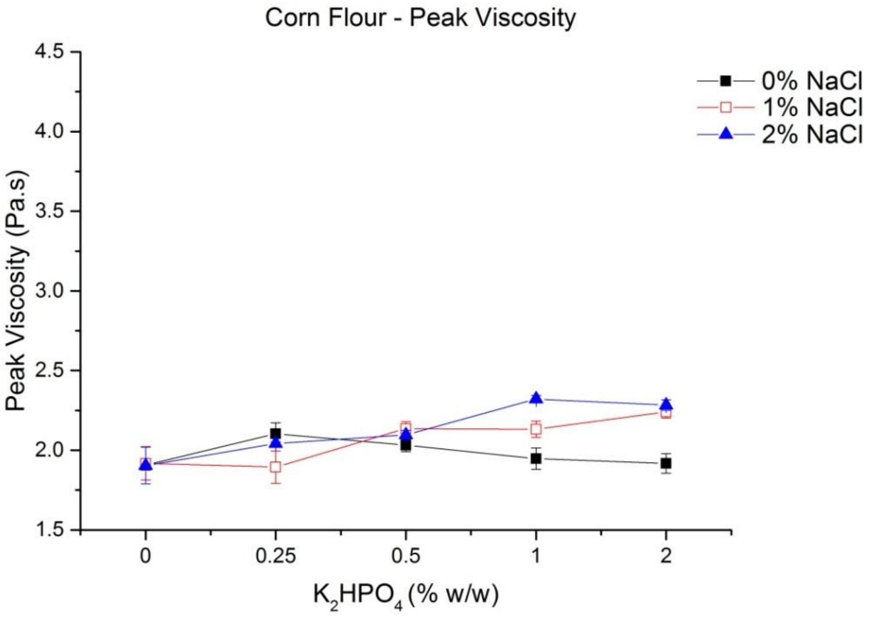

Figure 3: Peak Viscosity (mean N=3, ± standard deviation) of Corn flour as a function of K_2HPO_4 and NaCl

These results conform to the Donnan potential theory. However, at 2% NaCl, the peak viscosity increased with the addition of K_2HPO_4. This also follows the Donnan potential theory since at high amounts of salts; the Donnan potential is reduced letting the anions in the solution to penetrate the starch granules. At 0% NaCl, the peak viscosity increased at the addition of 0.25% K_2HPO_4 then decreased with further addition. This can be related to the high amylose content of corn flour (27.59 %) or amorphous part of the starch granule which makes it harder for the cation K^+ to penetrate the crystalline part especially since K^+ is a big ion, but does not have an effect over the divalent anion HPO_4^{2-}.

In point of fact, rice has a higher content of amylopectin hence the higher viscosity. Nevertheless, when K_2HPO_4's amount in solution increased the viscosity decreased because of the ability of the divalent HPO_4^{2-} to form bonds with the free water and decreased its availability for gelatinization. Oosten (1982) utilized corn starch to hypnotize and test the Donnan Potential. However, our data show that with the increase in NaCl, the peak viscosity increased. Similar results were reported by Alamri et al. (2015). The authors stated that NaCl prevented amylose-amylose interaction, causing an increase in peak viscosity. The peak viscosity increased at 0% NaCl when 0.25% K_2HPO_4. Since corn flour has a high amount of amylose, and since K^+ is considered a big cation, at low concentrations it did not penetrate the starch and act as a protective agent for the starch granules. However, at 1 and 2% NaCl, the peak viscosity decreased at first then increased. This is due to the fact that Na^+ is a smaller cation that has a higher penetration potential than K^+, and was able at low concentrations to increase the Donnan potential and hence the peak viscosity.

2. *Peak Time*

- Rice Flour

As Table 5 shows, at 0% NaCl, the peak time increased significantly when 1% of K_2HPO_4 was added to rice flour. However, at 1% NaCl, the amount of K_2HPO_4 needed to increase the peak time significantly declined to 0.5%. Moreover, at 2% NaCl, the peak time significantly increased at the addition of only 0.25% K_2HPO_4. This highlights the synergistic effect NaCl and K_2HPO_4 had on the peak time of rice flour. This may be related to the reduction in water available for the granules to absorb and swell, HPO_4^{2-} bonded with free water molecules, which retarded the hydration and dissociation of starch molecules. In addition, when the amount of salt in the solution was elevated, the mobility of water decreased.

Table 2: Reported Peak time (min) of Rice flour at different concentrations of NaCl and K_2HPO_4 (mean ± standard deviation)

%K_2HPO_4 %NaCl	0	0.25	0.5	1	2
0	8.50 ± 0.00^{a}	$8.50 \pm 0.00^{a,1}$	$8.50 \pm 0.00^{a,1}$	$8.67 \pm 0.00^{a,b}$	8.76 ± 0.00^{b}
1	8.50 ± 0.00^{a}	$8.50 \pm 0.00^{a,1}$	$8.76 \pm 0.00^{b,2}$	8.76 ± 0.00^{b}	8.76 ± 0.00^{b}
2	8.51 ± 0.00^{a}	$8.76 \pm 0.00^{b,2}$	$8.75 \pm 0.00^{b,2}$	8.76 ± 0.00^{b}	8.92 ± 0.14^{b}

Different letters indicate significant differences between groups per column ($P<0.05$) (ANOVA, Tuckey's HSD).
Different numbers indicate significant differences between groups per row ($P<0.05$) (ANOVA, Tuckey's HSD).

- Wheat Flour

For wheat flour, when no NaCl was added, at 0.5 and 1% K_2HPO_4 the peak time increased (Table 6), since when low concentrations of salt were added the Donnan potential attracted cations and repelled anions that are considered a gelatinizing agent. However, when 2% K_2HPO_4 were added, the peak time decreased (Table 6); this is explained by the decrease in the Donnan potential with the increase of salt concentration.

At 1% NaCl, when low concentrations of K_2HPO_4 were added, the peak time was not affected (Table 6), since the HPO_4^{2-} ions were forming bonds with gluten. However, with the increase in K_2HPO_4, more HPO_4^{2-} was available and hence formed hydrogen bonds with free water delaying the hydration of starch granules.

At 2% NaCl, the concentration of the water-salt solution was high enough, with the addition of K_2HPO_4, water for granular swelling decreased significantly and increased the time needed for the granules to swell and reach peak viscosity. Chen et al. (2010) observed a downward trend in peak time with the increase of gluten. This means, when K_2HPO_4 increased, more bonds formed with gluten reducing its effect as a gelatinization agent and on peak time. However, Zaidul et al. (2007)'s results contradicted the results of this study. The authors observed a decrease in peak time with the increase in phosphates.

Table 3: Reported Peak time (min) of Wheat flour at different concentrations of NaCl and K_2HPO_4 (mean ± standard deviation)

$\%K_2HPO_4$ / %NaCl	0	0.25	0.5	1	2
0	8.51 ± 0.00^{a}	$8.50 \pm 0.00^{a,1}$	$8.76 \pm 0.00^{b,1}$	$8.76 \pm 0.00^{b,1}$	$8.50 \pm 0.00^{a,1}$
1	8.51 ± 0.00^{a}	$8.50 \pm 0.00^{a,1}$	$8.50 \pm 0.00^{a,2}$	$9.01 \pm 0.00^{b,2}$	$9.01 \pm 0.00^{b,2}$
2	8.50 ± 0.00^{a}	$8.75 \pm 0.00^{b,2}$	$8.76 \pm 0.00^{b,1}$	$9.01 \pm 0.00^{c,2}$	$9.01 \pm 0.00^{c,2}$

Different letters indicate significant differences between groups per column ($P<0.05$) (ANOVA, Tuckey's HSD).
Different numbers indicate significant differences between groups per row ($P<0.05$) (ANOVA, Tuckey's HSD).

- <u>Corn Flour</u>

For corn flour, as the results in Table 7 show, the increase in K_2HPO_4 increased the time for the starch granules to hydrate and swell. This can be explained by the increase in the salt concentration and decrease in the free water available. Additionally, as mentioned before, the amylose content of corn flour (amorphous part) was high enough which also made it harder for water to affect amylopectin the crystalline part of granules.

Table 4: Reported Peak time (min) of Corn flour at different concentrations of NaCl and K_2HPO_4 (mean ± standard deviation)

$\%K_2HPO_4$ / %NaCl	0	0.25	0.5	1	2
0	8.01 ± 0.00^{a}	8.25 ± 0.00^{b}	$8.42 \pm 0.14^{b,c}$	8.51 ± 0.00^{c}	8.76 ± 0.00^{d}
1	8.00 ± 0.00^{a}	8.26 ± 0.00^{b}	8.25 ± 0.00^{b}	8.50 ± 0.00^{c}	8.76 ± 0.00^{d}
2	8.00 ± 0.00^{a}	8.25 ± 0.00^{b}	8.25 ± 0.00^{b}	8.50 ± 0.00^{c}	8.76 ± 0.00^{d}

Different letters indicate significant differences between groups per column (P<0.05) (ANOVA, Tuckey's HSD).
Different numbers indicate significant differences between groups per row (P<0.05) (ANOVA, Tuckey's HSD).

3. *Final Viscosity*

- Rice Flour

The final viscosity of rice flour, at different concentrations of NaCl, followed the same trend (figure 4). Tuckey test showed that at low concentrations of K_2HPO_4 that is at 0.25 and 0.5%, the final viscosity decreased significantly, which is also obvious in Figure 4. As explained in the general discussion, at low concentrations of salt, the cations replaced H^+ ions in the amylopectin molecules since they were attracted by the negative Donnan potential. Amylopectin with a cation linked to it dissociated faster and more efficiently. This rendered the ability of amylopectin to regain or rearrange into a more crystalline form weaker, which explains the decreased final viscosity. However, at high concentrations of K_2HPO_4 (1 and 2%), the final viscosity increased. This is also a consequence of the Donnan potential which increased with the increase in anions in solutions and the decrease of cations gradient between the starch and the water. However, with the rise in K_2HPO_4, the final viscosity increased (Figure 4). The higher driving force of anions repelled the amylopectin helping in forming a more rigid gel; hence the gelatinization process was much more efficient. Anions repelled the starch and stabilized its crystalline form upon cooling, increasing the final viscosity. Correspondingly, W.S. WU1 (2011) reported a decrease in final viscosity with low concentrations of phosphates. At 0% K_2HPO_4, the change in NaCl concentration did not affect the final viscosity. However, with the rise in K_2HPO_4, the final viscosity increased.

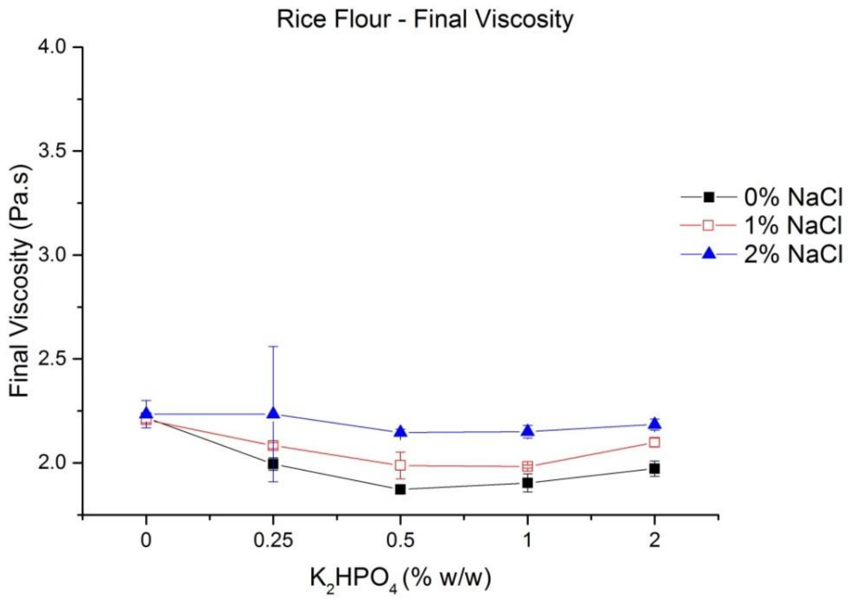

Figure 4: Final Viscosity (mean N=3, ± standard deviation) of Rice flour as a function of K_2HPO_4 and NaCl

- Wheat Flour

The data reported in Figure 9 show an increase in final viscosity with the increase in K_2HPO_4. When K_2HPO_4, was first added to wheat flour, HPO_4^{2-} ions formed bonds with the gluten. According to Chen et al. (2010); when gluten increased, the final viscosity decreased. Thus, the addition of K_2HPO_4 inhibited the downtrend of final viscosity caused by the gluten. According to Ragaee and Abdel-Aal (2006), soft wheat had a higher final viscosity, in contrast hard wheat had a lower final viscosity. In addition, HPO_4^{2-} bonded with water, decreasing the mobility of water and increasing the bonding between amylopectin molecules hence increasing the final viscosity even more. Likewise, Zaidul et al. (2007) observed an increase in final viscosity with the increase in phosphates.

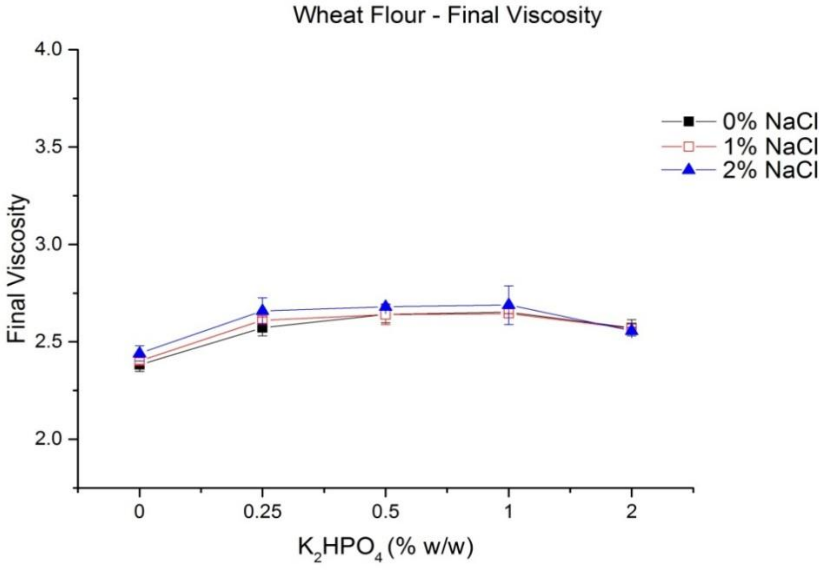

Figure 5: Final Viscosity (mean N=3, ± standard deviation) of Wheat flour as a function of K2HPO4 and NaCl

- Corn Flour

The final viscosity of corn flour decreased with the increase in K_2HPO_4 (Figure 6). K_2HPO_4 bonded with water decreasing its mobility and the amount of free water molecules. But at high concentrations of K_2HPO_4, the concentration of the divalent anion HPO_4^{2-} increased the repulsion between the negatively charged ions and gave place to amylopectin-amylopectin hydrogen bonding. As Figure 6 shows, increasing the amount of NaCl increased the final viscosity, and at 2% K_2HPO_4 the final viscosity gained an upward trend. Thus, adding salt especially NaCl and high amounts of K_2HPO_4, disrupted the amylose-amylose network making place to more amylopectin-amylopectin hydrogen bonding. Similarly, Alamri et al. (2015) reported an increase in final viscosity with the increase in NaCl.

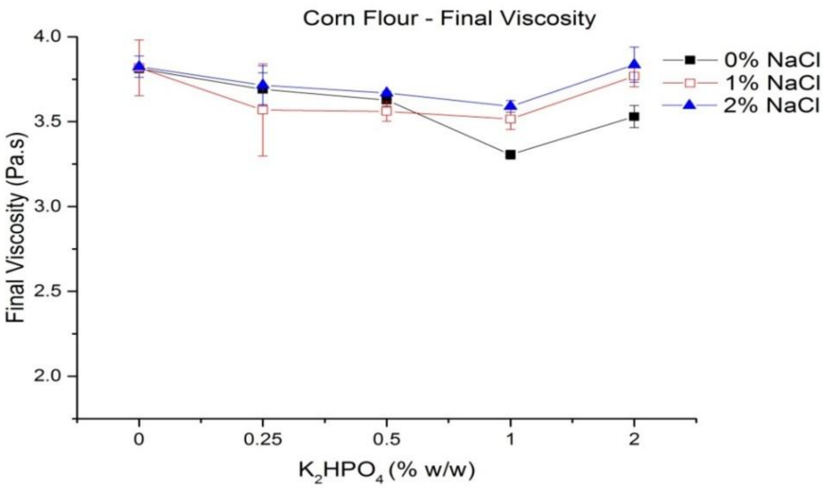

Figure 6: Final Viscosity (mean N=3, ± standard deviation) of Corn flour as a function of K2HPO4 and NaCl

4. *Setback Viscosity*

- Rice flour

The setback viscosity is a pasting property that reflects the re-crystallization of amylopectin into a more ordered structure by exclusion of water. As Figure 7 shows, at 0 and 1% NaCl, the setback of rice flour peaked at 0.25% K_2HPO_4, however at 2% NaCl the setback peaked at 0.5% K_2HPO_4 added.

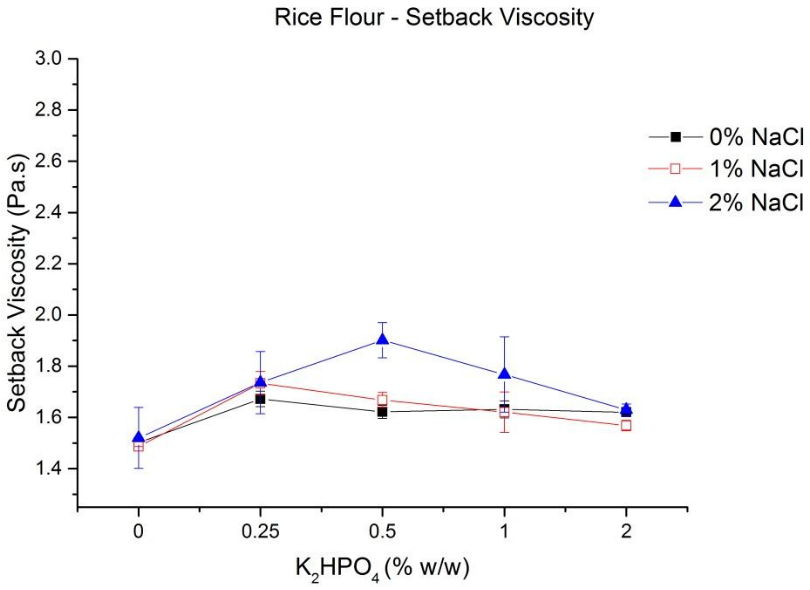

Figure 7: Setback Viscosity (mean N=3, ± standard deviation) of Rice flour as a function of K2HPO4 and NaCl

At 0% and 1% NaCl, the setback viscosity of rice flour followed the same trend with the increase in K_2HPO_4. However we can see a similar trend between three curves, when K_2HPO_4 was added the setback increased until it reached a peak then decreased (Figure 7).

At 0% NaCl, the retrogradation of rice flour increased significantly in the presence of phosphates. This correlates with the results of Lu et al. (2012), that the increase in phosphate ions increased the setback. The retrogradation is the re-crystallization of amylopectin into a new ordered structure, the presence of phosphates at low concentrations, enhanced the reordering process of amylopectin by bonding with water molecules and increasing the bonds between amylopectin. However, our data show a downward trend when the amount of salts were increasing (Figure 7). This can be due to the increased amount of cations especially Na^+, and the increase in HPO_4^{2-}, which helps in repelling Na^+ into the starch. This increased the solvation of amylopectin in water and decreased the hydrogen bonding of amylopectin. These data contradict the data observed by Samutsri and Suphantharika (2012), where the setback decreased with the addition of either NaCl or $CaCl_{2.}$

- Wheat Flour

The results of the wheat flour for the setback viscosity are shown in figure 8. At different concentrations of NaCl, the setback viscosity increased slightly with the increase in K_2HPO_4 until 2% were added, then the setback viscosity decreased. Thus, K_2HPO_4 can act as a staling agent inhibitor for wheat flour. In addition, during cooling starch mobility decreased, the negatively charged ions and their columbic repulsion repelled starch molecules and bonded to water helping the retrogradation of amylopectin. This in turn decreased the salvation of amylopectin and enhanced its re-crystallization into a rigid gel. Zaidul et al. (2007)'s, results showed that a higher content of phosphorus produced a more rigid and well-formed gel and enhanced the retrogradation as well. However, this may be related to the fact that higher contents of phosphorus in potato starch are usually related to high amount of amylopectin with long chains, which are responsible for the increase in retrogradation. As in Rice flour, the setback at high amounts of salts exhibited a downward trend (Figure 8).

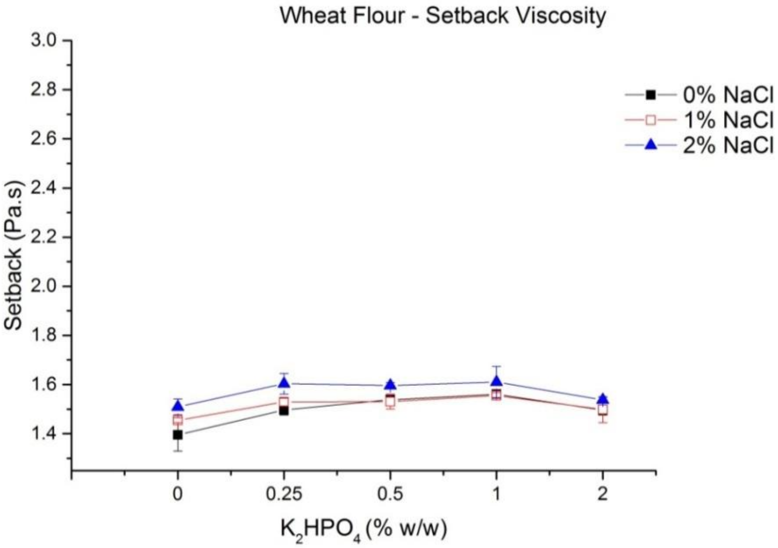

Figure 8: Setback Viscosity (mean N=3, ± standard deviation) of Wheat flour as a function of K_2HPO_4 and NaCl.

- <u>Corn flour</u>

As Figure 9 shows, in the case of corn flour, at different NaCl concentrations, the setback decreased with the increase in K_2HPO_4, until 2% is reached. This can be attributed to the high amylose content of corn flour (around 27.59% of total starch). In the case of corn flour, the amylose/amylopectin ratio played a crucial role in determining the pasting properties of corn flour. In fact, amylopectin is the polymer that contributes to the gelling of starch, thus the high amylose content, that is the low amylopectin content, could have been the source of the decrease in setback viscosity, decreasing also the effect of the divalent anions HPO_4^{2-} that bind to water molecules and repel the starch to help in the formation of hydrogen bonding between amylopectin polymers. The increase in salt content and the high amylose content decreased the mobility of water. Alamri et al. (2015) also observed a drop in setback with the increase in NaCl.

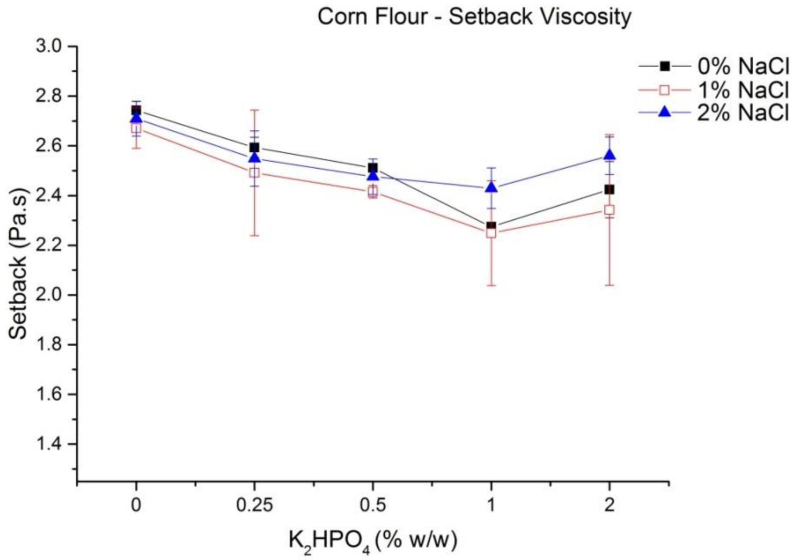

Figure 9: Setback Viscosity (mean N=3, ± standard deviation) of Corn flour as a function of K_2HPO_4 and NaCl

Note: The Breakdown and Pasting Temperature of the three flours did not exhibit a specific trend when adding Salts.

CHAPTER 5
CONCLUSION

Pasting properties provide a better understanding on the behavior of flours and starches during processing. In fact, the pasting temperature is the minimum temperature required to cook the starch in question; the peak viscosity shows the water-binding ability of the mixture as well as the power/torque required during manufacturing; the peak time is the time needed to initiate starch gelatinization; whilst the setback viscosity is an indication for retrogradation and syneresis usually correlated with quality of the end product, and its performance during freezing and thawing cycles. Furthermore, the final viscosity is associated to the quality and stability of the gel formed by the starch being tested. The pasting and gelatinization of starch can be affected by many factors such as the source of the starch, processing conditions, chemical modifications and added ingredients (i.e. salts). Based on the results of this study, it is clear that adding di-potassium phosphate with or without the presence of sodium chloride did not have any significant effect on all the pasting properties of wheat, corn, and rice flours. It is worthy to mention that although the changes are not significant ($P>0.05$) there seems to be a clear trend where the changes (especially in peak viscosity and final viscosity) are depending on the concentrations of di-potassium phosphate added. Additionally, sodium chloride and di-potasssium phosphate had synergistic effect increasing the peak time significantly. Moreover, the setback viscosity was observed to increase in wheat and rice flours with the increase in di-potassium phosphates added, however the trend was reversed in corn flour.

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