Production of spray-dried honey powder and its application in bread

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PRODUCTION OF SPRAY-DRIED HONEY POWDER AND ITS APPLICATION IN BREAD

A Thesis

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science

In

The Department of Food Science

by

Ahalya Kosal Ram
B.Tech., Vellore Institute of Technology University, 2009
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Dedicated to

Amma, Appa and Thangamal paatti
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ABSTRACT

Honey is a natural sweetener with various beneficial properties including anti-oxidant and anti-microbial activity. Retrograded starch is known to have hypocholesterolemic effects and act to inhibit fat accumulation. Honey powder produced using retrograded starch can be used as an alternative to sucrose in many bakery products like bread. The objectives of this study were to produce a honey powder containing retrograded starch, characterize the powder and use it as an alternative to sucrose in bread formulations. The honey powder was produced by spray drying honey using retrograded starch as a drying agent. The spray dried honey powder was characterized for moisture and sugar contents and color. Three bread formulations were prepared with (1) 100% liquid honey (HNY), (2) 50% substitution of sugar with honey powder (SHP) and (3) 100% honey powder (HP). A bread formulation prepared with only sugar was used as a control (S). Breads produced from all four formulations were analyzed for loaf volume, weight loss, density, specific volume, moisture content, texture, and freezable water. Triplicate experiments were conducted and data were statistically analyzed at α = 0.05. The dried honey powders contained glucose between 10.39±0.35 and 11.58±0.29 g/100g, fructose 12.07±0.49 and 15.14±0.29g/100g, sucrose 0.05±0.01 and 0.21±0.13g/100g and maltose 0.60±0.08 and 1.27±0.62g/100g. Among the bread samples HP showed highest loaf volume (mL) at 1462±45 while SHP, HNY and control showed decreasing loaf volumes at 1303±199, 1155±91 and 1100±66, respectively. All bread samples showed an increase in firmness and HP had a lower rate of staling than the other bread samples during storage. Control bread samples contained more freezable water (g/g solid) at 0.21±0.003 than HNY, SHP, and HP which had 0.20±0.003, 0.19±0.01 and 0.13±0.01, respectively. The study demonstrated that spray dried honey powder with retrograded starch could be used as a substitute for sucrose in baking bread.
CHAPTER 1 LITERATURE REVIEW

1.1 Honey: General Introduction

The Codex Alimentarius Commission (1981) defines honey as “the natural sweet substance produced by honey bees from nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living part of plants, which honey bees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature”. Honey is the oldest and only available unique natural sweetener to mankind and is the last of natural unprocessed food to be consumed (Bogdanov and others 2008; Ouchemoukh and others 2010). The mention of honey dates back to as long as 2100-2000 B.C in a Sumerian tablet which proclaims the use of honey as a drug and ointment. The Bible also mentions King Solomon’s words “Eat honey my son, because it is good” (Old Testament, proverb 24:13). Bee honey has significant nutritional and prophylactic-medicinal value (Juszczak and Fortuna 2006). Honey can be produced from the nectar of flowers or from honeydew and in some cases can even be a combination of both (Juszczak and Fortuna 2006). When derived from the nectar of flowers honey is known as nectar or blossom honey and can be further categorized as mono/unifloral honey and multifloral honey whereas honey produced from honeydew is known as honeydew honey (Ouchemoukh and others 2007). Nectar honeys are classified as monofloral and multifloral based on the pollen content analyzed by microscopic analysis which is known as mellisopalynological studies. Monofloral honeys are those whose pollen frequency from a single plant species is above 45% unless the pollen grains are “under- or over-represented” in which case around 10-46% is accepted as in Lavender, Citrus and Rosemary honeys (Felsner and others 2004). Honey produced from Eucalyptus, Castanea and Myosotis the pollen content is over-represented for a monofloral honey (Ouchemoukh and others 2007).
Honeydew honeys on the other hand are produced when bees ingest honeydew which is a sugar containing substance that is excreted by other insects which feed on plants (Ouchemoukh and others 2007).

The annual production of honey globally in the year 2008 was 1.2 million tons which was less than 1% of the sugar production. China is the largest producer of honey though its consumption is not the highest. The European Union countries have the largest per capita consumption of honey with Germany, Austria, Switzerland, and Poland (1 – 1.8 kg) leading within the continent where as Italy, France, and Great Britain have a moderate (0.3 – 0.4 kg) per capita consumption. USA, Canada and Australia have a per capita consumption of 0.8-0.9 kg (Bogdanov and others 2008). In the year 2009 144.1 million pounds of honey was produced in the United States of America with Louisiana producing 3.8 million pounds, an increase of 24% from 2008 (NASS, USDA 2011).

1.1.1 Honey Composition and Characteristics

Honey is an extremely complex mixture of carbohydrates that is found naturally (Swallow and Low 1990) with almost 70-80% sugars, 10-20% water and other minor constituents such as organic acids, mineral salts, vitamins, proteins, phenolic compounds, lipids and free amino acids (Gomes and others 2010; Ouchemoukh and others 2007). Honey maturity, the manner of production, processing and storage climatic conditions of the region of production and the nectar source have a substantial influence on the quality, composition and biochemical properties of honey (Guler and others 2007; Anupama and others 2003). The composition of honey in turn influences its physicochemical properties such as viscosity, hydrosopicity and granulation (Lazaridou and others 2004).
Sugars predominate the composition of honey and among them glucose and fructose are the prominent monosaccharides (60-85% of honey solids) which account for 85-95% of the honey carbohydrates (Swallow and Low 1990; Lazaridou and others 2004). Generally fructose is present in higher concentrations than glucose with the exception of honeys produced from plants like rape (Brassica napus), dandelion (Taraxacum officinale) and blue curls (Trichostema lanceolatum) (White 1976). The ratio of fructose to glucose on an average is 1.2:1 (Rodriguez and others 2004). Granulation of honey occurs due to spontaneous crystallization of the predominant sugars with glucose crystallizing first due to its lower solubility in water thus producing nucleation seeds in the form of glucose monohydrate (Venir and others 2009; Lazaridou and others 2004). Crystallization leads to an increase in the water content of the honey which causes an increase in the water activity, sometime over 0.60 which is the critical threshold for microbial stability (Venir and others 2009). This change in water activity allows the osmophylic yeasts present in honey to multiply thereby causing fermentation of honey and a decrease in its shelf life (Cui and others 2008). Crystallization affects the quality and textural properties of honey making it undesirable and in some cases could also cause corrosion of metal containers in which honey is stored (Lazaridou and others 2004; Cui and others 2008). The
important disaccharides present are maltose and sucrose (7-10%) (Cui and others 2008). An increase in sucrose content in the honey can occur when bees are over fed with sucrose by bee keepers (Anklam 1998). Azeredo and others (2003) reported that sucrose content also increased when the honey is harvested very early in fall thereby not giving the enzyme invertase enough time to convert sucrose to fructose and glucose. Honey also contains other low molecular weight oligosaccharides like melezitose, trehalose panose and turanose (Cui and others 2008; Bogdanov 2008).

Proteins account for 0.5-1% of the honey composition with proline constituting 50-80% of the total amino acids (Ouchemoukh and others 2007). Proline is produced during the conversion of nectar to honey by the honeybee and serves as an indicator of honey ripeness, and saccharose and glucose oxidase activities (Hermosin and others 2003). There are 26 other amino acids present in honey whose concentrations depend on the floral source in case of nectar honey and also if the honey is of honeydew origin (Hermosin and others 2003). The important enzymes present in honey are diastase (amylase), invertase (α-glucosidase) and glucose oxidase while catalase and acid phosphatase are present in lower amounts (National Honey Board 2005). Estevinho and others (2008) reported that the phenolic compounds present in honey are flavonoids and phenolic acids which can also serve as markers for determining the botanical origin of honey. They also reported that the phenolic acids were divided into two subclasses: substituted benzoic acids and cinnamic acids while the flavoids were divided into three classes with structural similarity namely flavonols, flavones and flavanones. The phenolic compounds contribute to the beneficial properties of honey due to their anti-oxidant nature (Estevinho and others 2008).
By international legislation the moisture content of honey should be less than 20-21% (Silva and others 2009; Mendes and others 1998) and generally average moisture content of honeys from different parts of the world ranges between 16-19% (Yanniottis and others 2006; Al-Khalifa and Al-Arify 1999; Juszczak and Fortuna 2006). The low moisture of honey causes a decrease in its water activity as water activity shows a linear dependency on water content (Abramovic and others 2008). Generally water activity of honey is within the range of 0.5-0.65 which is very low for most micro-organisms to grow since molds need around 0.70, yeast around 0.80 and bacteria 0.90 to grow (Gleiter and others 2006). Natural micro-flora of honey includes osmophillic yeasts of which the Saccharomyces ssp. are dominant, and have the ability to grow at low water activities of around 0.61-0.62 (Zamora and others 2005). When the water content of honey increases during storage due to granulation, the yeasts start fermenting the honey by acting on the glucose and fructose to produce ethanol and carbon dioxide (Zamora and others 2005; Gleiter and others 2006). The pH of acids is also a limiting factor to the growth of micro-organisms since generally honeys are of acidic pH in the range of 3.6-5 (Al-Khalifa and Al-Arify 1999; Ahmed and others 2007; Gomes and others 2010). The acidity of honey is contributed to the presence of organic acids, mostly gluconic acid and inorganic acids ions such as phosphate and chloride (Ouchemoukh and others 2007). However honey does have several sources of microbial contamination, primarily pollen, the digestive tract of honey bees, dust, air, and secondary sources maybe during handling and processing of honey (Snowdon and Cliver 1996). These micro-organisms include fungi such as Penicillium and Mucor, yeasts such as Saccahromyces, Schizosaccharomyces and Torula, molds and spores of Bacillus spp. and Clostridium spp. (Migdal and others 2000). Not many studies are devoted to the microbial contamination of honeys and most of them concentrate on Clostridium botulinum (Gomes and others 2010) since
the presence of Clostridium spores pose a risk of contracting botulism in infants who consume the honey (Finola and others 2007).

Viscosity of honeys is an important property that has been studied by various researchers (Sopade and others 2002; Junzheng and Changying 1998; Yoo 2004; Yanniotis and others 2006; Bhandari and others 1999; Lazaridou and others 2004). Viscosity has an influence on the physico-chemical and sensory properties of honey (Juszczak and Fortuna 2006) and knowledge about the rheological properties of honey is useful in its processing, handling and storage (Ahmed and others 2007). Viscosity of honey depends on factors such as temperature, water content, chemical constitution, amount and size of crystals, and types of colloids present in it (Juszczak and Fortuna 2006; Yoo 2004). Effect of temperature on viscosity is generally documented since a variety of temperature ranges are encountered during the processing, storage and handling of honey (Yoo 2004). Generally honey is considered to exhibit Newtonian behavior (Al-Malah and others 2001; Sopade and others 2002; Bhandari and others 1999; Zaitoun and others 2001) but there are a few reports about non-Newtonian behavior of honey (Ahmed and others 2007; Juszczak and Fortuna 2006).

1.1.2 Honey Beneficial Properties

The benefits of honey are many and it has been long used both as a source of nutrients and also as a medicine (Bogdanov and others 2008). A branch of medicine known as apitherapy has developed in recent years which utilize honey and its product in stimulation of wounds and burn healing and also in gastric and ulcers treatment (Bogdanov and others 2008; Ouchemoukh and others 2007). Anti-oxidant properties of honey are due to the presence of compounds such as pinocembrin, pinobanksin, chrysin and galagin (Cui and others 2008). Anti-oxidants are believed to protect against oxidation which is important for the prevention of chronic diseases (Ames and
others 1993). Studies conducted by Schramm and others (2003) and Al-Waili (2003) showed that consumption of honey increased antioxidant levels and decreased serum levels and they also increased Vitamin C concentration by 47%, β-carotene by 3%, uric acid by 12% and glutathione reductase by 7%. However even the antioxidant activity of honeys is heavily influenced by the botanical origin of honey (Al-Mamary and others 2002).

Glycemic index (GI) is an important indicator of the carbohydrate level in a food which bears a relation with the blood glucose level (Bogdanov and others 2008). A lower value of glycemic index is indicative of the ability to induce only a small increase in blood glucose level and vice versa. The only available data on honey GI is that of Australian honeys which was determined to be an average of 58 (Brand-Miller 1995). However this was of blended honeys (nectar and honeydew honey) rather than individual honeys from floral sources. Arcot and Brand Miller (2005) carried out studies to determine the GI of nectar honeys and reported that most nectar honeys were within the low GI range (55 or less) or intermediate GI range (56-69). However some blend honeys were in the high GI range (70 and above). The study also reported that the GI value of a honey was negatively correlated to its fructose content and hence honeys with higher fructose showed lower GI values. Fructose is absorbed more slowly than glucose from the gastrointestinal tract which causes only a minimal rise in the blood sugar levels (Jeffrey and Echazarreta 1996). Also the glycemic index of fructose is 19 which is very low when compared to that of sucrose which is 68 (Bogdanov 2010). Studies conducted by various researchers have shown that honey could be tolerated by patients afflicted with either types of diabetes i.e. type I and type II (Katsilambros and others 1988; Samanta and others 1985).

Honey also exhibits antimicrobial, antiviral, antiparasitic, antimutagenic and anti-inflammatory properties (Bogdanov 2008). Honey was seen to affect the levels of antibodies produced against
thymus dependent antigens in mice and sheep (Al-Waili and Haq 2004). Al-Waili 2003 reported that consumption of honey by humans on a 1.2g/kg body weight daily showed an increase of the following in their blood serum: monocytes (50%), iron (20%), copper(33%), and a slight increase in lymphocytes, eosinophils, zinc, magnesium, and hemoglobin. The study also reported a reduction in ferritin (11%), immunoglobulin E (34%), aspartate transaminase (22%), alanine transaminase (18%), lactic acid dehydrogenase (41%), creatine kinase (33%) and fasting sugar (5%).

Application of honey in food products is attributed to its properties such as antimicrobial and antioxidant nature. They prevent the spoilage of meat due to microbial growth or lipid oxidation as in the case of meats (Antony and others 2000; Nagai and others 2006). Honey also has the ability to prevent enzymatic browning of sliced fruits such as apple (Oszmianski and Lee 1990), in raisins (Mclellan and others 1995) and in vegetables (Chen and others 2000) too. They are used for clearing fruit juices and fruit drinks (Lee and others 1990; Oszmianski and Lee 1990). Shin and Ustunol (2005) reported that the presence of honey in yogurt not only enhanced the growth of indigenous bifidobacteria in the GI tract but also inhibited the growth of C. perfringes and E. aerofaciens. Honey has a diverse application in the bakery, cereal and confectionary industry (Bogdanov 2010). It is assumed that the advantages of adding honey to a product include moisture retention, good texture, improved baking, flavor and sensory properties (Bogdanov 2010).

1.2 Resistant Starch: Properties and Benefits

Resistant starch (RS) was first recognized due to the complication that it rendered during the determination of total dietary fiber levels by the Prosky method since it was clear that any starch that interfered with the assay was not a traditional fiber (Englyst and others 1987). Resistant
starch maybe defined as the non-digestible fraction of starch and starch products which are not hydrolyzed to D-glucose in the small intestine within 120 minutes of ingestion but instead ferments in the colon (Fuentes-Zaragoza and others 2010). RS is a linear chain molecule of α-1,4-D-glucan which is derived from retrograded amylase fraction and has low molecular weight of around 1.2x10^5 Da (Tharanathan 2002). RS has a low water holding capacity which helps processing and it enhances organoleptic qualities of food (Sozer and others 2002). Due to its increased expansion, enhanced crispiness and reduced oil pick up in deep fried foods (Tharanathan 2002) when used as a replacement for or as a complement to natural fiber in which case it would be labeled ‘dietary fiber’ (Sozer and others 2007). RS does not have a discernible effect generally on the sensory properties of the final product which is an added advantage when it comes to consumer acceptability of the product (Perez-Alvarez 2008a). The inability of RS to be digested is due to various factors and is dependent on these factors that resulting in the following categories:

i. RS1: the compact molecular structure of RS1 restricts the digestive enzymes from reaching and digesting them (Haralampu 2000).

ii. RS2: the starch granules are so structured that digestive enzymes are prevented from catabolizing them in the ungelatinized form (Nugent 2005). The high density and partial crystallinity render them resistant to enzyme degradation (Gallant and others 1992)

iii. RS3: the starch granules are first disrupted by heating during gelatinization during which stage they are accessible for digestion by enzymes. Then by rapid cooling the gels form starch crystals which are indigestible by the enzyme and these starches are known as retrograded starches (Fuentes-Zaragoza and others 2010).
iv. RS4: certain starches are resistant to digestion due to chemical modification by various processes such as etherization, esterization or cross-bonding (Lunn and Buttris 2007).

RS3 is studied more due to its thermal stability which allows it to be applied in a wide range of cooked products at varying temperatures in conventional foods (Haralampu 2000). RS3 comprises retrograded amlyose whose prolonged intake improves fasting triglyceride and cholesterol levels as opposed to a parallel amlopectin-rich diet (Mikulikova and others 2008). RS3 can form B-type crystalline structure when wheat starch is gelatinized fast at 68°C and then retrograded, while a slower crystallization at 100°C yielded A-type RS3 (Eerlingen and others 1993). Since RS3 is composed mainly of amlyose its melting temperature would correspond to that of high molecular weight crystalline amlyose which is around 150°C (Shamai and others 2003). This is the reason RS3 is an extremely heat stable pre-biotic starch that can be used in baked or high temperature cooked foods.

RS is physiologically beneficial to human health due to its pre-biotic nature. Prebiotics are non-digestible food ingredients that help selectively stimulate the growth of one or more micro-organisms in the gastro-intestinal tract thereby conferring beneficial health effects to the host (Marteau 2001). Studies have shown that when the fermentation occurs in the colon the starch produces short chain fatty acids including acetate, propionate and butyrate and results in the increase in fecal bulk and lowers colonic pH (Casterline and others 1997; Silvester and others 1995; Philips and others 1995). The butyrate produced can reduce the risk of malignant changes in cells as they have been shown to arrest one of the phases of the cell cycle (G1) (Sharma and others 2008). Studies conducted by Liu and Xu (2008) have shown that the presence of RS during the promotion phase in the middle and distal colon can retard the growth or development of neoplastic lesions in the colon thus re-emphasizing the benefits of RS as a preventive measure.
for colonic cancer. RS is also being studied to verify its ability to inhibit the accumulation of fat and have hypocholesterolemic effects in humans since in rats it is known to raise the cecal size and absorption of short-chain fatty acids, lower plasma cholesterol and triglyceride levels. There was also a lower concentration of cholesterol in the lipoprotein fraction as well as decreased triglyceride concentration in the triglyceride rich lipoprotein fraction (Sajilata and others 2006; Nugent 2005). RS also has a low glycemic response which can help in decreasing overall glycemic load of a food if it is replacing the existing carbohydrate (Nugent 2005). RS must be consumed at a concentration of at least 14% of total starch intake in order to have the glycemic and insulinaemic responses in the host (Brown and others 2003; Higgins 2004).

1.3 Spray Drying

Spray drying is a method for convective drying of liquids (Strumillo and Kudra 1986) which has been in operation for over a century but is still an active field of interest for continuous innovation given the demand for better quality product (Vehring and others 2007). Spray drying was first recorded in a patent by Samuel Perry in 1872 (Bhandari and others 2008). The process involves the transformation of feed slurry into a dried particulate form by spraying in a hot drying medium which is generally air (Goula and Adamopoulos 2005). This technology has application in varied fields including food and dairy industries, pharmaceutical, agrochemical, light and heavy chemicals, detergent, pigment, biotechnology and ceramics (Vehring and others 2007). Masters 1996 reported that there were more than 15000 industrial size spray dryers in the world and almost double of that number in pilot plants and laboratories. According to Bhandari and others (2008) some of the key advantages of spray drying are

i. Particles of predetermined characteristics (moisture, size, density etc) and type (particles, granules and agglomerates) can be produced.
ii. Heat spoilage is relatively small in the product due to the extremely short exposure time, the cooling effects in a critical drying period and also due to evaporation of solvent phase at a temperature lower than its normal boiling point. Thus heat sensitive and heat resistive products can be spray dried using the machine.

iii. The process is versatile with the ability to use the same equipment for a variety of different products.

iv. Spray drying is a continuous process with high production rates which makes it economical and also the product is produced in the desired powdered form thereby requiring no additional grinding.

The disadvantages of the process includes high cost of sophisticated atomizing and dust collecting devices and large dimensions of installment.

1.3.1 Principle

Masters (1972) stated that the basic physical principle of spray drying can be summed in the phrase “evaporation causes cooling”. The homogeneity of the spray followed by atomization and high rate of moisture evaporation enable the dried product to be at a temperature that is significantly lower than that of the air that leaves the drying temperature. Thus the product does not undergo extremely high heating temperatures and when it separates from the drying air it leaves so without any heat degradation. Dryer design and operation along with chemical and physical characteristics of food determine the characteristics of the final product.

1.3.2 Working

The process of spray drying consists of the following four fundamental steps (Gharsallaoui and others 2007):

i. Atomization
ii. Droplet-hot air contact

iii. Evaporation of droplet water

iv. Dry product–humid air separation

Atomization involves the formation of a spray due to pressure or centrifugal force and establishing contact between the spray and the drying air. The next stage can occur in two different ways: co-current or counter current. In co-current the spray and hot air have the same direction of flow which results in powders being exposed to moderate temperatures. However in counter current the flow of spray and hot air are in the opposite directions which causes the powder to be exposed to higher temperatures which limits the application of this method to heat stable products. However the latter is more economic in terms of energy consumption when compared to the former. The third stage is the critical stage where the actual drying takes place. Initially moisture from the surface gets lost due to heat and moisture from the inside of the droplet moving towards the surface to make up for the lost moisture. This continues till no more moisture can be lost from the surface and drying starts occurring with a crust formation. Then moisture moves along this crust and dries the droplet as it does so. Finally a dried powder is obtained. This powder gets separated in the final step into two collection points. The first point is right below the drying chamber where the particles settle down and the next collecting point is below a cyclone separator where the lighter particles get separated from the dust and settle down. Spray dryers are also fitted with filters known as “bag houses” that remove the finest powders and chemical scrubbers that remove any remaining powders or volatile pollutants. (Masters 1972; Strumillo and Kudra 1986).
1.4 Bread: General Introduction

Bread is an essential basic dietary product that dates back to the Neolithic era with the first bread being made around 10,000 BC or over 12,000 years in the past (Mondal and Datta 2008). The importance of bread is due to its contribution to a well balanced diet given its starch and complex carbohydrate content (Altamirano-Fortoul and Rosell 2011). Bread has been used to exercise political influence for at least 2000 years and even in modern times a shortage of bread is synonymous with difficult times whereas a promise of bread is used as an indication for an enriched life (Scanlon and Zghal 2001). Bread plays an important role in different countries for both cultural and dietary reasons; for example 30% of the daily requirement of calories and proteins is provided by bread in the Russian diet (Samsonov and Petrasov 1993) while in Ghana bread is eaten daily as part of the cultural practice (Ellis and others 1997).

1.4.1. Bread Ingredients and Function

Bread is a solid that is “soft” by nature (Scanlon and others 2000) and comprises two main phases at the macroscopic level – air (fluid) and cell wall material (solid) (Scanlon and Zghal 2001). Water and flour affect the texture and crumb the most in bread and hence are considered the most important ingredients (Mondal and Datta 2008). The flour is always 100% and all other ingredients are added on a percentage weight basis of that amount by weight. Flour characteristics is an important parameter that affects bread type and though this largely depends on the type of wheat crop, the flour should possess certain characteristics to be considered good for baking bread. Flour contains two proteins which when they come into contact with water that help in formation of gluten. There should be at least 11% of gluten to make bread with good baking quality. Water used for making dough should be of medium hardness as soft water gives sticky doughs with poor gas retention and should constitute at least 50% of the flour amount.
Most artisan bread formulas have 60% to 75% of water added to them. An increase in the water percentage also leads to an increase in the levels of CO$_2$ produced in yeast breads and also a coarser bread crumb.

Other ingredients added to bread are leavening agent yeast (2%), sugar (4%), salt (2%), and a shortening agent such as margarine or butter (3%). The yeast which is a commercial baker’s yeast *Saccharomyces cerevisiae* causes fermentation of the sugar in the dough and converts it to moisture and CO$_2$ (Mondal and Datta 2008). The moisture and CO$_2$ is incorporated into the gluten structure of the dough and during the final proofing (rising of dough) the dough increases in volume and gives a porous and light product. The expansion of water and CO$_2$ occurs due to high temperatures and this acts as an insulating agent and prevents a high rate of temperature increase in the bread crumb thus preventing excessive loss of moisture from occurring. Sugar in some cases is added for sweetening but usually it is present in order to provide a substrate for the yeast to act upon. However if large volumes of sugar is added it causes the yeast cells to dehydrate and reduce dough volume (Vaclavik and Christian 2003). Addition of salt besides contributing to flavor also causes dehydration of yeast cells which in turn controls their growth and CO$_2$ production (Vaclavik and Christian 2003). The shortening agent helps increase the slicability of the final bread product (Mondal and Datta 2008). Fresh bread is characterized by an appealing brownish and crunchy crust with a pleasant aroma, good slicing characters and a soft and moist crumb texture (Giannou and others 2003).

Milk is also added in some bread formulations due to its ability to enrich and improve the nutritional value of food. Other ingredients that maybe added are certain emulsifiers, enzymes, soy flour, oxidants and reductants (Kent and Evers 1994) due to an increase in consumer demand for high quality and longer shelf life baked product with added health benefits (Stampfli and
These ingredients improve the machinability, rate of hydration, crumb structure, gas holding capacity and keeping quality of bread (Mondal and Datta 2008; Leon and others 2002).

1.4.2 Bread Baking

Baking is a complex physicochemical process that involves a set of processes which must be carried out in a specific period of time and in a specific sequence (Fu 2006). It begins with the mixing and kneading of the dough, followed by fermentation of the dough which allows biochemical and chemical modifications to occur to the polymers in dough. During fermentation the CO₂ produced causes leavening (Scanlon and Zghal 2001). This is then followed by the moulding and proofing step where the dough is allowed to rise and expand in volume (Kent and Evers 1994). This is followed by baking where a lot of reactions and changes occur simultaneously. The proteins present in the flour and any other ingredient aggregate causing them to harden (Scanlon and Zghal 2001) while simultaneously gelatinization of the starch molecules occurs due to moisture absorption (Vaclavik and Christian 2003). Evaporation of water occurs while creating an outer crust which turns brown due to the Maillard browning reaction (Vaclavik and Christian 2003).

Mondal and Datta (2008) reported in their review 3 methods by which baked products including bread is produced:

1. Straight dough method: in this method all the ingredients are mixed in a single step and kneaded together in the dough.

2. Sponge and dough method: in this method the ingredients of the dough are mixed in two different steps where in the first step only the leavening agent is mixed into the flour.
along with a certain amount of water and the dough is left to rise and develop for a couple of hours. In the second step the rest of the ingredients are mixed into the flour.

3. **Chorleywood method**: in this method all the ingredients are mixed together in a single step but the mixing is done for a few minutes using an ultrahigh mixer.

The process of making dough and baking bread have received attention since it is believed that modifications in these processes may affect the keeping quality of bread and delay its staling while simultaneously exploring the relationships between bread quality and temperature and holding time (Giannou and others 2003).

### 1.4.3 Staling

Staling is the collective physical, chemical and sensory changes that occur after baking during the storage period which is characterized by the change in flavor, an increasing “stale” odor and crumb firming (Gellynck and others 2009; Altamirano-Fortoul and Rosell 2011). Contrary to other food products, in bread, staling is of major concern rather than spoilage due to microorganisms or endogeneous enzyme activity (Barcenas and Rosell 2005). Staling represents one of the primary reasons for economic losses in the bread industry due to consumer apprehension in using such products. It is estimated that in a market where 20 billions of bread is produced annually at least 600million (3%) is lost due to staling problems (Lai and Lin 2006). The phenomenon of staling has been under investigation for around 150 years (Gerrard and others 1996) and yet its complex mechanism hasn’t been fully understood and remains a subject of intensive study (Lodi and others 2007; Ribotta and Bail 2007; Curti and others 2011; Le-Bail and others 2009).

Crumb firming is the major indicator of staling from a consumer’s perception. However staling occurs both, at the crust and crumb. Crust staling is characterized by a soft leathery appearance
due to moisture migration from the crumb to crust (Lai and Lin 2006). Crumb staling is also characterized by the loss of suppleness and increased opaqueness. The crumb becomes more granular and grainy to touch with the ability to crumble easily and a decreased tendency to be pleasant to chew given the fact that it is less easily moistened (Calvel and others 2001). Bread staling is attributed to many factors with the major ones including starch retrogradation specifically amylopectin, water migration and interaction between starch gluten and gluten proteins (Lai and Lin 2006). Starch retrogradation is only partially responsible for staling with water distribution playing a major role both at the macroscopic and microscopic level. At the macroscopic level it involves the migration of water from the crumb to the crust whereas at the molecular level it may be integrated into the retrograded amylopectin matrix or may be the decreasing freezable water content (as determined by DSC), or migrate from the gluten to starch which causes elasticity loss in the bread crumb (Curti and others 2011). A uniform water distribution in the bread matrix has been shown to decrease staling rate and could have an impact on slowing the amylopectin retrogradation rate as the process requires water (Lodi and others 2007). Understanding this phenomenon is important and many techniques have been used to try explain it like DSC (Ribotta and Bail 2007), NMR (Curti and others 2011), and MRI (Lodi and others 2007).

Numerous studies have been devoted to the retardation of staling bread and prolonging its keeping quality with the addition of anti-staling agents being central to most of the studies (Altamirano-Fortou and Rosell 2011; Mandala and others 2007; Moayedallaie and others 2010; Stampfli and Nersten 1995). Anti-staling agents include lipids and emulsifiers (sodium stearoyl-2-lactylate and diacetyl tartaric acid esters of monoglycerides), pentosans, alcohol (ethanol) and sugars (Pateras 2007). However rather than adding additives it is advisable to adjust the bread-
making process by studying the role of each ingredient and their interaction with each other thus leading to an improved baking performance (Schiraldi and Dimitrios 2000).
CHAPTER 2 CHARACTERIZATION OF LOUISIANA HONEY AND PRODUCTION OF SPRAY-DRIED HONEY POWDER

2.1 Introduction

Honey is a natural food product sweet to taste, with a yellowish or brown color, and a viscous nature (Ahmed and others 2007; Abu-Jdayil and others 2002). Honey is known for both its nutritional value and medicinal properties (Juszczak and Fortuna 2006). Honey is produced either from the nectars of flowers or from honeydew. When obtained from a floral source honey can be categorized as mono-floral or multi-floral depending on the species of flowers from where the nectar was collected. It can also be categorized depending on its color. Honey is a complex mixture of carbohydrates and contains organic acids and some amino acids, as well as certain micro- and macro-elements, and it is a rich source of many biologically active compounds (Gomes and others 2010; Juszczak and Fortuna 2006; Ahmed and others 2007). These bioactive compounds include phenols such as flavonoids and phenolic acids which in some research has shown to be a more potent anti-oxidant than vitamin C or E (Cao and others 1997). The anti-oxidant activity of honey is due to the abundance of both enzymatic and non-enzymatic anti-oxidants such as glucose-oxidase, catalase, flavonoids, ascorbic acid, phenolic acids and carotenoids (Liviu Al and others 2009). Though the carbohydrate content may vary depending on the floral source the major sugars in honey are always monosaccharides, namely glucose and fructose. Additionally, small amounts of disaccharides like maltose, sucrose, trechalose, isomaltose, nigerose, turanose, kojibiose and other are present (Bhandari and others 1999).

Characterization of honey in terms of its chemical, rheological and sensory properties has received a lot of attention with numerous publications being released in a single year over the same topic in different countries (Corbella and Cozzolino 2006; Juszczak and Fortuna 2006;
Ouchemoukh and others 2007; Yanniotis and others 2006). All of these properties are intertwined and vary with plant species type, climate, region of collection, and honey maturity. (Anupama and others 2003). Of these, the rheological properties of honey are very important as they play an influential role in handling, processing, storage and quality control (Yoo 2004; Kayacier and Karaman 2008). The rheological properties of honey also serve as an important factor in providing information about the structural organization of food and also about its fluid and heat transfer properties (Ahmed and others 2007). The rheological properties of honey depend on 3 major factors — temperature, composition and water content (Abu-Jdayil and others 2002; Ahmed and others 2007). Viscosity of honey is seen to decrease with an increase in temperature and in most literature has been reported as a liquid exhibiting Newtonian behavior when steady shear viscosimetry is applied (Abu-Jdayil and others 2002; Juszczak and Fortuna 2006; Recondo and others 2006). However certain studies have been published wherein the higher sensitivity of the rheometer has led to the detection of some amount of yield stress being present in those foods previously accepted as Newtonian in nature (Ahmed and others 2007). Commonly, the Arrhenius equation is used to describe the temperature dependency in honey samples.

Liquid honey poses multiple problems in handling during processes involving mass production due to its viscous and sticky nature thereby leading to a growing demand for dried honey powder by both consumers and the food industry. Honey powder with its low moisture content has the ability to be easily blended with other ingredients apart from other advantages including convenience, ease of handling, reduced storage space, sanitation and storage for a longer period. Various methods of drying honey have been used such as tunnel drying, vacuum drying, spray drying and solidification into blocks by crystallization (Cui and others 2008). Drying of honey
however poses many problems such as low recovery rates due to its high sugar content (Wang and Langrish 2009) and also utilization of at least 50-70% of additives to obtain a dried powder (Cui and others 2008). Spray drying is a unit operation (Gharsallaoui and others 2007) that is gaining popularity due to its application in a variety of fields such as food and dairy industries, pharmaceutical, agrochemical, light and heavy chemicals, detergent, pigment, biotechnology and ceramics (Vehring and others 2007). Spray drying of high sugar content liquids such as juices and honey involves the use of additives that serve as drying agents such as maltodextrin and gum Arabic (Cano-Chauca and others 2005; Wang and Langrish 2009). However commercially available honey powders have up to 63% of maltodextrin which can cause an alteration in flavor and texture which may be undesirable to consumers (Wang and Langrish 2009). Sahu (2008) used three different drying aids - maltodextrin, glycerol monostearate (emulsifier) and tricalcium phosphate (anti-caking agent) to produce honey powder from each by vacuum drying. Resistant starch type 3, i.e. retrograded starches, in comparison to the above mentioned drying aids confer health benefits and have the added advantage of being able to withstand high cooking temperatures. Retrograded starch causes malabsorption of starches from the food thereby leading to a lower rise in blood glucose level which could prove beneficial for diabetic patients. this malabsorption of starch also implies a long-term benefit in controlling hyperlipaemia (Haralampu 2000). Therefore utilization of such a starch as an additive for drying may be an interesting area to explore.

Louisiana is one of the leading producers of honey in the United States yet, except for its melissopalynological studies (Lieux 1972), there is little information about its properties in literature. The objectives of this study were to: 1) characterize Louisiana honey based on its physic-chemical properties and study its flow behavior 2) spray dry the honey so characterized
using retrograded starch as the drying agent to produce honey powder 3) characterize the spray-dried honey powder.

### 2.2 Materials and Methods

Four honey samples were purchased in batches of three from local honey producers based in Denham Springs, Bossier city, St. Marksville and Breaux bridge respectively. The samples were of multi-floral origin with tallow and willow being among the major sources of pollen. USDA color designation was determined for each sample using a honey color analyzer (Hannah Instruments, HI 83221, RI, USA). The color was designated by measuring the optical density and expressing it in terms of millimeters (Pfund scale). The corresponding grade was then read from the manual provided with the instrument and the honey samples were labeled based on the Pfund value of each. Thus honey sample with Pfund scale 80, 65, 79 and 77 mm were labeled LH1, LH2, LH3 and LH4 respectively (table 2.2.1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pfund scale</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH1</td>
<td>80</td>
<td>Light amber</td>
</tr>
<tr>
<td>LH2</td>
<td>65</td>
<td>Light amber</td>
</tr>
<tr>
<td>LH3</td>
<td>79</td>
<td>Light amber</td>
</tr>
<tr>
<td>LH4</td>
<td>77</td>
<td>Light amber</td>
</tr>
</tbody>
</table>

The Pfund scale is a USDA 1985 established measure of the color of honey. The purpose of this is to ensure uniformity in the grading process for honeys from different floral origins. The Pfund scale depends upon the optical density of the honey measured and is expressed in millimeters. All of the honey samples fell under the USDA color designation of Light amber which includes the Pfund scale range of 50mm to 85mm.
2.2.1 Physico-chemical Characterization of Liquid Honey

2.2.1.1 pH, °Brix, Moisture, Specific gravity and Color

The pH was measured by dissolving 10 g of honey in 75 mL of water (Gomes and others 2010), using a digital pH meter (SB70P, Symphony™, VMR Inc., Beverly, MA, USA).

The °Brix was measured using a digital handheld refractometer (AR200, Reichert Inc., Depew, NY, USA). The readings were taken at 20°C in triplicate and were reported as the mean value along with the standard deviation.

The moisture content was determined using AOAC 969.38b, 1995 method using a forced air convection oven. Three grams of each sample were weighed in triplicate and then left in the oven at 105°C for 24 h. The results were reported as the mean of the triplicate measurements along with standard deviations.

Specific gravity was determined by calculating the ratio of the mass of honey to the mass of an equal volume (25 mL) of distilled water measured in a volumetric flask.

\[
\text{Specific Gravity} = \frac{\text{Mass of specific volume of honey}}{\text{Mass of an equal volume of distilled water}}
\]

Results were reported as the mean of triplicate measurements along with standard deviations.

The color was measured using the HunterLab Labscan XE colorimeter (Labscan XE, Hunter Associates laboratory Inc., Reston, Virginia, USA) in triplicate of samples weighing 8 grams each. The colorimeter was standardized using black and white tiles. The results were reported as \(L^*\) (lightness), \(a^*\) (redness and greenness), and \(b^*\) (yellowness and blueness). The measurements were made in triplicate and the means and standard deviations were reported.
2.2.1.2 HPLC Analysis for Sugar Content

The sugar content of the honeys was determined using an HPLC technique. HPLC equipment was composed of a solvent delivery system (Waters 600, Waters, Milford, MA, USA), work station (M32, Waters Corporation.). One percent solution of the honey samples was prepared by dissolving 1 g of honey powder in 100g of distilled water, and 15 μL of the dilute honey was injected into HPLC column (Waters). For analysis of the glucose and fructose, the CarboPac PA10 column (4 x 250 mm) was used with 112mMNaOH as the mobile phase at ambient room temperature. They were detected using an EC detector system. The contents of glucose, fructose, sucrose and maltose in liquid honey were calculated based on the ratio of integrated peak areas using commercial glucose, fructose, sucrose and maltose (Sigma–Aldrich, St. Louis, MO, USA) as the standard compounds. Triplicate analysis was conducted for each sample and results reported as the mean along with the standard deviation.

2.2.1.3 Rheological Study

The rheological measurements were performed using a controlled shear stress rheometer (AR 2000ex, TA Instruments, New Castle, DE) with computer control. A parallel plate geometry with a plate radius of 40mm was used and the gap was set at 500 μm. The temperature was maintained by the Peltier temperature control system. The viscosity was measured at the shear rate 100s⁻¹ for 20, 30, 40, 50 and 60°C. The measurements were done in triplicate for each temperature and the data was obtained from the company supplied software, Advantage software, Version 2.3.

The Power law was used to fit the stress-shear data and is given by the following equation:

\[ \sigma = K(\gamma)^n \]
Where \( \sigma \) is the shear stress (Pa), \( \gamma \) is the shear rate (s\(^{-1}\)), \( K \) is the consistency coefficient (Pas\(^n\)) and \( n \) is the flow behavior index (dimensionless).

The Arrhenius equation was used to study the effect of temperature on viscosity of the honey samples which is given as follows

\[
\mu = \mu_0 \exp\left(\frac{E_a}{RT}\right)
\]

Where \( \mu \) is the viscosity (Pas), \( \mu_0 \) the Arrhenius constant, \( E_a \) the activation energy (kJ/mol), \( R \) is gas constant (8.314 J/mol K), and \( T \) is the temperature (Kelvin). A linear graph of \( \ln(\mu) \) versus \( (1/T) \) was plotted and activation energy was obtained from it. Results were reported as the mean along with the standard deviation of triplicate measurements.

### 2.2.2 Spray Drying of Liquid Honey Using Retrograded Starch as Drying Agent

A solution comprising 20% honey, 30% retrograded corn starch and 50% water (by weight) was prepared (at room temperature). The proportions were determined based on preliminary studies. The solution was prepared by mixing continuously using a magnetic stirrer until the honey dissolved completely in the solution (10 mins). The mixture was then sonicated using a laboratory scale ultrasonic processor (Model Model CPX 500, Cole-Parmer Inc., Vernon Hills, IL, USA) for 5 minutes at 80% amplitude (pulse on 2 and pulse off 1). The solution was placed in an ice bath to prevent any temperature rise in the solution. Sonication was done to ensure that the particles were smaller in size and well dispersed in the solution. The honey solution was spray dried into dry powder using the FT80 tall form spray dryer (Armfiled Limited, Ringwood, Hampshire, England). The honey solution was pumped at a flow rate of 9 mL/min and spray dried at 200 °C. The spray dried honey powder was stored at 4 °C until analyzed. All data were obtained by triplicate experiments.
2.2.3 Characterization of Spray-dried Honey Powder

The spray-dried honey powders were labeled based on the honey they were spray-dried from. Thus honey powder produced was labeled HP1, HP2, HP3 and HP4 to signify its production from LH1, LH2, LH3 and LH4 honey samples respectively.

2.2.3.1 Color

The color of the honey powder was measured as per mentioned in Section 2.2.1.2. The measurements were made in triplicate and the means and standard deviations were reported.

2.2.3.2 Moisture Content

The moisture of the honey powders was measured using a Moisture analyzer (System 5, CEM Corporation Ltd., Matthews, NC, U.S.A) and reported as the means of the triplicate measurements along with the standard deviations.

2.2.3.3 HPLC Analysis for Sugar Content

The sugar content of the honey powders was determined using an HPLC technique. HPLC equipment was composed of a solvent delivery system (Waters 600, Waters, Milford, MA, USA), work station (M32, Waters Corporation.). Half percent solution of the honey powder samples was prepared by dissolving 0.5 g of honey powder in 100g of distilled water. Of this solution, 15 µL was injected into HPLC column (Waters). For analysis of the glucose and fructose, the CarboPac PA10 column (4 x 250 mm) was used with 112mMNaOH as the mobile phase at ambient room temperature. They were detected using an EC detector system. The contents of glucose, fructose, sucrose and maltose in liquid honey were calculated based on the ratio of integrated peak areas using commercial glucose, fructose, sucrose and maltose (Sigma–
Aldrich, St. Louis, MO, USA) as the standard compounds. Triplicate analysis was conducted for each sample and results reported as the mean along with the standard deviation.

### 2.2.3.4 SEM Analysis for Particle Morphology

The morphology of honey powders was determined using Scanning Electron Microscopy (SEM) (JSM-6610LV, JEOL Ltd., Japan) using an acceleration voltage of 5 kV. The honey powder samples were mounted on aluminum SEM stubs and coated with gold: palladium (60:40) in an Edwards S150 sputter coater (Edwards High Vacuum International, Wilmington, MA). The mounted powders were then imaged with the SEM and particle diameter was also determined.

The statistical significance of observed differences among formulation means was evaluated by analysis of variance (ANOVA) (SAS, Version 9.2, SAS Institute Inc., Cary, NC., USA) followed by the post-hoc Tukey’s studentized range test (SAS 2002).

### 2.3 Results and Discussion

#### 2.3.1 Physico-chemical Characterization of Louisiana Honey

All of the honey samples were acidic in nature with the pH ranging between 3.89 and 4.10 (Table 2.3.1). Similar results have been observed in honey from different parts of the world including Algeria (3.49 - 4.43) (Ouchemoukh and others 2007), India (3.8 – 5.0) (Ahmed and others 2007), and Brazil (3.56 – 4.00) (Azeredo and others 2003). The acidity of honey has been attributed to the presence of organic acids especially gluconic acid as well as inorganic ions such as phosphate and chloride (Ouchemoukh and others 2007). Gluconic acid is a mild organic acid and is found naturally in fruit juices (0.25%) as well as in honey (1%). It is used in the pickling of foods as well as in baking (component of leavening agent) and sherbets (flavoring)
The total solid content of the honey samples, represented by °Brix, was found to be within the range of 79.9% and 80.3% which is close to that found in Indian honeys (76 - 81%) (Anupama and others 2003) but lower than that found in Australian honeys (82.4 – 83.3%) (Bhandari and others 1999). This is because honey characteristics such as pH, moisture, viscosity etc. are affected by various factors including floral origin, geographical location and maturation time (Ahmed and others 2007; Juszczak and Fortuna 2006).

### Table 2.3.1 pH, °Brix, %moisture and specific gravity of liquid honey

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>°Brix</th>
<th>%Moisture</th>
<th>Specific gravity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH1</td>
<td>4.10±0.03&lt;sup&gt;A&lt;/sup&gt;</td>
<td>80.33±0.21&lt;sup&gt;A&lt;/sup&gt;</td>
<td>18.75±1.22&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.42±0.01&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>LH2</td>
<td>3.89±0.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td>80.17±0.32&lt;sup&gt;A&lt;/sup&gt;</td>
<td>17.72±2.12&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.42±0.01&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>LH3</td>
<td>3.99±0.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>79.93±0.12&lt;sup&gt;A&lt;/sup&gt;</td>
<td>19.19±0.39&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.43±0.01&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>LH4</td>
<td>3.93±0.02&lt;sup&gt;B&lt;/sup&gt;</td>
<td>79.90±0.10&lt;sup&gt;A&lt;/sup&gt;</td>
<td>17.33±1.20&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.41±0.02&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. <sup>A-B</sup> Means with different letters in each column are significantly different (P>0.05).

The results indicated that all of the honey samples analyzed were similar in moisture content (Table 2.3.1). The moisture content was well within the acceptable range of 17 – 20% as found in most honeys and indicated a proper range of maturity (Al-Khalifa and Al-Arify 1999; Silva and others 2009). Moisture content also varies according to floral origin, geographical location and maturation time as indicated by the different ranges obtained in studies conducted in different regions of the world. For example Brazilian honeys have reported moisture content in the range determined in this study, while Polish honeys have 14.7 – 18.0% (Juszczak and Fortuna 2006), Indian honeys have 17 – 22.6%, Algerian honeys have 14.64 – 19.04% (Ouchemoukh and others 2007) and Greek honeys have 13 – 18.9% (Lazaridou and others 2004). Specific gravity was found to be between 1.41 and 1.43 which seems to be close to those reported for Indian honeys (Ahmed and others 2007).
The CIE color values (Table 2.3.2) were for most part similar to each other with significant differences only among a few samples. In terms of lightness LH3 was the lightest at 36.28 whereas LH 1 was the darkest at 32.57. a* values, which represent redness, were considerably lower at a range of 0.69 – 7.11 than the b* values, representative of yellowness, which ranged from 34.24 to 42.35. This indicates that all of the honey samples tended to be more yellowish in color than red and visually the honeys were golden yellow with a tinge of brown. Similar trends were reported by Ahmed and others (2007) where Indian honey samples exhibited much lower a* values than b* values. However, the Indian honey samples were much lighter with L* values ranging between 40.96 and 50.04. The difference in lightness generally depends on the ash content of the honey with a higher ash content indicating a darker sample while lower ash content is indicative of a lighter honey sample (Gomes and others 2010). Color values are also related to the botanical origin of the honey due to the minerals and other minor components (National Honey Board, 303) as well as phenolics and hydroxymethylfurfural content of the honey (Lazaridou and others 2004).

Table 2.3.2 Color

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>c*</th>
<th>h*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH1</td>
<td>32.57±0.40&lt;sup&gt;B&lt;/sup&gt;</td>
<td>42.97±0.61&lt;sup&gt;A&lt;/sup&gt;</td>
<td>85.44±0.35&lt;sup&gt;C&lt;/sup&gt;</td>
<td>7.11±0.32&lt;sup&gt;A&lt;/sup&gt;</td>
<td>42.35±0.58&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>LH2</td>
<td>34.53±1.99&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>34.49±2.49&lt;sup&gt;B&lt;/sup&gt;</td>
<td>94.53±1.47&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.69±0.84&lt;sup&gt;C&lt;/sup&gt;</td>
<td>34.24±2.50&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>LH3</td>
<td>36.28±0.37&lt;sup&gt;A&lt;/sup&gt;</td>
<td>35.41±0.46&lt;sup&gt;B&lt;/sup&gt;</td>
<td>92.44±0.47&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.82±0.30&lt;sup&gt;C&lt;/sup&gt;</td>
<td>35.25±0.46&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>LH4</td>
<td>33.68±1.10&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>40.92±0.47&lt;sup&gt;A&lt;/sup&gt;</td>
<td>89.99±0.54&lt;sup&gt;B&lt;/sup&gt;</td>
<td>5.08±0.35&lt;sup&gt;B&lt;/sup&gt;</td>
<td>40.59±0.50&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. <sup>AC</sup> Means with different letters in each column are significantly different (P>0.05).

The prominent sugars in honey are glucose and fructose with the ratio of fructose to glucose in honey being 1.2:1 (Rodriguez and others 2004). All the samples showed an average of 65.65% of reducing sugars comprising glucose and fructose thus indicating that a majority of the honey composition and its solid contents is sugar (Table 2.3.3).
Table 2.3.3 Sugar Content (20°C)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose(g/100g)</th>
<th>Fructose(g/100g)</th>
<th>Sucrose(g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH1</td>
<td>29.54±0.89&lt;sup&gt;A&lt;/sup&gt;</td>
<td>34.04±0.80&lt;sup&gt;A&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>LH2</td>
<td>29.67±0.12&lt;sup&gt;A&lt;/sup&gt;</td>
<td>36.12±0.23&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.12±0.01&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>LH3</td>
<td>31.99±0.76&lt;sup&gt;A&lt;/sup&gt;</td>
<td>34.68±1.32&lt;sup&gt;A&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>LH4</td>
<td>31.06±0.28&lt;sup&gt;A&lt;/sup&gt;</td>
<td>35.48±0.35&lt;sup&gt;A&lt;/sup&gt;</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND-Not detected
Values are means ± SD of 3 determinations. <sup>A</sup> Means with same letter in each column are not significantly different (P>0.05).

Sucrose and maltose though present were not in adequate concentration so as to be quantified with the HPLC used and only in LH2 was the quantification possible. Low sucrose concentrations are generally believed to be due to the honey being in an advanced stage of ripening (Lazaridou and others 2004). Honey was in its early stage of ripening contains more higher concentrations of sucrose since the sucrose hasn’t been converted into glucose and fructose by the enzyme invertase yet (Gomes and others 2010).

Apparent viscosity values (Table 2.3.4) at 20°C were lowest for LH2 at 7.79 Pa s while LH4 showed the highest apparent viscosity 9.15 Pa s. LH3 and LH2 showed intermediate values of viscosity indicating that the general viscosity range could be between 7.79 and 9.15 Pa s. Difference in viscosity is attributed to various factors with pollen source, moisture content and chemical composition including the presence of colloids and crystals being the predominant ones (Juszczak and Fortuna 2006; Yanniotis and others 2006). The presence of monosaccharides and disaccharides is also an important consideration with disaccharides contributing more to viscosity for the same mass fraction of monosaccharides (Bhandari and others 1999). Munroe (1943) reported that the effect of a 1% change in moisture content on viscosity equates to the effect that a 3.5°C change in temperature would have on the same. Though all the honeys were of multi-floral origin with tallow and willow being the predominant pollen source with no significant difference in moisture content, variations in chemical composition could contribute to
the difference in viscosity values. This is well established by the studies on Australian honeys with the viscosity values reported by Sopade and others (2002) being different from those reported in earlier studies by Bhandari and others (1999) even though the floral source of the honeys were the same.

Table 2.3.4 Apparent viscosity (Pa s) values of honey samples at different temperatures (20-60°C)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>LH1</th>
<th>LH2</th>
<th>LH3</th>
<th>LH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>8.43±0.05BC,a</td>
<td>7.79±0.46C,a</td>
<td>8.79±0.20AB,a</td>
<td>9.15±0.15A,a</td>
</tr>
<tr>
<td>30</td>
<td>2.61±0.04C,b</td>
<td>2.42±0.03D,b</td>
<td>2.79±0.03B,b</td>
<td>2.93±0.01A,b</td>
</tr>
<tr>
<td>40</td>
<td>0.93±0.03B,c</td>
<td>0.90±0.01B,c</td>
<td>1.03±0.01A,c</td>
<td>1.07±0.01A,c</td>
</tr>
<tr>
<td>50</td>
<td>0.43±0.02AB,d</td>
<td>0.39±0.01B,dc</td>
<td>0.42±0.02AB,d</td>
<td>0.46±0.03A,d</td>
</tr>
<tr>
<td>60</td>
<td>0.22±0.01AB,c</td>
<td>0.18±0.01B,d</td>
<td>0.22±0.004A,d</td>
<td>0.25±0.03A,e</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-C Means with different letters in each row are significantly different (P>0.05). a-e Means with different letters in each column are significantly different (P>0.05).

Apparent viscosity showed a significant drop in values with an increase in temperature as expected. The drop in values of viscosity were extremely large between 20°C and 30°C and though there was significant difference in values at temperatures above 30°C the difference in values were not very large. This is because the effect of temperature on the viscosity of honey is more pronounced at temperatures below 30°C and isn’t as much at temperatures above 45°C (Yanniotis and others 2006; Juszczak and Fortuna 2006; Yoo 2004). The fall in viscosity values is attributed to the decrease in molecular friction as well as the hydrodynamic force (Mossel and others 2000).

Literature mostly reports honey as a Newtonian fluid (Abu-Jdayil and others 2002; Bhandari and others 1999; Lazaridou and others 2004; Juszczak and Fortuna 2006). However Mossel and others (2000) cited some papers which have reported thixotropic behavior in honey from heather (Calluna vulgaris), buckwheat (Fagopyrum esculentum), white clover (Trifolium repens) and Indian Karvi (Carvia callosa) while dilatant behavior was observed in honeys originating in
Nigeria. The Power law model (Table 2.3.5) showed flow behavior value almost close to unity at all temperatures which indicate Newtonian behavior, with consistency index that decreased with an increase in temperature. The consistency index significantly decreased for each sample over the temperature range (20-60°C). This fits best for Newtonian type fluid behavior which is the expected behavior for honey.

Table 2.3.5 Consistency index, K (Pa s^n) and flow behavior index, n (dimensionless) values for Power law model

<table>
<thead>
<tr>
<th>Temperature(°C)</th>
<th>LH1</th>
<th>LH2</th>
<th>LH3</th>
<th>LH4</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.99±0.00A,a</td>
<td>0.99±0.011A,a</td>
<td>0.99±0.001A,a</td>
<td>0.99±0.00A,a</td>
</tr>
<tr>
<td>30</td>
<td>0.99±0.002A,a</td>
<td>0.99±0.002A,ab</td>
<td>0.99±0.001A,a</td>
<td>0.99±0.002A,a</td>
</tr>
<tr>
<td>40</td>
<td>0.99±0.004A,a</td>
<td>0.98±0.002B,b,ab</td>
<td>0.99±0.001A,a</td>
<td>0.99±0.003A,a</td>
</tr>
<tr>
<td>50</td>
<td>0.99±0.01A,a</td>
<td>0.98±0.001A,b</td>
<td>0.99±0.01A,a</td>
<td>0.99±0.01A,a</td>
</tr>
<tr>
<td>60</td>
<td>0.99±0.003A,a</td>
<td>0.99±0.002A,ab</td>
<td>0.99±0.004A,a</td>
<td>0.99±0.004A,a</td>
</tr>
<tr>
<td>20</td>
<td>8.37±0.07AB,a</td>
<td>7.66±0.34C,a,b</td>
<td>8.71±0.17AB,a</td>
<td>9.19±0.14A,a</td>
</tr>
<tr>
<td>30</td>
<td>2.69±0.03C,b,ab</td>
<td>2.42±0.04D,b,ab</td>
<td>2.82±0.02B,b</td>
<td>2.99±0.02A,b</td>
</tr>
<tr>
<td>40</td>
<td>1.07±0.02C,c</td>
<td>0.96±0.01D,c</td>
<td>1.11±0.01B,c</td>
<td>1.18±0.01A,c</td>
</tr>
<tr>
<td>50</td>
<td>0.49±0.003B,d,ab</td>
<td>0.44±0.002C,d</td>
<td>0.51±0.01B,d</td>
<td>0.55±0.02A,d</td>
</tr>
<tr>
<td>60</td>
<td>0.25±0.002C,e</td>
<td>0.23±0.004D,d</td>
<td>0.27±0.01B,c</td>
<td>0.28±0.01A,d</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-D Means with different letters in each row are significantly different (P>0.05). a-e Means with different letters in each column are significantly different(P>0.05)

Figure 2.1 Graphical representation of Arrhenius equation
LH2 had the highest value of activation energy at 75.92 kJ/mol while LH1 had the least 74.57 kJ/mol, though the four samples showed no significant difference in the activation energy values (Table 2.3.8). Activation energy is an indicator of the sensitivity of the viscosity to changes in temperature with higher value of activation energy indicating a higher sensitivity of the honey viscosity to the change in temperature (Bhandari and others 1999). Activation energy can help determine changes in behavior of honey during processing and storage. This could serve as a helpful tool in determining process conditions and in modeling mass transfer processes such as dehydration (Mirzaee and others 2009). Activation energy was comparable to very few of the honeys of Greek origin though the latter showed much higher values of activation energy (72.69 – 93.75 kJ/mol) for most samples as compared to Louisiana honey samples (Lazaridou and others 2004). Honeys of Jordan origin also showed extremely high values of activation energy ranging between 95.6 kJ/mol and 97.7 kJ/mol (Al-Malah and others 2001). The correlation coefficient, R², value for all the samples was 0.99 indicating a very high dependence of the viscosity on the temperature (Lazaridou and others 2004).

Table 2.3.6 Activation energy values of honey samples at different temperatures (20-60°C)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Arrhenius equation</th>
<th>Activation energy E_a (kJ/mol)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH1</td>
<td>ln(μ) = 8906.3x – 28.38</td>
<td>74.57±0.89 A</td>
<td>0.99</td>
</tr>
<tr>
<td>LH2</td>
<td>ln(μ) = 9155.2x – 29.28</td>
<td>75.92±0.65 A</td>
<td>0.99</td>
</tr>
<tr>
<td>LH3</td>
<td>ln(μ) = 9073.9x – 28.88</td>
<td>75.15±0.80 A</td>
<td>0.99</td>
</tr>
<tr>
<td>LH4</td>
<td>ln(μ) = 8862.3x – 28.13</td>
<td>74.89±0.75 A</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A Means with same letter in each column are not significantly different (P>0.05).

2.3.2 Characterization of Spray-dried Honey Powder

The L* values of the 4 honey powder samples ranged between 85.62 and 91.29 which indicates an extremely high degree of lightness as compared to the very dark colored honey (Table 2.3.9). This is because the dark colored honey when combined with the resistant starch which is whitish
in color resulted in a color profile that was intermediate. The addition of starch increased the lightness while decreasing the redness (increase in a* values). The b* values are indicative of yellowness appearance in keeping with the decrease in redness values. Sablani and others (2008) reported similar findings in spray dried date powder.

Table 2.3.7 Color values of honey powder samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>L*</th>
<th>c*</th>
<th>h*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP1</td>
<td>90.22±0.29A</td>
<td>13.71±0.32B</td>
<td>99.24±0.65AB</td>
<td>-0.82±0.70A</td>
<td>13.69±0.26B</td>
</tr>
<tr>
<td>HP2</td>
<td>91.29±0.53A</td>
<td>11.79±0.72C</td>
<td>101.76±2.87A</td>
<td>-1.22±0.09C</td>
<td>11.50±0.70C</td>
</tr>
<tr>
<td>HP3</td>
<td>87.71±0.85B</td>
<td>12.07±0.73C</td>
<td>101.98±0.69A</td>
<td>-1.29±0.04D</td>
<td>12.12±0.19C</td>
</tr>
<tr>
<td>HP4</td>
<td>84.62±0.38C</td>
<td>19.62±0.45A</td>
<td>96.96±3.49B</td>
<td>-1.10±0.02B</td>
<td>19.28±0.44A</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-D Means with different letters in each column are significantly different (P>0.05).

The moisture for the honey samples ranged between 3.83% and 5.53% (Table 2.3.10). Moisture content of spray dried powders is affected by many factors including the feed flow rate (Tonon and others 2008), the inlet temperature, dry air as well as the amount of drying agent added (Kha and others 2010). In this study spray drying conditions such as feed rate and inlet temperature were constant. Thus the variation in moisture content of the honey samples could be due to fluctuation of humidity in inlet air which also affects the moisture content of the final product (Pu and others 2011). As the samples were spray dried on different days changes in humidity of air could have been a possible reason for the difference in moisture content. Vacuum dried honey powders with no additives have moisture contents values of 2.5% or less to ensure better keeping qualities since pure honey when dried without additives produces very hygroscopic powders (Cui and others 2008).
Table 2.3.8 % Moisture content of honey powder samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>% Moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP1</td>
<td>4.61±0.25(^B)</td>
</tr>
<tr>
<td>HP2</td>
<td>5.53±0.37(^A)</td>
</tr>
<tr>
<td>HP3</td>
<td>4.14±0.18(^{BC})</td>
</tr>
<tr>
<td>HP4</td>
<td>3.83±0.19(^C)</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. \(^{A-C}\) Means with different letters in each column are significantly different (P>0.05).

The honey powders in this study were produced using 30% resistant starch and spray dried at a feed rate of 9 mL/min at an inlet temperature of 200°C which was found to be the optimum conditions. Any further increase in resistant starch would have lowered the quality the powders and decreased the nutritional content obtained from honey (Quek and others 2007).

The sugar content was concentrated when spray dried and they were all from the honey as the starch had less than 0.05% sugars (Table 2.3.11). Detection of sucrose and maltose was possible in the spray dried powders as they were concentrated. In all the samples fructose was still higher than the glucose and HP2 showed the highest sucrose content. This correlates to the initial sucrose determination in LH2. The encapsulation by resistant starch was similar in nature to maltodextrin which is capable of altering surface stickiness of low molecular sugars such as glucose, fructose and sucrose thereby facilitating drying and reducing stickiness of powders (Quek and others 2007). Cui and others (2008) reported that for honey powder prepared without any additives and dried by vacuum drying an increase in the glucose and fructose content was seen whereas there was a decrease in the sucrose and maltose content. Since the current study used a drying aid this change in sucrose and maltose content was not noticed.
Table 2.3.9 Sugar content of honey powder samples by HPLC

<table>
<thead>
<tr>
<th>Sample</th>
<th>Glucose (g/100g)</th>
<th>Fructose (g/100g)</th>
<th>Sucrose (g/100g)</th>
<th>Maltose (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP1</td>
<td>10.39±0.35&lt;sup&gt;B&lt;/sup&gt;</td>
<td>12.54±0.19&lt;sup&gt;C&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.60±0.08&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP2</td>
<td>10.68±0.06&lt;sup&gt;B&lt;/sup&gt;</td>
<td>13.34±0.07&lt;sup&gt;B&lt;/sup&gt;</td>
<td>0.09±0.02&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.64±0.03&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP3</td>
<td>11.58±0.29&lt;sup&gt;A&lt;/sup&gt;</td>
<td>12.07±0.49&lt;sup&gt;C&lt;/sup&gt;</td>
<td>0.09±0.01&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>0.61±0.09&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP4</td>
<td>11.55±0.44&lt;sup&gt;A&lt;/sup&gt;</td>
<td>15.14±0.29&lt;sup&gt;A&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;AB&lt;/sup&gt;</td>
<td>0.89±0.01&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. <sup>A-C</sup> Means with different letters in each column are significantly different (P>0.05).

In all of the SEM images (Figure 2.2) it was seen that most of the particles were smooth and rounded and more or less dispersed with the exception of HP2. HP2 showed some dented and rough particles.

![SEM images](image)

Figure 2.2 SEM images at 1000X magnification of (A) HP1 (B) HP2 (C) HP3 (D) HP4

This occurrence can be explained by the induction of crystallization of sugars during the spray drying (Cano-Chauca and others 2005). Harnkarnsujarit and Charoenrein (2011) also reported...
that crystallization of sugars may cause the surface roughness of the dried powders. Among the four samples HP2 showed the highest presence of sugars (glucose, fructose and sucrose) thereby adhering to the expected roughness in appearance as well as the segregation of particles. The dents could also be caused due to a slower evaporation rate in some particles as well as other factors such as atomization and feed rate (Pu and others 2011). Smaller droplets tend to be heavier in terms of mass and higher heat transfer rates than larger atomized droplets and thus have less dented particles. Given the SEM images it could be said that the spray drying conditions used produced powders with acceptable qualities for a good powder.

2.4 Conclusion

All of the honeys were acidic in nature, with expected moisture content and a high percentage of total solids. Variations in characteristics were likely explained by the difference in floral source and region of origin primarily. All of the samples showed a high monosaccharide content with the concentration of fructose being greater than that of glucose. The honey samples showed the best fit for the Power Law model which is indicative of Newtonian behavior in liquids. Activation energy was lower than those reported for honeys from different regions such as Jordan and Greece indicating that the samples were less sensitive to temperature changes which have a direct effect on storage and handling of honey. Resistant starch proved to be an effective drying agent and helped form honey powder by spray drying. The spray dried powder had good morphology and were dispersed rather than being sticky and aggregated which is generally not preferred in a powder. Powders also showed presence of both monosaccharides and disaccharides and seemed to have the potential for further use in processing such as baking.
CHAPTER 3 DEVELOPMENT OF A BREAD FORMULATION WITH HONEY POWDER AND ITS CHARACTERIZATION

3.1 Introduction

Bread is a globally consumed baked product and is a central constituent of many well balanced diets because of its rich starch and complex carbohydrate content (Altamirano-Fortoul and Rosell 2011; Hathorn and others 2008). The baking industry has been dynamically changing in the past 150 years (Mondal and Datta 2008) in an attempt to optimize the technology so as to cope with various issues that stem from various reasons such as socioeconomics, market competition, changing consumer preferences, needs and attitudes, and changes in the production and quality of the basic ingredients (Hathorn and others 2008). Health authorities worldwide recommend an increase in cereal intake as it is an important source of dietary fiber. This has lead to the development of several types of bread whose flour composition is substituted with soy flour, potato flour etc. (Wang and others 2002). Whole grain breads with non-traditional cereals in them have shown an increasing demand due to their nutritional benefits (Svec and Hruskova 2010). Sucrose in bread serves as substrate to yeasts primarily for fermentation purposes and contributes to the calorie content of the product with no added nutritional benefits. They also affect texture of bread. Honey contains fructose which is more hygroscopic than sucrose and thus bread baked with honey is expected to be moister (Vaclavik and Christian 2003). However adding honey or honey powder may affect the quality of bread. The primary two attributes that consumers look for to determine quality of bread are its appearance and physical texture (Scanlon and Zghal 2001). However after baking, the freshness of bread begins to deteriorate rapidly due to various physical and chemical reactions which all together are called staling (Stampfli and Nersten 1995; Barcenas and Rosell 2005).
As opposed to other food products, staling in bread is of major concern rather than spoilage due to micro-organisms or endogeneous enzyme activity (Barcenas and Rosell 2005). It is estimated that in a market where 20 billion pounds of bread are produced annually at least 600 million (3%) is lost due to staling problems (Lai and Lin 2006). Though a lot of literature has been published regarding staling, the process itself remains complex and is not fully understood (Ribotta and Bail 2007). Crumb firming is the most important parameter that is linked by consumers to staling (Lai and Lin 2006). Staling is attributed to many reasons with the prominent ones being starch retrogradation, mainly that of amylopectin (Mandala and others 2007), interactions between starch and gluten proteins (Patel and others 2005), and the loss and redistribution of water (Barcenas and Rosell 2005).

Honey is known to be high in sugars such as fructose and glucose (80-90%) which also improve browning due to Maillard reactions and also retains moisture when used in baked products (Bogdanov 2010). The bread produced using honey or honey powder in this study is expected to have qualities similar to those breads baked using sucrose. The objectives of this study were to use honey or honey powder containing retrograded starch as an alternative to sucrose in bread formulations and to characterize the bread and study textural changes during a storage period of 12 days.

3.2 Materials and Methods

3.2.1 Bread Formulation

The bread was prepared using the Straight dough method for white pan bread followed by American Institute of Baking (AIB). The basic bread formulation per 100 g of flour was 62 g
water, 3 g margarine, 7 g granulated sugar, 2 g salt and 2 g yeast. The four formulations for the bread are illustrated in Table 3.2.1.

**Table 3.2.1 Bread formulations**

<table>
<thead>
<tr>
<th></th>
<th>Control (with sucrose)</th>
<th>Substitution with liquid honey (HNY)</th>
<th>Substitution with 50% honey powder (SHP)</th>
<th>Substitution with 100% honey powder (HP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
<td>100 g</td>
</tr>
<tr>
<td>Yeast</td>
<td>2 g</td>
<td>2 g</td>
<td>2 g</td>
<td>2 g</td>
</tr>
<tr>
<td>Sucrose</td>
<td>7 g</td>
<td>-</td>
<td>3.5 g</td>
<td>-</td>
</tr>
<tr>
<td>Honey</td>
<td>-</td>
<td>10.6 g</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Honey powder</td>
<td>-</td>
<td>-</td>
<td>14 g</td>
<td>28 g</td>
</tr>
<tr>
<td>Salt</td>
<td>2 g</td>
<td>2 g</td>
<td>2 g</td>
<td>2 g</td>
</tr>
<tr>
<td>Margarine</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
<td>3 g</td>
</tr>
<tr>
<td>Water</td>
<td>62 g</td>
<td>62 g</td>
<td>62 g</td>
<td>62 g</td>
</tr>
</tbody>
</table>

A bread formulation prepared with only sugar (S) was used as a control. Three bread formulations were prepared with (1) 100% liquid honey (HNY), (2) 50% substitution of sugar with honey powder (HP2) (SHP) and (3) 100% honey powder (HP). HP2 was selected as the honey powder to substitute sucrose as it had the moisture content (5.53%) closest to that of the sucrose (5.51%) used in this study.

### 3.2.2 Characterization of Flour Mixture

#### 3.2.2.1 Rapid Visco Analyzer (RVA)

The RVA parameters – peak viscosity, minimum viscosity and final viscosity were determined using a RVA 4 (Newport Scientific, Australia) and RVA data were analyzed using the software ThermoCline for Windows, Version 3.1. Above mentioned four formulations without salt, yeast, margarine and water were prepared. Flour only (with no added ingredient) was used as the
control. Moisture content of each sample as well as the flour was determined using the moisture analyzer (CEM – 5) and according to the moisture content, 3.5 g of sample was weighed and mixed with 25 mL of water. Each flour mixture with water was held at 50°C for 1 min and then heated to 95°C at the rate of 12.2°C/min. The heated suspension was then held at 95°C for 2.5 min. The cooling cycle then began at the rate of 11.8°C/min till the temperature of 50°C was attained after which it was held at the same temperature for 2 min. The values of peak viscosity, minimum viscosity and final viscosity were obtained from the graph generated and these values were used to compute the breakdown and total setback values.

\[
\text{Breakdown} = \text{Peak viscosity} - \text{Minimum viscosity}
\]

\[
\text{Total Set Back (TSB)} = \text{Final viscosity} - \text{Minimum viscosity}
\]

### 3.2.3 Bread Preparation

All of the ingredients were mixed in a Kitchen Aid mixer at speed 2 for 4 minutes. After mixing the dough was divided into 6 pieces and placed in rectangular pans. Proofing was done for 60 minutes at 81°F and then for 10 minutes at 110°F. The breads were then baked at 440°F for 20 minutes. The breads were individually stored in Ziploc bags at 20°C for further analysis and storage study.

### 3.2.4 Bread Characterization and Storage Study

#### 3.2.4.1 Loaf volume, Specific Volume and Density

Loaf volume of bread was determined an hour after baking on day 0 by the bean displacement method (Greene and Bovell-Benjamin 2004; Wang and others 2002). Beans were poured so as to cover the bottom of a container of known volume. The bread loaf was then placed and the
remainders of the bean seeds were poured into the container. The beans were leveled on the surface of the container using a spatula. The beans that were not required to fill the container were measured in a graduated cylinder and represented the volume of the loaf. The results were expressed as means of triplicate values along with standard deviation.

Specific volume was calculated as the ratio of the loaf volume to the loaf mass determined an hour after baking according to the method of Penfield and Campbell (1990).

\[
Specific\ volume\ (cm^3/g) = \frac{Loaf\ volume\ of\ bread}{Mass\ of\ bread}
\]

Bread density was calculated as the ratio of the loaf mass to the loaf volume (Shogren and others 2003).

\[
Density\ (g/cm^3) = \frac{Mass\ of\ bread}{Loaf\ volume\ of\ bread}
\]

### 3.2.4.2 Weight Loss

Weight of the dough and the bread baked were measured and the % weight loss was calculated as follows

\[
% Weight\ loss = \frac{Weight\ of\ dough - Weight\ of\ baked\ bread}{Weight\ of\ dough} \times 100
\]

### 3.2.4.3 Crumb and Crust Color

Color values for crust and crumb were measured at three different locations on the same loaf using the HunterLab Labscan XE colorimeter (Labscan XE, Hunter Associates laboratory Inc., Reston, Virginia, USA) in triplicates of samples weighing 8gm each. The results were reported
as $L^*$ (lightness), $a^*$ (redness and greenness), and $b^*$ (yellowness and blueness). The measurements were made in triplicate and the means and standard deviations were reported.

### 3.2.4.4 Crumb and Crust Moisture

Moisture for the crust and crumb was determined by AOAC 969.38b, 1995 method using a forced air convection oven. Three grams of crust and crumb each from 3 different locations on the bread were heated at 105°C for 24hrs. Triplicate measurements were done and values were reported as means along with standard deviations.

### 3.2.4.5 Analysis of Texture

Texture was analyzed using a texture analyzer (TA-XT plus) with a 51mm diameter cylindrical probe at test speed of 10 mm/s and a 5 kg load. Bread slices used for testing were cut from the center of the loaf and were 25 mm thick. Firmness, cohesiveness, springiness and resilience values were determined. Chewiness was calculated as follows:

$$Chewiness = Firmness \times Cohesiveness \times Springiness$$

Triplicate measurements were done and results were expressed as means along with standard deviations.

### 3.2.4.6 Condition for Ageing Tests and Model Used to Describe Staling

Ageing tests were carried out at 20°C for 12 days according to Le-Bail and others (2009). The texture analysis was conducted on day 0, 1, 3, 6, 9 and 12. First order kinetic model was used to model the crumb hardening based on the following equation

$$E(t) = E_\infty + (E_0 - E_\infty) e^{-\frac{t}{\tau}}$$
Where $E_0$ and $E_\infty$ represent the Young’s modulus at initial time and final storage respectively. The characteristic time constant, $\tau$ was used to characterize and compare the phenomenon of crumb hardening.

### 3.2.4.7 Differential Scanning Calorimetry (DSC)

A 10 mg of the core of the bread samples was placed in the hermetically sealed aluminum pans and analyzed using the DSC (TA Advantage). The samples were first cooled from 25°C to -50°C and then slowly heated at a ramp of 2°C/min from -50°C to 100°C. Condensation in the measurement cell was minimized using dry nitrogen gas flow. The onset ($T_0$), peak ($T_p$) and conclusion ($T_c$) temperatures are obtained. The amount of freezable water was determined from the relationship between transition enthalpy of ice melting and latent heat of ice melting (334J/g) (Ribotta and Le Bail 2007). The measurements were done in duplicate.

The statistical significance of observed differences among formulation means was evaluated by analysis of variance (ANOVA) (SAS, Version 9.2, SAS Institute Inc., Cary, NC., USA) followed by the post-hoc Tukey’s studentized range test (SAS 2002).

### 3.3 Results and Discussion

#### 3.3.1 Physico-chemical Characterization of read

The peak viscosity values (Table 3.3.1) of all of the flour mixture samples were lower than the peak viscosity value of the flour alone. This held true for minimum viscosity, final viscosity, breakdown and TSB values. This is attributed to the fact that any added ingredient to a flour base always depreciates its gluten forming characteristics due to the absence of proteins in starch which are required for binding especially in the case of SHP and HP flour samples due to the presence of added resistant starch. Lei and others (2008) studied specifically the impact of
resistant starch on the physical properties of wheat flour and reported that increasing levels of resistant starch in a resistant starch-wheat flour mixture (up to 20%) showed a marked decrease in peak viscosity, breakdown and total setback (TSB) values. However they did not affect the pasting temperature or peak time which was also observed in this study.

Table 3.3.1 RVA analysis for flour samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Peak viscosity (x10^3 Pa s)</th>
<th>Minimum viscosity (x10^3 Pa s)</th>
<th>Final viscosity (x10^3 Pa s)</th>
<th>Breakdown (x10^3 Pa s)</th>
<th>TSB (x10^3 Pa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flour</td>
<td>995±22.27^A</td>
<td>183±18^A</td>
<td>528.67±15.95^A</td>
<td>812±5.29^A</td>
<td>345.67±13.50^A</td>
</tr>
<tr>
<td>Control</td>
<td>798±74.84^B</td>
<td>139.33±13.65^B</td>
<td>416±27.71^B</td>
<td>658.67±60.91^B</td>
<td>276.67±14.22^A</td>
</tr>
<tr>
<td>HNY</td>
<td>712.67±17.90^B</td>
<td>118.67±6.51^BC</td>
<td>375±10^BC</td>
<td>594±12.50^B</td>
<td>256.33±3.51^BC</td>
</tr>
<tr>
<td>SHP</td>
<td>537.67±13.65^C</td>
<td>98.67±4.51^C</td>
<td>322.33±22.85^CD</td>
<td>439±9.17^C</td>
<td>223.67±18.77^C</td>
</tr>
<tr>
<td>HP</td>
<td>394±15.87^D</td>
<td>92.33±15.31^C</td>
<td>279.67±22.48^D</td>
<td>301.67±6.03^D</td>
<td>187.33±11.85^D</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. ^A-D Means with different letters in each column are significantly different (P>0.05). Control – sugar; HNY – liquid honey; SHP – 50% substitution with honey powder; HP – honey powder; TSB – Total Setback.

The breakdown in viscosity is associated with the holding period (95°C) where the sample is subjected to mechanical stress at a high temperature which results in the breakdown of starch granules with amyllose leaching and realignment. The capacity of starch to withstand high temperatures and mechanical stress is an important factor in many processes (Newport Scientific 1998). High values of breakdown viscosity correspond to high peak viscosities and this correlates to the degree of swelling of the starch granules. The high degree of swelling in turn causes the starch to reach its maximum viscosity very fast while causing breakdown to also occur rapidly due to weak intermolecular forces thus causing them to be sensitive to high temperatures and mechanical stress (Raggae and Aal 2006; Zaidul and others 2007). Therefore it can be concluded that starch granules broke down very easily in descending order of the control, HNY, SHP and HP thereby making the HP sample more resistant to high temperatures. It is also
an indication of the denser crystalline structure of HP as compared to the other samples (Lei and others 2008).

Total setback, on the other hand, represents the period of cooling which indicates the value obtained due to rearrangement of excreted amylose molecules from starch granules after swelling. This is related to retrogradation with a higher setback value relating to a higher degree of retrogradation (Lei and others 2008). Thus among the samples since HP significantly showed lesser TSB values it indicates that degree of retrogradation is the lowest in this sample thereby implicating that bread made from this formulation would be the softest. This was followed in ascending order of SHP, HNY and finally the control.

Loaf volume (Table 3.3.2) was the highest for HP and was significantly higher than the control bread and HNY while SHP was an intermediate to both. However no significant differences in density, specific density or weight loss were observed. Hathorn and others (2008) reported that when bread dough was supplemented with sweet potato flour as well as dough enhancers it caused an increase in loaf volume while increasing concentrations of sweet potato flour alone caused a decrease in loaf volume. This was attributed to the presence of the protein sporamin instead of gluten since it is the latter that is required for forming the structural framework in bread. However the retrograded starch does not contain any protein to contribute to gluten formation and hence higher loaf volume could be due to the presence of a variety of sugars for the yeast to work on. The bread samples had loaf volumes that ranged from 1100±65.66 mL to 1461.56±45.05 mL which is within range of that reported for supplemented breads especially those with replaced flour (Hathorn and others 2008; Borla and others 2004).
Table 3.3.2 Loaf volume, Density, Specific density and %Weight loss of bread samples (Day 0)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Loaf volume (mL)</th>
<th>Density (g/cm³)</th>
<th>Specific volume (cm³/g)</th>
<th>% Weight loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1100.00±65.66B</td>
<td>0.44±0.06A</td>
<td>2.29±0.27A</td>
<td>9.14±1.08A</td>
</tr>
<tr>
<td>HNY</td>
<td>1155.44±90.9B</td>
<td>0.42±0.07A</td>
<td>2.41±0.35A</td>
<td>11.21±1.97A</td>
</tr>
<tr>
<td>SHP</td>
<td>1303.33±198.52AB</td>
<td>0.39±0.09A</td>
<td>2.68±0.60A</td>
<td>12.16±2.19A</td>
</tr>
<tr>
<td>HP</td>
<td>1461.56±45.05A</td>
<td>0.35±0.03A</td>
<td>2.86±0.22A</td>
<td>12.98±2.21A</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-B Means with different letters in each column are significantly different (P>0.05)

Specific volume is an important parameter as it is associated with dough inflating ability and oven spring and extremes in its values affect crumb structure (Yi and others 2009). Smaller values of specific density are associated with compact, dense and closed grain structure while larger values indicate open grain airy structures (Sharadanant and Khan 2003). Density gives an indication of the size and ratio of air cells to solid product while the % weight loss is associated with the loss of moisture and entrapped CO₂ from the dough matrix (Hathorn and others 2008).

Shogren and others 2003 reported density values of 0.29-0.73 g/cm³ for whole wheat bread supplemented with varying concentrations of soy flour which is comparable to the bread samples in this study.

The color of bread crust is mostly attributed to Maillard browning and also caramelization due to the presence of sucrose. Though a clear pattern could not be discerned in terms of lightness of crumb it was seen that in all cases the lightness was higher at the end of 12 days as compared to day 0 (Table 3.3.3). Redness indicated by a* values decreased over time indicating a lightening of the crumb. HP showed lowest L* values for the crust which was followed by SHP, HNY and control in ascending order (Table 3.3.4). This indicates that HP had a much darker crust as compared to the other samples which could be attributed to the presence of more types of sugars in the honey powder that contributed to more Maillard browning. Mohamed and others (2010)
reported similar findings for bread made with banana flour which was high in sugar content. The crust of bread supplemented with 30% banana flour was much darker as compared to the breads containing 10% banana flour and no banana flour. Irregularity in patterns over storage may be due to differences in sampling region and lack of uniformity in browning of the crust.

Table 3.3.3 Crumb color L*, a* and b* values of bread during storage

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.64±</td>
<td>54.57±</td>
<td>59.91±</td>
<td>60.63±</td>
<td>68.15±</td>
<td>72.32±</td>
</tr>
<tr>
<td></td>
<td>0.02C,</td>
<td>0.17D,b</td>
<td>0.05C,c</td>
<td>0.08E,b</td>
<td>0.05B,a</td>
<td>0.52A,a</td>
</tr>
<tr>
<td>HNY</td>
<td>65.53±</td>
<td>62.98±</td>
<td>65.16±</td>
<td>57.79±</td>
<td>67.55±</td>
<td>68.35±</td>
</tr>
<tr>
<td>L*</td>
<td>0.01C,a</td>
<td>0.06E,a</td>
<td>0.01D,a</td>
<td>0.10F,d</td>
<td>0.03B,b</td>
<td>0.02A,b</td>
</tr>
<tr>
<td>SHP</td>
<td>56.74±</td>
<td>54.13±</td>
<td>60.28±</td>
<td>58.13±</td>
<td>65.82±</td>
<td>70.94±</td>
</tr>
<tr>
<td></td>
<td>0.01E,d</td>
<td>0.02F,c</td>
<td>0.01D,b</td>
<td>0.21C,c</td>
<td>0.01B,d</td>
<td>0.06A,ab</td>
</tr>
<tr>
<td>HP</td>
<td>63.98±</td>
<td>43.30±</td>
<td>49.69±</td>
<td>62.36±</td>
<td>65.64±</td>
<td>71.78±</td>
</tr>
<tr>
<td></td>
<td>0.04C,b</td>
<td>0.02E,d</td>
<td>0.21E,d</td>
<td>0.01D,a</td>
<td>0.02B,c</td>
<td>0.03A,a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.54±</td>
<td>1.07±</td>
<td>0.67±</td>
<td>0.64±</td>
<td>0.34±</td>
<td>0.30±</td>
</tr>
<tr>
<td></td>
<td>0.01A,b</td>
<td>0.01B,c</td>
<td>0.02C,d</td>
<td>0.02C,c</td>
<td>0.01D,d</td>
<td>0.02D,d</td>
</tr>
<tr>
<td>HNY</td>
<td>1.24±</td>
<td>1.04±</td>
<td>0.89±</td>
<td>0.73±</td>
<td>0.56±</td>
<td>0.48±</td>
</tr>
<tr>
<td>a*</td>
<td>0.01A,d</td>
<td>0.01B,d</td>
<td>0.01C,c</td>
<td>0.01D,b</td>
<td>0.01A,a</td>
<td>0.01F,c</td>
</tr>
<tr>
<td>SHP</td>
<td>1.44±</td>
<td>1.36±</td>
<td>1.34±</td>
<td>0.97±</td>
<td>0.73±</td>
<td>0.69±</td>
</tr>
<tr>
<td></td>
<td>0.01A,c</td>
<td>0.01B,b</td>
<td>0.01B,b</td>
<td>0.01C,a</td>
<td>0.01D,b</td>
<td>0.01E,b</td>
</tr>
<tr>
<td>HP</td>
<td>1.74±</td>
<td>1.67±</td>
<td>1.43±</td>
<td>0.98±</td>
<td>0.86±</td>
<td>0.81±</td>
</tr>
<tr>
<td></td>
<td>0.01A,a</td>
<td>0.01B,a</td>
<td>0.01C,a</td>
<td>0.01D,a</td>
<td>0.01B,a</td>
<td>0.01F,a</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.58±</td>
<td>24.96±</td>
<td>20.42±</td>
<td>19.64±</td>
<td>20.88±</td>
<td>21.67±</td>
</tr>
<tr>
<td></td>
<td>0.01B,c</td>
<td>0.03A,</td>
<td>0.05E,d</td>
<td>0.04F,d</td>
<td>0.02B,d</td>
<td>0.01C,c</td>
</tr>
<tr>
<td>b*</td>
<td>0.05D,d</td>
<td>0.01C,d</td>
<td>0.01A,a</td>
<td>0.01B,a</td>
<td>0.01F,c</td>
<td>0.01E,b</td>
</tr>
<tr>
<td>SHP</td>
<td>22.67±</td>
<td>24.38±</td>
<td>22.50±</td>
<td>21.18±</td>
<td>21.54±</td>
<td>23.03±</td>
</tr>
<tr>
<td></td>
<td>0.02B,b</td>
<td>0.02A,</td>
<td>0.01C,c</td>
<td>0.01E,c</td>
<td>0.02B,c</td>
<td>0.01D,b</td>
</tr>
<tr>
<td>HP</td>
<td>23.10±</td>
<td>24.51±</td>
<td>23.05±</td>
<td>22.88±</td>
<td>23.37±</td>
<td>24.04±</td>
</tr>
<tr>
<td></td>
<td>0.02D,a</td>
<td>0.03A,</td>
<td>0.02D,b</td>
<td>0.01E,b</td>
<td>0.02C,a</td>
<td>0.01B,a</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-F Means with different letters in each row are significantly different. a-d Means with different letters in each column are significantly different (P>0.05)
Moisture content of food is an indicator of the quality of the product and has a potential impact on the sensory, physical and microbial properties of bread in particular (Hathorn and others 2008). In terms of crumb moisture it was seen that HP was the highest on day 0 from the other three samples which were comparable to each other (Table 3.3.5). Higher moisture content as long as it is in the acceptable range has shown to positively increase the loaf volumes of bread (Gallagher and others 2003). The four bread samples on all days showed moisture content values that were comparable to those reported in literature (Mandala and others 2007; Hathorn and others 2008). All of the breads showed an expected trend of decrease in crumb moisture over the
12 day storage period but the control did not. The decrease in crumb moisture corresponded to an increase in the crust moisture (Table 3.3.6) over the 12 days. Altamirano-Fortoul and Rosell (2011) reported the same and also contributed any increase in moisture during storage to absorption of water from the atmosphere due to the moisture gradient between crumb and crust. This moisture gradient varied with each bread sample even though storage conditions remained the same. Primo-Martin and others (2007) contributed the loss of crispiness of crust during storage primarily due to its increase in water content since water acts as a plasticizer and starch retrogradation was only secondary as it sets in only 2 days later.

Table 3.3.5 Moisture content (%) values of bread crumb

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.06±</td>
<td>40.85±</td>
<td>40.38±</td>
<td>38.71±</td>
<td>39.57±</td>
<td>38.64±</td>
</tr>
<tr>
<td></td>
<td>0.76A,b</td>
<td>0.99A,a</td>
<td>0.98A,a</td>
<td>0.02A,a</td>
<td>0.74A,ab</td>
<td>0.87A,a</td>
</tr>
<tr>
<td>HNY</td>
<td>42.14±</td>
<td>41.53±</td>
<td>38.94±</td>
<td>37.35±</td>
<td>40.21±</td>
<td>38.71±</td>
</tr>
<tr>
<td></td>
<td>2.62A,ab</td>
<td>1.56A,B,a</td>
<td>1.58A,B,ab</td>
<td>1.18B,a</td>
<td>1.62A,B,a</td>
<td>0.10A,B,a</td>
</tr>
<tr>
<td>SHP</td>
<td>39.17±</td>
<td>38.71±</td>
<td>36.82±</td>
<td>34.05±</td>
<td>36.15±</td>
<td>33.33±</td>
</tr>
<tr>
<td></td>
<td>3.90A,ab</td>
<td>0.01A,B,ab</td>
<td>1.16A,BC,ab</td>
<td>1.24A,BC,a</td>
<td>1.16B,BC,b</td>
<td>0.96C,b</td>
</tr>
<tr>
<td>HP</td>
<td>50.07±</td>
<td>37.23±</td>
<td>34.74±</td>
<td>34.01±</td>
<td>31.86±</td>
<td>31.11±</td>
</tr>
<tr>
<td></td>
<td>4.26A,a</td>
<td>1.6B,b</td>
<td>0.64B,c</td>
<td>1.39B,a</td>
<td>1.70B,BC,b</td>
<td>1.92B,b</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-B Means with different letters in each row are significantly different. a-c Means with different letters in each column are significantly different (P>0.05)

Table 3.3.6 Moisture content (%) values of bread crust

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.71±</td>
<td>27.96±</td>
<td>29.68±</td>
<td>29.75±</td>
<td>31.11±</td>
<td>31.18±</td>
</tr>
<tr>
<td></td>
<td>1.86B,ab</td>
<td>1.86A,B,ab</td>
<td>0.56A,a</td>
<td>0.56A,ab</td>
<td>1.92A,a</td>
<td>1.86A,a</td>
</tr>
<tr>
<td>HNY</td>
<td>27.71±</td>
<td>28.98±</td>
<td>30.75±</td>
<td>32.58±</td>
<td>30.43±</td>
<td>31.54±</td>
</tr>
<tr>
<td></td>
<td>2.52A,a</td>
<td>2.29A,a</td>
<td>1.30A,a</td>
<td>1.75A,a</td>
<td>1.65A,a</td>
<td>2.23A,a</td>
</tr>
<tr>
<td>SHP</td>
<td>21.48±</td>
<td>24.19±</td>
<td>28.24±</td>
<td>29.35±</td>
<td>29.67±</td>
<td>30.78±</td>
</tr>
<tr>
<td></td>
<td>1.33C,b</td>
<td>1.40A,B,a</td>
<td>1.37A,B,ab</td>
<td>1.49A,B,ab</td>
<td>0.56A,a</td>
<td>2.25A,a</td>
</tr>
<tr>
<td>HP</td>
<td>20.21±</td>
<td>22.58±</td>
<td>24.46±</td>
<td>25.27±</td>
<td>26.09±</td>
<td>29.64±</td>
</tr>
<tr>
<td></td>
<td>1.57C,b</td>
<td>0.01BC,b</td>
<td>1.68BC,b</td>
<td>1.73BC,b</td>
<td>0.50AB,b</td>
<td>0.49A,a</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-C Means with different letters in each row are significantly different. a-b Means with different letters in each column are significantly different (P>0.05)
The extremely high values of firmness, resilience, cohesiveness, springiness and chewiness as compared to those reported generally in literature is attributed to the fact that the probe size used in this study was 51mm in diameter as opposed to the 25mm that is generally used. This was due to lack of availability of the 25mm probe. High values similar to the one in this study was reported by Mohamed and others (2010) as the probe used was 35mm in diameter.

The four bread samples showed increased firmness (Appendix, Table A.1.1) with an increase in days of storage and there was a significant change in the firmness values over the 12 days which is attributed to staling, mainly the phenomenon of amylopectin retrogradation. On day 1 Control, HNY and SHP showed no significant difference in their firmness values and were significantly higher only in comparison to HP. However day 2 saw a marked increase in HP bread which was comparable to SHP but significantly higher than control. On days 3, 6 and 9 however, HP and control did not show any significant difference in values in comparison to each other though they were significantly lower than the HNY and SHP. On day 12 all of the four samples did not show any significant difference in firmness values. The decrease in crumb moisture values correspond to the increase in firmness though the differences in firmness were much more significant that the corresponding moisture content. This is due to the fact that amylose leaches out during baking thus causing retrogradation to occur quickly during cooling leading to crumb firming while the longer storage period is characterized by amylopectin retrogradation as the main ageing factor (Korus and others 2009). Presence of increased sugar levels is attributed to increasing bread firmness as it affected water distribution as well as trapped moisture within the bread structure (Mohamed and others 2010). However the presence of fibers helps decrease the firmness (Mohamed and others 2008) thus explaining why HP showed the least firmness among all four samples. The lack of significant difference on day 12 has been observed even in a study
involving addition of banana flour to the bread formulation where after 7 days of storage at 25°C the samples differing in the level of banana flour concentration showed no significant difference (Mohamed and other 2010). The difference in the number of days for firmness to stabilize could be due to the difference in the temperature at which storage study was conducted.

![Figure 3.1 Firmness changes during 12 day storage of control, HNY, SHP and HP breads](image)

![Figure 3.2 Resilience changes during 12 day storage of control, HNY, SHP and HP breads](image)

Cohesiveness (Appendix, Table A.1.3) for the control bread decreased overall during the storage period though there was no clear trend. However, HNY showed no significant change in cohesiveness during storage while SHP and HP showed an increase in cohesiveness values. On all days HP showed lower values of cohesiveness than the control while HNY and SHP were for
most part comparable to the control values. Springiness values (Appendix, Table A.1.4) for all samples were highest on day 0 and decreased from day 1 onwards though the decrease after day 1 was not significant. However, on each day the springiness of HP and control did not significantly differ from each other and they were lower than those of SHP and HNY which were again not significantly different from each other. Firmness, springiness and cohesiveness are the indicators of bread freshness (Charoenthaikij and others 2010). Charoenthaikij and others 2010 reported a similar trend of increasing firmness with a decrease in both cohesiveness and springiness values over storage time in wheat flour bread substituted with germinated rice flour. Thus a decreasing cohesiveness value and springiness value adds to the firming of bread. From the above it can be concluded that overall the degree and extent of firmness of control bread and HP was almost the same.

Figure 3.3 Cohesiveness changes during 12 day storage of control, HNY, SHP and HP breads
Figure 3.4 Springiness changes during 12 day storage of control, HNY, SHP and HP breads

Figure 3.5 Chewiness changes during 12 day storage of control, HNY, SHP and HP breads

Chewiness is given as the energy required for masticating a solid food (Stable Micro Systems, Texture Exponent Analysis). The four bread samples showed increase in chewiness values (Appendix, Table A.1.5) over the 12 days with a significant difference between day 0 and day 12 in all samples. Day 1, 3, 6 and 9 did not show very significant changes in chewiness generally though they were significantly different from the values obtained on day 0 and 12 except for day 1 and 9 for the control and HNY.
3.3.2 Study of Staling During Storage

The Young’s modulus (Appendix, Table A.1.6) showed a steady increase over the storage period and in case of HNY and SHP almost stabilized at day 9. This is expected though in the other 2 samples there was significant difference between day 9 and 12. Young modulus is supposed to have stabilized after day 8 though it can be safely assumed that day 12 represents time infinity. Stabilization time can vary and in some cases depending on baking conditions has been even 6 days (Le-Bail and others 2009). The time constant obtained from the values of the Young modulus indicate that HP showed the highest value of the same and was significantly different from SHP. However control and HNY showed intermediate results to both the control and HP. The time constant value of HP was almost twice that of SHP and this is an indication of the staling rate of SHP being much faster than that of HP. However that of control and HNY was closer to that of HP thereby giving bread of almost comparable or similar quality with a slower retrogradation rate.

Figure 3.6 Evolution of Young’s modulus of bread samples during storage
Table 3.3.7 Time constant values of bread samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>( \ln(E_\infty - E_t) = \ln(E_\infty - E_0) - \frac{t}{\tau} )</th>
<th>Time constant</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>( 7.713 - 0.1807t )</td>
<td>5.45±0.95(^{AB})</td>
<td>0.86</td>
</tr>
<tr>
<td>HNY</td>
<td>( 7.708 - 0.2114t )</td>
<td>4.69±0.51(^{AB})</td>
<td>0.99</td>
</tr>
<tr>
<td>SHP</td>
<td>( 7.545 - 0.2417t )</td>
<td>3.29±0.70(^{B})</td>
<td>0.99</td>
</tr>
<tr>
<td>HP</td>
<td>( 7.899 - 0.1365t )</td>
<td>7.37±1.31(^{A})</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. \(^{A-B}\) Means with different letters in each row are significantly different.

The onset temperature (Appendix, Table A.2.1) of control bread decreased over the storage period though it varied significantly only between day 1 and day 12 (-12.64±1.07 to -16.67±0.52°C) while SHP showed significant difference after day 3 (-13.16±0.34 to -17.04±0.83°C). HNY (-12.95±0.49 to -14.91±0.07°C) and HP (-15.29±0.001 to -17.57±0.29°C) showed no significant change among the values. Day 1 showed significant difference only between control and HP while day 3 and 12 showed a significant difference between HNY and HP with HNY showing the lower value. On all other days there was no significant difference between the temperatures. The peak temperature (Appendix, Table A.2.1) decreased for all samples and significant difference was only seen between day 1 and day 12 for the control and between day 3 and day 6 for SHP. HNY and HP showed no significant change over the 12 days. Among the samples significant difference was seen on day 0 between control and HP, on day 3 between HNY and HP where HP had the lowest value. All other days showed no significant difference among the samples. The conclusion temperature, \( T_e \) (Appendix, Table A.2.1) showed a similar trend as \( T_o \), the glass transition temperature \( T_g \) (Appendix, Table A.2.1) showed significant difference between day 0 and day 12 for the control, between day 0 and day 9 for SHP but there was no difference during the storage period for HNY and HP. On day 0 HP showed significant difference from the other samples. Day 3 showed no significant difference...
between control and SHP though they were significantly different from HNY and HP. Day 6 showed significant difference between HNY and HP while day 12 showed a significant difference between HNY against SHP and HP. In all the cases HP showed the lowest values.

Figure 3.7 Changes in onset temperature ($T_o$) of bread samples over 12 day storage period

Figure 3.8 Changes in peak temperature ($T_p$) of bread samples over 12 day storage period
Figure 3.9 Changes in conclusion temperature ($T_e$) of bread samples over 12 day storage period

![Graph showing changes in conclusion temperature ($T_e$) over 12 days for different samples.](image)

Figure 3.10 Changes in glass transition temperature ($T_g$) of bread samples over 12 day storage period

![Graph showing changes in glass transition temperature ($T_g$) over 12 days for different samples.](image)

The transition enthalpy ($\Delta H$) (Appendix, Table A.2.2) on day 0 was highest for the control and differed significantly from SHP and HP where HP had the lowest transition enthalpy. All samples showed significant difference in transition enthalpy over the 12 days with the enthalpy decreasing and this decreased enthalpy was reflected the decreased amounts of freezable water (FW) fraction. The decrease in freezable water content (Appendix, Table A.2.1) suggests that more water was becoming immobilized and bound in the bread matrix with increasing time due to staling (Baik and Chinachoti 2000). Roos (1995) and Mohamed and others (2010) reported...
that concentration of solutes such as sugars and salts caused a depression of the freezing temperature of the water phase which in this case could be seen by the decrease in FW which could cause an increase in the concentration of solutes thereby decreasing the ice-melting temperatures as seen in figure 3.9. The decrease in FW is thus contributed to moisture migration from the crumb to crust as well as its incorporation into the starch crystalline structure during staling as loss of crumb moisture only accounts for 38% of the reduction in FW (Ribotta and Le Bail 2007).

![Figure 3.11 Changes in enthalpy (ΔH) of bread samples over 12 day storage period](image1)

![Figure 3.12 Changes in freezable water content (FW) of bread samples over 12 day storage period](image2)
3.4 Conclusion

A bread formulation using a 50% and 100% substitution of sugar with honey powder was developed. Physico-chemical and texture analysis of bread samples over a 12 day storage period have shown favorable and comparable characteristics of HP to the control bread. Loaf volume of HP was the highest in comparison with SHP and HNY as well as control. Staling rate was comparable between HP and control while SHP showed the highest staling rate. In terms of firmness HP was closer to the control on all days of storage while SHP and HNY showed extremely high values of firmness on all days. In terms of freezable water content HP had the least value as compared to the other three samples. More research is needed to understand the phenomenon of moisture migration. Overall, substitution of sucrose with 100% honey powder proved to be a viable option given the favorable characteristics it transferred to the bread as well as due to its characteristics being closest to the control.
CHAPTER 4 SUMMARY AND CONCLUSION

In this study honey obtained locally in Louisiana was characterized based on its physico-chemical characteristics. The characterized honey was further converted to a dried powder by spray drying using retrograded corn starch as a drying agent. The retrograded starch is a resistant starch. The honey powder so produced was used as an alternative sweetener to sucrose in a bakery product – bread. The honeys obtained were found to be acidic in nature, with high levels of fructose and glucose as expected and with extremely low levels of maltose and sucrose. The honeys were golden yellow in color and had high amounts of solids. Viscosities of the honey ranged between 7.79 and 9.15 Pa s and showed the presence of slight amounts of yield stress. However the honey samples showed Newtonian behavior at higher temperatures with flow behavior index close to unity. Activation energy of the samples ranged between 74.57 and 75.92 kJ/mol. The samples were spray dried and the powders obtained were studied using SEM as well as by physico-chemical characterization. Good quality, well dispersed powders were obtained.

A comparison of the breads baked with sucrose (control), liquid honey (HNY), 50% honey powder (SHP) and 100% honey powder (HP) showed that bread with NHY and SHP showed faster and greater firming than that of the bread containing sucrose or honey powder. This was indicated by the higher time constant of the HP and the control compared to SHP. Texture analysis also revealed a higher firmness of HNY, SHP than the control and HP. RVA analysis of the flour mixtures also showed that total setback increased in the same order as above indicating a lower rate of retrogradation for HP as compared to control. Loaf volume was significantly higher for HP for the same formulation. DSC analysis of the four samples revealed lower freezable water in HP than the other samples. Overall it is clear that HP showed comparable characteristics to the control cases and in most cases was better than SHP AND HNY. Thus this
study showed that honey powder could be used successfully to replace wholly or partly sucrose as an alternative sweetener.
REFERENCES


Bogdanov S. Honey as a Nutritional and Functional food in Book of honey. 2010.


Hathorn CS, Biswas MA, Gichuhi PN, Bovell-Benjamin AC. 2008. Comparison of chemical, physical, micro-structural, and microbial properties of breads supplemented with sweetpotato flour and high gluten dough enhancers. LWT 41: 803-815.


National Honey Board, 303: 776-2337.


Figure A.1.1 Graphical representation of RVA data

Table A.1.1 Firmness (g) values of bread samples over 12-day storage period

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>714.70±</td>
<td>863.02±</td>
<td>1183.88±</td>
<td>1502.05±</td>
<td>2290.26±</td>
<td>2401.20±</td>
</tr>
<tr>
<td></td>
<td>71.65&lt;sup&gt;D,a&lt;/sup&gt;</td>
<td>28.23&lt;sup&gt;CD,c&lt;/sup&gt;</td>
<td>116.51&lt;sup&gt;BC,c&lt;/sup&gt;</td>
<td>115.60&lt;sup&gt;B,b&lt;/sup&gt;</td>
<td>235.31&lt;sup&gt;A,b&lt;/sup&gt;</td>
<td>166.28&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HNY</td>
<td>745.42±</td>
<td>938.36±</td>
<td>1599.20±</td>
<td>2458.88±</td>
<td>2775.07±</td>
<td>2666.96±</td>
</tr>
<tr>
<td></td>
<td>61.35&lt;sup&gt;C,a&lt;/sup&gt;</td>
<td>68.53&lt;sup&gt;C,bc&lt;/sup&gt;</td>
<td>25.67&lt;sup&gt;B,ab&lt;/sup&gt;</td>
<td>112.52&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>204.16&lt;sup&gt;A,a&lt;/sup&gt;</td>
<td>259.56&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SHP</td>
<td>683.22±</td>
<td>1200.68±</td>
<td>1775.03±</td>
<td>2164.46±</td>
<td>2842.87±</td>
<td>2775.41±</td>
</tr>
<tr>
<td></td>
<td>56.01&lt;sup&gt;E,a&lt;/sup&gt;</td>
<td>98.14&lt;sup&gt;D,ab&lt;/sup&gt;</td>
<td>77.69&lt;sup&gt;C,a&lt;/sup&gt;</td>
<td>121.76&lt;sup&gt;B,a&lt;/sup&gt;</td>
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<td>158.47&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HP</td>
<td>333.60±</td>
<td>1223.64±</td>
<td>1408.08±</td>
<td>1531.662±</td>
<td>2049.21±</td>
<td>2672.85±</td>
</tr>
<tr>
<td></td>
<td>46.06&lt;sup&gt;D,b&lt;/sup&gt;</td>
<td>77.62&lt;sup&gt;C,a&lt;/sup&gt;</td>
<td>64.61&lt;sup&gt;C,bc&lt;/sup&gt;</td>
<td>136.04&lt;sup&gt;C,b&lt;/sup&gt;</td>
<td>31.98&lt;sup&gt;B,b&lt;/sup&gt;</td>
<td>190.44&lt;sup&gt;A,a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A–E Means with different letters in each row are significantly different. a–c Means with different letters in each column are significantly different (P>0.05)
Table A.1.2 Resilience values of bread samples over 12-day storage period

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.69±</td>
<td>0.67±</td>
<td>0.74±</td>
<td>0.75±</td>
<td>0.75±</td>
<td>0.68±</td>
</tr>
<tr>
<td></td>
<td>0.003^{ABC,a}</td>
<td>0.04^{C,ab}</td>
<td>0.02^{AB,a}</td>
<td>0.03^{A,ab}</td>
<td>0.02^{A,ab}</td>
<td>0.01^{BC,d}</td>
</tr>
<tr>
<td>HNY</td>
<td>0.68±</td>
<td>0.72±</td>
<td>0.74±</td>
<td>0.77±</td>
<td>0.78±</td>
<td>0.76±</td>
</tr>
<tr>
<td></td>
<td>0.02^{D,a}</td>
<td>0.001^{C,a}</td>
<td>0.01^{CB,a}</td>
<td>0.003^{AB,a}</td>
<td>0.01^{A,a}</td>
<td>0.01^{AB,b}</td>
</tr>
<tr>
<td>SHP</td>
<td>0.59±</td>
<td>0.64±</td>
<td>0.68±</td>
<td>0.73±</td>
<td>0.75±</td>
<td>0.80±</td>
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<td>0.03^{D,b}</td>
<td>0.01^{C,bc}</td>
<td>0.02^{C,b}</td>
<td>0.01^{B,bc}</td>
<td>0.01^{A,b}</td>
<td>0.01^{A,a}</td>
</tr>
<tr>
<td>HP</td>
<td>0.58±</td>
<td>0.61±</td>
<td>0.67±</td>
<td>0.68±</td>
<td>0.71±</td>
<td>0.73±</td>
</tr>
<tr>
<td></td>
<td>0.02^{E,b}</td>
<td>0.01^{D,c}</td>
<td>0.01^{C,b}</td>
<td>0.01^{BC,c}</td>
<td>0.01^{AB,b}</td>
<td>0.002^{A,c}</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-D Means with different letters in each row are significantly different. a-c Means with different letters in each column are significantly different (P>0.05)

Table A.1.3 Cohesiveness values of bread samples over 12-day storage period

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.93±</td>
<td>0.93±</td>
<td>0.95±</td>
<td>0.94±</td>
<td>0.92±</td>
<td>0.88±</td>
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<tr>
<td></td>
<td>0.002^{B,a}</td>
<td>0.01^{AB,a}</td>
<td>0.01^{A,a}</td>
<td>0.01^{AB,a}</td>
<td>0.01^{B,a}</td>
<td>0.01^{C,c}</td>
</tr>
<tr>
<td>HNY</td>
<td>0.94±</td>
<td>0.92±</td>
<td>0.93±</td>
<td>0.93±</td>
<td>0.92±</td>
<td>0.93±</td>
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<tr>
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<td>0.004^{A,a}</td>
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<td>0.01^{A,b}</td>
<td>0.01^{A,a}</td>
<td>0.02^{A,a}</td>
<td>0.01^{A,ab}</td>
</tr>
<tr>
<td>SHP</td>
<td>0.88±</td>
<td>0.92±</td>
<td>0.91±</td>
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<td>0.94±</td>
<td>0.94±</td>
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<td>0.01^{D,b}</td>
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<td>0.003^{A,a}</td>
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<tr>
<td>HP</td>
<td>0.57±</td>
<td>0.84±</td>
<td>0.89±</td>
<td>0.90±</td>
<td>0.91±</td>
<td>0.92±</td>
</tr>
<tr>
<td></td>
<td>0.02^{D,c}</td>
<td>0.01^{C,b}</td>
<td>0.01^{B,d}</td>
<td>0.01^{AB,b}</td>
<td>0.01^{AB,a}</td>
<td>0.01^{A,b}</td>
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</tbody>
</table>

Values are means ± SD of 3 determinations. A-D Means with different letters in each row are significantly different. a-d Means with different letters in each column are significantly different (P>0.05)

Table A.1.4 Springiness values of bread samples over 12-day storage period

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.95±</td>
<td>0.94±</td>
<td>0.87±</td>
<td>0.85±</td>
<td>0.87±</td>
<td>0.83±</td>
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<tr>
<td></td>
<td>0.003^{A,ab}</td>
<td>0.01^{A,ab}</td>
<td>0.01^{B,a}</td>
<td>0.01^{BC,ab}</td>
<td>0.004^{B,b}</td>
<td>0.02^{C,b}</td>
</tr>
<tr>
<td>HNY</td>
<td>0.96±</td>
<td>0.96±</td>
<td>0.87±</td>
<td>0.86±</td>
<td>0.89±</td>
<td>0.86±</td>
</tr>
<tr>
<td></td>
<td>0.01^{A,ab}</td>
<td>0.02^{A,a}</td>
<td>0.01^{B,a}</td>
<td>0.01^{A,b}</td>
<td>0.02^{B,b}</td>
<td>0.002^{B,a}</td>
</tr>
<tr>
<td>SHP</td>
<td>0.97±</td>
<td>0.91±</td>
<td>0.86±</td>
<td>0.85±</td>
<td>0.97±</td>
<td>0.84±</td>
</tr>
<tr>
<td></td>
<td>0.01^{A,a}</td>
<td>0.02^{B,b}</td>
<td>0.02^{A,b}</td>
<td>0.01^{C,ab}</td>
<td>0.01^{A,a}</td>
<td>0.004^{C,ab}</td>
</tr>
<tr>
<td>HP</td>
<td>0.92±</td>
<td>0.86±</td>
<td>0.84±</td>
<td>0.83±</td>
<td>0.87±</td>
<td>0.83±</td>
</tr>
<tr>
<td></td>
<td>0.03^{A,b}</td>
<td>0.002^{B,c}</td>
<td>0.02^{B,a}</td>
<td>0.01^{B,b}</td>
<td>0.01^{B,b}</td>
<td>0.01^{B,b}</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-B means with different letters in each row are significantly different. a-b Means with different letters in each column are significantly different (P>0.05)
Table A.1.5 Chewiness values of bread samples over 12-day storage period

<table>
<thead>
<tr>
<th>Samples</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>629.98±</td>
<td>756.21±</td>
<td>920.47±</td>
<td>1189.69±</td>
<td>1833.82±</td>
<td>1746.24±</td>
</tr>
<tr>
<td></td>
<td>65.85C,a</td>
<td>21.51C,b</td>
<td>97.03B,c</td>
<td>98.40B,b</td>
<td>78.14A,cb</td>
<td>82.21A,b</td>
</tr>
<tr>
<td>HNY</td>
<td>677.55±</td>
<td>836.68±</td>
<td>1287.25±</td>
<td>1974.71±</td>
<td>2263.43±</td>
<td>2120.43±</td>
</tr>
<tr>
<td></td>
<td>64.40C,a</td>
<td>40.30B,ab</td>
<td>91.18A,a</td>
<td>255.21A,ab</td>
<td>200.95A,a</td>
<td></td>
</tr>
<tr>
<td>SHP</td>
<td>584.50±</td>
<td>1008.02±</td>
<td>1380.85±</td>
<td>1700.56±</td>
<td>2566.02±</td>
<td>2172.25±</td>
</tr>
<tr>
<td></td>
<td>52.37F,a</td>
<td>63.84D,a</td>
<td>74.27C,a</td>
<td>229.95A,a</td>
<td>42.11B,a</td>
<td></td>
</tr>
<tr>
<td>HP</td>
<td>175.58±</td>
<td>885.18±</td>
<td>1044.14±</td>
<td>1152.28±</td>
<td>1614.23±</td>
<td>2035.97±</td>
</tr>
<tr>
<td></td>
<td>43.12D,b</td>
<td>124.20C,ab</td>
<td>108.81C,b</td>
<td>63.33B,c</td>
<td>150.35A,ab</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-D means with different letters in each row are significantly different. a-d means with different letters in each column are significantly different (P>0.05)

Table A.1.6 Young’s modulus (Pa) values of bread during storage study

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 6</th>
<th>Day 9</th>
<th>Day 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>405.22±</td>
<td>488.92±</td>
<td>664.25±</td>
<td>1169.53±</td>
<td>1918.97±</td>
<td>2246.83±</td>
</tr>
<tr>
<td></td>
<td>18.33E,a</td>
<td>44.20D,E,b</td>
<td>89.30D,b</td>
<td>151.74C,b</td>
<td>20.91B,b</td>
<td>124.10A,b</td>
</tr>
<tr>
<td>HNY</td>
<td>455.72±</td>
<td>640.06±</td>
<td>1307.18±</td>
<td>1949.69±</td>
<td>2208.57±</td>
<td>2544.59±</td>
</tr>
<tr>
<td></td>
<td>89.69D,a</td>
<td>19.58D,ab</td>
<td>158.57C,a</td>
<td>94.14B,a</td>
<td>203.22A,ab</td>
<td>273.07A,ab</td>
</tr>
<tr>
<td>SHP</td>
<td>441.79±</td>
<td>741.49±</td>
<td>1390.43±</td>
<td>1843.93±</td>
<td>2321.80±</td>
<td>2289.10±</td>
</tr>
<tr>
<td></td>
<td>78.25E,a</td>
<td>32.75D,a</td>
<td>69.21C,a</td>
<td>120.03B,a</td>
<td>120.21A,a</td>
<td>43.35A,b</td>
</tr>
<tr>
<td>HP</td>
<td>216.33±</td>
<td>695.17±</td>
<td>1197.68±</td>
<td>1630±</td>
<td>2207.48±</td>
<td>2951.04±</td>
</tr>
<tr>
<td></td>
<td>16.98E,b</td>
<td>121.30E,a</td>
<td>133.10D,a</td>
<td>214.18C,a</td>
<td>83.52B,a</td>
<td>176.34A,a</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-F Means with different letters in each row are significantly different. a-b Means with different letters in each column are significantly different (P>0.05)
Figure A.1.2 Graph for determination of time constant from staling equation using Young’s modulus
APPENDIX 2 RESULTS OF DSC ANALYSIS

Table A.2.1 Onset temperature (T_o), Peak temperature (T_p), Conclusion temperature (T_c) and Glass transition (T_g) for control, HNY, SHP and HP breads as obtained from DSC

<table>
<thead>
<tr>
<th></th>
<th>DAY 0</th>
<th>DAY 1</th>
<th>DAY 3</th>
<th>DAY 6</th>
<th>DAY 9</th>
<th>DAY 12</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T_o (°C)</td>
<td>T_p (°C)</td>
<td>T_c (°C)</td>
<td>T_g (°C)</td>
<td>T_o (°C)</td>
<td>T_p (°C)</td>
</tr>
<tr>
<td>Control</td>
<td>-12.64±</td>
<td>-10.79±</td>
<td>-15.21±</td>
<td>-15.24±</td>
<td>-15.65±</td>
<td>-16.67±</td>
</tr>
<tr>
<td></td>
<td>1.07±A,a</td>
<td>0.77±A,a</td>
<td>0.49±AB,bc</td>
<td>1.19±AB,a</td>
<td>0.30±AB,a</td>
<td>0.52±B,b</td>
</tr>
<tr>
<td>HNY</td>
<td>-12.95±</td>
<td>-12.02±</td>
<td>-12.28±</td>
<td>-13.43±</td>
<td>-16.78±</td>
<td>-14.91±</td>
</tr>
<tr>
<td></td>
<td>0.49±A,b</td>
<td>0.66±A,a</td>
<td>0.20±A,a</td>
<td>1.04±A,a</td>
<td>2.83±A,a</td>
<td>0.07±A,a</td>
</tr>
<tr>
<td></td>
<td>0.34±A,b</td>
<td>0.78±A,a</td>
<td>0.02±AB,ab</td>
<td>1.11±B,a</td>
<td>0.20±A,a</td>
<td>0.83±B,b</td>
</tr>
<tr>
<td>HP</td>
<td>-15.29±</td>
<td>-15.01±</td>
<td>-16.44±</td>
<td>-17.25±</td>
<td>-17.39±</td>
<td>-17.57±</td>
</tr>
<tr>
<td></td>
<td>0.001±A,b</td>
<td>1.70±A,a</td>
<td>0.71±A,c</td>
<td>0.37±A,a</td>
<td>0.01±A,a</td>
<td>0.29±A,b</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-C Means with different letters in each row are significantly different. a-c Means with different letters in each column are significantly different (P>0.05)
Table A.2.2 Enthalpy (ΔH) and freezable water (FW) fraction of bread samples

<table>
<thead>
<tr>
<th></th>
<th>DAY 0</th>
<th>DAY 1</th>
<th>DAY 3</th>
<th>DAY 6</th>
<th>DAY 9</th>
<th>DAY 12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ΑH</td>
<td>71.65±</td>
<td>52.12±</td>
<td>46.19±</td>
<td>43.06±</td>
<td>38.99±</td>
<td>27.81±</td>
</tr>
<tr>
<td>(J/g)</td>
<td>1.04A,a</td>
<td>0.77B,b</td>
<td>0.19C,b</td>
<td>0.51C,b</td>
<td>0.45D,a</td>
<td>1.69E,b</td>
</tr>
<tr>
<td>HNY</td>
<td>67.66±</td>
<td>66.44±</td>
<td>56.85±</td>
<td>48.13±</td>
<td>46.23±</td>
<td>43.54±</td>
</tr>
<tr>
<td></td>
<td>1.18A,ab</td>
<td>1.79A,a</td>
<td>0.81B,a</td>
<td>0.62C,a</td>
<td>1.51C,a</td>
<td>0.36C,a</td>
</tr>
<tr>
<td>SHP</td>
<td>63.21±</td>
<td>61.15±</td>
<td>54.19±</td>
<td>39.84±</td>
<td>17.68±</td>
<td>17.67±</td>
</tr>
<tr>
<td></td>
<td>2.38A,b</td>
<td>0.62AB,a</td>
<td>2.90A,a</td>
<td>0.20BC,c</td>
<td>1.56C,b</td>
<td>2.33C,c</td>
</tr>
<tr>
<td>HP</td>
<td>44.72±</td>
<td>41.74±</td>
<td>31.82±</td>
<td>26.85±</td>
<td>24.45±</td>
<td>22.90±</td>
</tr>
<tr>
<td></td>
<td>2.27A,c</td>
<td>1.83AB,c</td>
<td>0.32BC,c</td>
<td>0.86C,d</td>
<td>4.32C,b</td>
<td>3.75C,bc</td>
</tr>
<tr>
<td>Control FW</td>
<td>0.21±</td>
<td>0.16±</td>
<td>0.16±</td>
<td>0.14±</td>
<td>0.13±</td>
<td>0.08±</td>
</tr>
<tr>
<td>(g/g solid)</td>
<td>0.003A,a</td>
<td>0.002B,b</td>
<td>0.002BC,c</td>
<td>0.001CD,b</td>
<td>0.002D,a</td>
<td>0.01E,b</td>
</tr>
<tr>
<td>HNY</td>
<td>0.20±</td>
<td>0.19±</td>
<td>0.12±</td>
<td>0.14±</td>
<td>0.14±</td>
<td>0.13±</td>
</tr>
<tr>
<td></td>
<td>0.003A,ab</td>
<td>0.01A,a</td>
<td>0.002B,a</td>
<td>0.002C,a</td>
<td>0.004C,a</td>
<td>0.001C,a</td>
</tr>
<tr>
<td>SHP</td>
<td>0.19±</td>
<td>0.18±</td>
<td>0.16±</td>
<td>0.12±</td>
<td>0.05±</td>
<td>0.05±</td>
</tr>
<tr>
<td></td>
<td>0.01A,b</td>
<td>0.001AB,a</td>
<td>0.01B,a</td>
<td>0.001C,c</td>
<td>0.005D,b</td>
<td>0.01D,c</td>
</tr>
<tr>
<td>HP</td>
<td>0.13±</td>
<td>0.12±</td>
<td>0.10±</td>
<td>0.08±</td>
<td>0.07±</td>
<td>0.07±</td>
</tr>
<tr>
<td></td>
<td>0.01A,c</td>
<td>0.01AB,c</td>
<td>0.001BC,c</td>
<td>0.002C,d</td>
<td>0.01C,b</td>
<td>0.01C,bc</td>
</tr>
</tbody>
</table>

Values are means ± SD of 3 determinations. A-E Means with different letters in each row are significantly different. a-c Means with different letters in each column are significantly different (P>0.05).
APPENDIX 3 SAMPLE GRAPHS OF DSC CURVES

Figure A.3.1 DSC curve showing transition of control, HNY, SHP and HP breads on day 0

Figure A.3.2 DSC curve showing transition of control, HNY, SHP and HP breads on day 12
Figure A.3.3 DSC curve showing transition occurring in control bread during 12 day storage

Figure A.3.4 DSC curve showing transition occurring in HP bread during 12 day storage
Ahalya Kosal Ram was born in July 1987 in Tamil Nadu, India. She completed her undergraduate studies at Vellore Institute of Technology, University in the year 2009 obtaining a Bachelor of Technology degree in Biotechnology. She enrolled for the master’s program in the Department of Food Science at LSU in August 2009. Ahalya is currently a Master’s degree candidate and will receive her degree in August 2011.