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Design and Development of Seed Hydration Analyzing Device and its Utilization in Studying Cereal and Legume Hydration

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I am submitting herewith a dissertation written by Vinay Kumar Mannam entitled "Design and Development of Seed Hydration Analyzing Device and its Utilization in Studying Cereal and Legume Hydration." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Food Science and Technology.

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Design and Development of Seed Hydration Analyzing Device and its Utilization in Studying Cereal and Legume Hydration

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DEDICATION

To My caring and supportive parents and grandparents
(am'ma, nānna, thathaiyyalu, am'mamma, na'namma)
Lovely Sister (chelli)

To My Teachers

My Harshitha

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ABSTRACT

Cereals and legumes are important sources of vegetable-based human nutrition. Together they account for 48.6% of protein and 8.7% carbohydrate consumption around the world. During preparation, majority of these agricultural staples are re-hydrated to aid in their digestibility, palatability and the bio-availability of the nutrients.

Study of hydration kinetics of cereals and legumes is an important and valuable necessity for industry and academia to understand and gain insights into seed hydration characteristics. An automatic seed hydration analyzing system is developed as a solution for lack of instruments with broad capabilities to study variety of seed properties. The device measures volume and weight with 97.87% and 99.08% accuracy respectively.

Utilizing the seed hydration analyzing device, studies were conducted to analyze different regional cultivars of navy beans, black beans and pinto beans. The rate of volume and weight uptake of different international navy bean cultivars is also studied. The data obtained showed considerable differences among both regional and international cultivars. Navy bean hydration is studied for impact its dependence on sodium and calcium salts concentrations in soak water, and optimum salt content is determined for maximum volume and weight increase during soaking.

Data obtained from the seed hydration analyzing device during soaking of dry beans, oil seeds and barley are utilized to analyze different empirical models that are used to study hydration kinetics of cereals and legumes by applying Theil error splitting method. Weibull distribution model worked better for all soak studies with certain limitations.

The steeping process in malt preparation using barley is analyzed by measuring volume and weight of the seeds. Data analysis showed that the device was able to predict germination time of

barley during steeping process and it is later utilized to evaluate different barley varieties are steeping the seed hydration analyzing device, with different steep water temperatures and air-rest periods.

The seed hydration analyzing device was designed, developed and tested to prove its capability as a fast and easy method to provide support for hydration kinetic studies of cereals and legumes and it is possible to improve their process and nutritional quality.

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CHAPTER I

Introduction and Literature Review

Cereals and legumes – a nutrition power house

Cereals grains and legumes are one of the primary plant based food source for majority of the world population (Leterme, 2002; Shridhar K Sathe, 2012). The term "legumes is used to define dry beans (e.g., navy, black, pinto, lentil, lima, kidney, chickpea, faba, pigeon pea) and oilseeds (e.g., soy beans, peanuts, lupin seeds) and the term 'cereal' is for seeds obtained from grass crops (e.g., rice, wheat, barley, maize, sorghum). Together, cereals and legumes constitute an important source of protein and energy uptake in the world. According to FAO food supply data, total world consumption of cereals as food is 146.7 kg/capita/year, and legumes is 6.6 kg/capita/year (Organization, 2013). The consumption in of cereals and legumes is higher in developing (152.3 and 11.9 kg/capita/year respectively) and under-developed (113.8 and 12 kg/capita/year respectively) countries, when compared to United States of America (108.2 and 4.5). Nutritionally, cereals are abundant in carbohydrates and minerals, while legumes are protein rich (Ford & Hewitt, 1979). Proteins are important nutrients obtained from legumes and are relatively cheap to produce when compared to protein obtained from animal sources (Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006). This prominence is more evident in under developed and developing countries, where lack of animal protein is compensated with legume rich diet. In these regions, more than two thirds of protein intake is acquired from legumes (40.9 g/capita/day out of 65 g/capita/day). In United States, proteins from cereals and legumes have found new recognition due to their higher nutrient density and protein to calorie ratio with better health benefits, resulting in 2010 dietary guidelines recommendation (40 grams of beans and 110 grams of whole grains) from department of Health and Human Services (USDHSS, 2010; Williams, Grafenauer, & O'Shea, 2008).

Importance of hydration in preparation of cereals and legumes

Availability of proteins in cereal and pulses depends on the processing method (Hotz & Gibson, 2007). Elimination of anti-nutrients from legumes and cereals is also an important function of processing, (Ayyagari, Rao, & Roy, 1989). While factors such as soil Ph, organic matter content, available nutrients and soil-water relationship effect initial crop nutritional value, it is the post-harvest processing that will enable the bio-availability of all the nutrients in finished product (Hornick, 1992). Refined and milled grains tend to loose valuable dietary fibers and important nutrients during processing. Traditionally, cereals and legumes are prepared in common households by soaking followed by cooking or thermal treatment. Different preparation methods for cereals and legumes and their effect for plant based diet are reviewed and discussed in depth elsewhere and are summarized in Table 1.1 (Egli, Davidsson, Juillerat, Barclay, & Hurrell, 2002; Hornick, 1992; Hotz & Gibson, 2007; Lewicki, 1998). A simple hydration followed by cooking or germination of cereal and legumes is considered more effective processing technique then other intrusive methods such as milling, roasting, and grinding (Tharanathan & Mahadevamma, 2003).

The type of hydration a seed receives in processing is tailored for specific cereal or legume considering the desired end product. Each method has its own particular advantages and disadvantages as summarized in Table 1.2. Hydration of cereals and legumes can be broadly classified into simple soaking and steeping. As mentioned in the table 1.2, soaking is generally accompanied with temperature to improve hydration rates, and steeping is carried out to promote germination.

Table 1.1. Different processing methods in preparation of cereals and legumes

Method	Description	Effect	Seeds	Reference
Thermal	Boiling Cooking Retorting High pressure cooking	Breaks structure by puncturing of cells walls to enable bioavailability of nutrients. Over exposure to thermal processing may result in loss of heat-liable and water soluble nutrients. Thermal processing is also carried out to eliminate harmful bacteria such as *Clostridium botulinum.*	Processi-ng dry beans by canning	Hotz, 2007 Rao, 1986 Perez, 2012
Mechanical	Mechanical shear forces Milling Grinding	Shear forces are used to remove barn/germ from cereals, which contains phytates, thus enhancing availability of iron, zinc and calcium. Cereal grains are also milled to make flour. Grinding and milling reduce valuable nutrients.	Rice, Wheat, Maize, Sorghu-m	Hotz, 2007 Velu, 2006 Gys, 2004
Soaking	Cereals and legumes soaked in water	Water soluble phytates diffuse passively, and cell wall is exposed making important nutrients bio-available. Over soaking can cause textural damages and loss of nutrients into water. Soaking is often used as secondary process aid to most of the plant based-food preparation methods.	Dry beans, Rice, Barley, Wheat, Maize	Hotz, 2007 Boajun, 2008 Mahatma, 2009 Sayar, 2001 Nissreen, 1998
Fermentation	Exposure of seeds to microbial enzymes in controlled environment	Phytates are hydrolyzed by microbial enzymes, which preserve protein content and increases digestibility of cereals. Fermentation is often preceded by soaking	Barley, Wheat, Millet, Maize, Rice	Hotz, 2007 Blandino, 2003 Inyang, 2008
Malting	Activating internal enzymes by allowing germination	Germination triggers internal enzymes in cereals and legumes which induce phytate hydrolysis. The rate of this activity can be controlled by temperature and Ph content.	Barley, Rye, Wheat	Hotz, 2007 Egli, 200

Table 1.2 Hydration methods and their impact on different seed varieties downstream processing (Abu-Ghannam, 1998; Mahatma, Bhatnagar, Solanki, & Mittal, 2009; Sayar, Turhan, & Gunasekaran, 2001; B. Xu & Chang, 2008) .

Cereal/ Grains	Type of Hydration	Effect	Product	Reference
Dry beans	Soaked in water prior to cooking, often at temperatures higher than 120^{o} F for short time	Softens seed, and removes phytic acid and enzyme inhibitors	Prepared beans such as baked beans	Siddiq 2011, Van der Poel 1990
Wheat, Barley, Rye, Sorghum, dry beans	Steeped in water prior to germination	Activate amylase enzymes that enable conversion of starch to sugars	Cereals are steeped for malt used for preparation of alcoholic beverages. Dry beans are steeping for sprouts	Briggs 1998 Mwikya 2000
Soy beans	Dry soybeans are hydrated for 3 -12 hours based on temperature of water	Hydration of powdered soybeans help extract soy slurry through wet grinding	Preparation of soy milk and tofu	Kiple 2007
Rice	Rice is soaked prior to cooking for 3-4 hours at room temperature, or in some cases for longer periods of up to 12-14 hours	Soaking prior to cooking softens seeds, and saves energy while cooking and longer soaking is preferred for better nutrient retention	cooked prepared rice	Isabelle 2005, Bello 2004, Chiang 2002

The common ingredient in all the hydration methods is water and it primarily softens texture and ensures easier usage of one or more hydration methods is recommended and adopted in food industry to get the best nutritional retention and reduction of anti-nutrients of each preparation protocol (Friedman, 1996; Sandberg, 2002). Factors such as time of exposure to water, properties of water and temperature impact the end product of the particular hydration method. Soaking or simple hydration being the starting step for most preparation methods makes it most studied and analyzed processing step for cereals and legumes. Better understanding of soaking and its effects on thermal processing, fermentation and germination/malting can lead to higher bioavailability of nutrients in cereals and legumes along with shorted processing times (Han & Baik, 2006; Hotz & Gibson, 2007).

Factors affecting hydration of cereals and legumes:

Cereal grains and legumes are dried after harvesting to preserve maximum quality and hinder growth of bacteria and fungus during storage. As discussed earlier, soaking removes anti-nutrients from seeds by passive diffusion phytate salts of Na, Mg, and K into water (Perlas & Gibson, 2002). The extent of reduction depends on the type of cereal or legume, water conditions and length of soaking. Rehydration is essential for bio-availability of nutrients in dried beans and cereals. It is referred to as ‘amount of water absorbed by dry seed’, but there is no consistent nomenclature and standard definition for rehydration, the term varies in usage with different seed (Lewicki, 1998). The factors affecting soaking in cereals and legumes are discussed below. Apart from internal mechanics, external variables play a prominent role in seed hydration (Resio, Aguerre, & Suárez, 2003; Sefa-Dedeh, Stanley, & Voisey, 1978). Studies have been carried out to analyze the effect of extrinsic parameters on hydration kinetics of seeds during soaking, and Table 1.3 summarizes some of the important work done (S. Xu, 2010).

Initial seed conditions:

Cereals and legumes are rarely processed and cooked immediately after harvesting. Postharvest conditions heavily impact hydration kinetics. During storage after harvest, moisture content of cereals and legumes is decreased to improve quality and reduce growth of microorganisms (Hornick, 1992; Molina, Fuente, & Bressani, 1975). Seeds that are stored for longer periods in drier environment tend to loose further moisture and are harder to hydrate than normally stored beans. Initial moisture content before hydration in most cereals and legumes is between 12-15% (Kashaninejad, Dehghani, & Kashiri, 2009; Resio, Aguerre, & Suárez, 2003). Lower moisture content in seeds increases the time required for seeds to achieve optimum water uptake and also rate of water uptake. Whereas, higher moisture content induces textural damages and kick starts germination while in storage (Nasar-Abbas, Siddique, Plummer, White, Harris, Dods, et al., 2009; Vorwald & Nienhuis, 2009). Also, temperature of seed before soaking effects the hydration rate, as lower seed temperature will hinder water diffusivity until equilibrium temperature is attained (Vorwald & Nienhuis, 2009). In cereal grains, de-hulling enables quicker rehydration rates (Lucas, 2010). Hard-to-cook seeds (seeds that hard to hydrate, often called steely seeds) are mainly associated with irregular moisture profiles and tough storage conditions (Reyes-Moreno, Paredes-López, & Gonzalez, 1993).Treatment of seeds with enzymes and chemicals, such as protease and $NaHCO_3$, alters cell wall structure in seeds to improve water diffusivity in steely seeds. Optimized pretreatments with care can reduce soak and cooking times and effect of the enzyme and chemical pretreatments on legumes are studied elsewhere (Sreerama, Sashikala, & Pratape, 2009).

Soaking time:

Soaking is often a slow process with minimal control parameters other than time. Based on type of grain or legume, the rate of diffusion varies during soaking (Hotz & Gibson, 2007). The relationship between rate of hydration and soak time is well understood, where water update increases as soak time is increased, while water diffusion rate decreases over time resulting in moisture content reaching a plateau (Marcelo Bello, Marcela P. Tolaba, & Constantino Suarez, 2004; Sefa-Dedeh, Stanley, & Voisey, 1978; P. A. Sopade, Ajisegiri, & Badau, 1992). Soak time is also dependent on other factors and it can be considerable reduced when high temperatures are used (Kashaninejad, Dehghani, & Kashiri, 2009). Over exposure of seeds to water can results in textural damage and leaching out of important nutrients.

Soak temperature:

Temperature of soaking medium is one of the critical parameters affecting diffusivity of water into grain. Increase in temperature has proven to increase water uptake and hydration rate (Abu-Ghannam, 1998; Resio, Aguerre, & Suárez, 2003). Controlled low temperatures are also important in soaking when germination of cereals or legumes has to be achieved for preservation of valuable nutrients (Khan, Gul, & Weber, 2000). Hydration of foods and effect of temperature can be been explained by diffusion kinetics such as Fick's Law, which states that diffusion of water increases as the temperature increases, leading to faster inhibition rate (Resio, Aguerre, & Suárez, 2003; S. Xu, 2010). The dependence of diffusivity on temperature is described by Arrhenius type equation (Prasad, Vairagar, & Bera, 2010; Turhan, Sayar, & Gunasekaran, 2002).

$$D_e = D_o e^{\left(E_a / R_g T\right)}$$

Where: D_e is Diffusion co-efficient (m^2/s), D_o is pre-exponential factor (m^2/s), E_a is activation energy (KJ mol^{-1}), R_g is universal gas constant (8.314 KJ $mol^{-1}K^{-1}$),T is absolute temperature (K).

The effect of soak water temperature and its relationship with hydration rate and water update is not inter-dependent, which means that as temperature increase the water holding capacity will decrease after certain point, while diffusivity keeps on increasing (Turhan, Sayar, & Gunasekaran, 2002).

Soak additives:

Chemistry of soak water impacts hydration of cereals and legumes. It is known that as pH increases, water uptake also increases (Haladjian, Fayad, Toufeili, Shadarevian, Sidahmed, Baydoun, et al., 2003). pH in soak water is altered by presence of monovalent ions such as Na^+ and K^+ ions in form of salt has shown to decrease soak time and increase protein retention (De León, Elias, & Bressani, 1992). Alkaline solutions are proven to improve water holding capacity of both normal seeds and hard-to-cook seeds (García-Vela & Stanley, 1989). Although salts help seeds hydrate, high concentrations known to destroy germination properties of seeds, whereas low salt concentrations (up to certain level) have proven to help germination, as uptake of monovalent ions increases, the chances of germination decreases (Tobe, Li, & Omasa, 2002, 2004). In literature, it was proven than both monovalent ions such as Na^+, K^+ compete with divalent ions such as Ca^{2+},Mg^{2+} in water and in soil. Research has shown that optimizing these two ions in water can quicken soaking time and help soften the seeds when cooking. Additives in form of salts like NaCl, $CaCl_2$, $NaHCO_3$ are added to improve the ionic concentration in soak medium to optimum conditions. Although higher amounts of salts changes the flavor of final

product, but the difference is found be minimal after cooking (De León, Elias, & Bressani, 1992). Thus, additives in soak water can play an important role in changing the pH of water and in turn enhancing water holding capacity. The exact mechanisms of these ions and their interactions are reviewed elsewhere (M. Siddiq, Butt, & Sultan, 2011).

Pressure:

It is evident that temperature can impact water uptake of seeds during soaking, and effect of pressure paired with temperature has been a keen interest. Pressure is extensively employed in processing of rice, which are hard to soak grains among cereals and legumes (Ahromrit, Ledward, & Niranjan, 2006; Marcelo Bello, Marcela P. Tolaba, & Constantino Suarez, 2004). Hydrostatic and osmotic pressures of seeds increase during processing which impact water absorption rate in seed. This mechanism is utilized processing of seeds using pressure to increase water uptake with lower temperatures (Ramaswamy, Balasubramaniam, & Sastry, 2005). Pressure can be used inactivate microorganisms at lower temperatures, which will help in preserving heat-liable protein in cereals and legumes (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007). It is widely reported that combination of thermal and pressure works best for foods (Knorr, 1999). Among cereals and legumes in industry, only rice and occasionally soy bean is processed with pressure, and it is very rarely utilized commercially in processing of dry beans and other cereal grains. There has been plenty of studies carried out to analyze viability of pressure of dry beans processing, and factors such as complete inactivation of microorganisms hinder its application (Estrada-Giron, Swanson, & Barbosa-Cánovas, 2005). The energy saving advantages of pressure by replacing thermal treatments, combined with better functional and nutritional retention of ingredients, with improved food quality parameters make it one of the exciting novel processing methods for cereals and legumes.

Physiological and nutritional changes during hydration

The primary objective of soaking in legumes and cereals is providing the seeds with water to enable required physical and chemical changes. Based on desired end product the extent of hydration and supporting environment is altered to achieve optimally soaked grain. As discussed in previous sections, hydration is primarily provided to cereals and legumes through simple soaking (continuous water exposure) or steeping (intermittent water exposure) processes. During both soaking and steeping, the physiological changes start with mass transfer of water through seeds and leaching out of solids into water. The process of mass transfer from seeds and into seeds continues until soaking/steeping is stopped and sometimes is impacted by temperature and water chemistry. The following sections review some of the primary nutritional and physiological changes that occur during soaking and steeping of cereals and legumes.

Soaking:

Legumes:

Legumes such as soy beans, navy beans, pinto beans, black beans, kidney beans, lima beans, faba beans, and lentils gain both weight and volume with water absorption during soaking. Moisture content of beans increases from 10-15% to 50-60% during soaking. Weight increase is proportional to moisture, whereas volume changes may vary across varieties (Barampama & Simard, 1995). Soaking in dry beans is performed to facilitate fast cooking and to facilitate quicker gelatinization of starch, along with enabling higher protein quality due to shorter thermal processing. Oligosaccharides such as raffinose, and sachyose are non-digestible, and 30% of them leach out during soaking process (Silva & Braga, 2006). Anti-nutrient components such as

phytic acid, tanins and trypsin inhibitors are decreased by 28.75%, 7.02% and 28.75% respectively during soaking (Deshpande & Cheryan, 1983).

Table 1.3 Summary of factors affecting soaking of cereals and legumes

Factor	Description	Effect	Sample	Reference
Initial conditions	Initial Storage conditions Pre-hydration treatments like de-hulling	Decrease in moisture content, and hard-to-cook phenomena *Seeds stored at high temperature and high humidity can become hard-to-cook, which is an important defect*	Dry beans Barley Chick-peas	Moreno, 1993 Kashaninejad, 2009 Abbas, 2009 Vorwald, 2009 Lucas, 2010
Soaking time	Time of exposure of seeds to water	Water uptake, nutrients, anti-nutrients leaching and degradation *Longer soak time increase water uptake*	Rice Wheat Black beans	(Sefa-Dedeh, Stanley, & Voisey, 1978) Molina, 1975 Marcelo, 2004
Temperature	Temperature of hydration medium	Rate of water uptake (diffusivity) and final water content (water holding capacity), *Higher temperatures increase water diffusivity and holding capacity until equilibrium is achieved*	Amaranth White beans Rice Red- beans Chick-pea	Andrea, 2004 Tao, 2011 Perez, 2012 Khan, 2000 Prasad, 2010 Nissreen, 1998 Sayar, 2001
Additives	Salts – effecting pH	Soak time, flavor *Presence of monovalent salts (Na+, K+) decreases cooking time, and slightly impacts flavor*	Dry beans Faba beans Pinto beans	De Leon, 1992 Haladjian, 2003 Pirhyati, 2009 Leonel, 2008 Stanley, 1989
Pressure	Pressure application in hydration container	water uptake, shorter soaking time, flavor, texture *Optimum pressure will increase water update, decreases solid loss, preserves flavor and texture*	Chick pea Green pea	Boajun, 2008 Estrada-Giron, 2005 Rastogi, 2007

Vitamins and minerals in dry beans are lost primarily through leaching out into water. Reduction in levels of vitamins (E, B12) and minerals (Ca, Mg) during soaking is highest at 50^{o} C-60^{o} C (Muhammad Siddiq & Uebersax, 2012). During soaking of soybeans, a 12% loss of iso-flavones is observed (Wang & Murphy, 1996).

Cereals:

Among cereals, rice is soaked prior to thermal processing to achieve adequate starch gelatinization and to decrease time of exposure at higher temperatures and save energy (Marcelo Bello, Marcela P. Tolaba, & Constantino Suarez, 2004). Moisture content of rice changes from 10-15% to 55-65% during soaking depending on the soak water temperature. Soaking proved to be effective in reducing phytate content in rice, maize, millet, rice and sorghum up to 28%, 21%, 4%, and 17%respectively. Minerals such as Fe and Zn are lost by 33% & 3% respectively in millet, 7% & 11% in maize, 41% &1.3% in sorghum and 60% & 30 % in rice (Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005).

Steeping:

Legumes:

Steeping of dry beans is carried out for production of sprouts that are consumed either whole or with minimal processing. Moth beans, soy beans, alfalfa, lentils, pea, chickpeas and mung beans are legumes that are steeped for sprouting. During steeping, enzymes are activated with optimum soak water temperatures, which prompt biochemical changes in seed towards germination. Germination through steeping is considered to be effective technology to retain maximum nutrients in legumes. Bioavailability of nutrients in legumes improved considerably after

sprouting. Steeping reduced enzyme inhibitors such as trypsin inhibitor by 50% in kidney beans and improve their digestibility (El-Hag, Haard, & Morse, 1978). Starch content of beans is also reduced by 55.6% during steeping process of kidney beans. Also, vitamin content (thiamin, riboflavin, niacin) of beans such as soy increased (20%, 50% and 330% respectively) during steeping as indicated by ascorbic acid content analysis (Abdullah & Baldwin, 1984).

Cereals:

Wheat, rye, sorghum and barley are mainly used for human consumption by steeping to obtain malt (although barley is most commonly malted grain). Barley and other cereals are converted to malt for beer preparation, salads, soups or to be consumed powdered as nutritional supplement (Lorenz & D'Appolonia, 1980). During steeping the bulk volume of cereals increases about 25%, with increase in width and depth of seeds, and no change in length (Briggs, 1998). Steeping of barley enables release of enzymes, which break down complex starches to simple sugars (e.g: glucose and maltose). Also proteins and nutrients are released through disruption of protein matrix (Bamforth, 2006). Absorption of water during steeping facilitates release of gibberellins (GAs) from embryo. Gas helps trigger release of hydrolytic enzymes such as amylase and convert starch present in endosperm to sugars. The sugars which are converted from starch during steeping are later utilized by yeast during fermentation The germination changes of other cereals, such as wheat, rye are similar to barley during steeping (Briggs, 1998).

Studies on hydration of cereals and legumes

Hydration of cereals and legumes has been studied to understand the impact of extrinsic and intrinsic factors on water uptake (Abu-Ghannam, 1998; Athanasia M. Goula & Konstantinos G. Adamopoulos, 2009; Hegarty & Scottish Horticultural Research Institute, 2012; Khazaei &

Mohammadi, 2009; Ramaswamy, Balasubramaniam, & Sastry, 2005; A. G. Taylor, Prusinski, Hill, Dickson, Taylor, Prusinski, et al., 1992). There are numerous studies that are carried out in food processing of cereals and legumes and in plant physiology, to understand water uptake for germination. The factors that are different among both cases are extrinsic. Where most of cereals and legumes that are used for processing foods are never germinated, there has been recent interest in developing functional foods using germinated seeds (Charalampopoulos, Wang, Pandiella, & Webb, 2002). Most of the studies are either experimental in nature or mathematical modeling of hydration rate of cereal or legume. The following section reviews both these experimental and mathematical studies of seed hydration for their accuracy and utility.

Experimental Studies

Experimental studies on cereals and legume are carried out to understand hydration rate as affected by changing extrinsic factors such as soak parameters, processing method, and seed conditions. Most of the reviews published on studies of cereals and legumes are to analyze nutritional changes during processing. While the role of hydration in seeds is well understand, there are no standard parameter or set of parameters that are measured. Moisture content is the primary indicator of water uptake in seeds, but 'the state of water' i.e., how tightly it is bound, is rarely studied. Recent advance in technologies has enabled to visualize water diffusion into seeds through NMR, and scanning electron microscopy (Muñoz, Cobos, Diaz, & Aguilera, 2012; Terskikh, Müller, Kermode, & Leubner-Metzger, 2011; Vashisth, Joshi, & Singh, 2012; W., C., & R., 2012). The following sections describe different techniques used to determine hydration kinetics of cereals and legumes

Moisture content:

Most legume and cereal moisture content during storage ranges from 12%-15%, while hydration, this is brought up to a range of 40-60%, depending on the desired end-product. The conventional method used to measure moisture content through complete drying of the hydrated sample and performing gravimetric analysis for their ease of application, as described in AOAC method 925.10 (Iqbal, Khalil, Ateeq, & Sayyar Khan, 2006). The type of drying method and accuracy varies by standard method employed (Bouraoui, Richard, & Fichtali, 1993). During soaking of cereals and legumes, moisture content is measured to determine the time required to reach equilibrium moisture content, which is varies from 50-65% (Resio, Aguerre, & Suárez, 2003). During steeping, moisture content of cereals and legumes are monitored to understand extent of hydration and correlate the data to germination times of seeds during micro-malting studies (Mayolle, Lullien-Pellerin, Corbineau, Boivin, & Guillard, 2011). For germination and better quality of malt, the seeds with quicker water uptake or initial moisture increase are considered better than those seeds that hydrate slowly (Briggs, 1998).

Volume:

Measurement of volume during soaking of cereals and legumes is essential when further processing is dependent up on the size of the seed, for example in thermal processing. Volume change varies across the spectrum of legumes and cereals, but in general, legumes tend to increase in volume more than cereals (A. Taylor, Prusinski, Hill, & Dickson, 1992). Volume change also correlates to moisture content of the seed based on seed properties, where legumes gain more than 100% of their volume, cereals gain about 25% (Thakor, Sokhansanj, Patil, & Deshpande, 1995). Methods employed to measure volume are both direct and indirect. In direct

methods, the volume of seeds are measured by Archimedes principle of displacement (Moreira, Chenlo, Chaguri, & Fernandes, 2008). While, in indirect methods, weight and density are measured to arrive at volume using pycnometer filled with toluene (Muramatsu, Tagawa, Sakaguchi, & Kasai, 2006). Numerous manual volume measurement techniques are employed, by approximating seed particle to ellipsoid or spheroid, but these fail to reflect volume of bulk particles with large sample. Even with assistance of imaging technology, the manual measurement had very little accuracy pertaining to its small sample size (Igathinathane, Pordesimo, Columbus, Batchelor, & Methuku, 2008; M. Shahin & Symons, 2005; M. A. Shahin, Symons, & Poysa, 2006).

Imaging techniques:

Modern imaging techniques have been used to understand diffusion of water into seeds (M. Shahin, Symond, Schepdael, & Tahir, 2006; M. Shahin & Symons, 2005; M. A. Shahin, Symons, & Poysa, 2006). Proton Nuclear Magnetic Resonance paired with Magnetic Resonance Imaging provides a non-invasive technique to study water uptake in seeds (Terskikh, Müller, Kermode, & Leubner-Metzger, 2011; Vashisth, Joshi, & Singh, 2012). These techniques provide good understand of water activity in seeds, but it is difficult to quantify the rate and amount of water uptake. The methods are widely employed to understand hard-to-cook phenomena in seeds (Aguilera & Lillford, 1997; Laurent, Ousman, Dzudie, Carl, & Emmanuel, 2010).

Mathematical models for seed hydration:

The difficulty and inaccuracy of experimental methods have led to the usage of mathematical models to describe hydration kinetics of seeds. Modeling of seed hydration has become a de facto standard in research to emphasize the rate and extent of hydration and to determine the

impact of temperature, and soak water parameters. Many models are proposed to describe hydration of seeds, both in food processing and plant sciences (Bradford, 2002; Athanasia M. Goula & Konstantinos G. Adamopoulos, 2009). Research groups have mainly used two approaches to describe hydration of porous biomaterials. One approach is based on Fick's law of diffusion, which constituted the earliest attempts to model water uptake in seeds (Waggoner & Parlange, 1976), while other approaches are based on empirical and semi-empirical methods (Athanasia M. Goula & Konstantinos G. Adamopoulos, 2009; Peleg, 1988). Empirical models like Peleg and Weibull are more widely employed to simulate hydration in foods, due to their simplicity, and having to work with fewer parameters (Weerts, Lian, & Martin, 2003). Of the models that are described below, the empirical models have proven to be best suited for hydration of foods, whereas, physical models are best utilized to describe the properties (e.g., diffusivity) and process conditions (e.g., temperature) of both the hydration medium and the rehydrating food particles.

Diffusion model

The diffusion model is based on following assumptions using Fick's second law of diffusion: 1. The water diffusion is constant and primary method of transfer, 2. Shape of the seed is spherical, 3. No external resistance to heat and mass transfer exists, 4. Surface concentration of water reaches saturation immediately after immersion (M. Bello, M.P. Tolaba, & C. Suarez, 2004).

The model equation is given as:

$$\frac{m - m_s}{m_0 - m_s} = \frac{6}{\pi^2} exp\left(-\frac{\pi^2 D_{ef} t}{R^2}\right)$$

Where m_s and m_0 are saturation and initial moisture contents (kg/kg, dry basis), t is the time (s), R the equivalent radius (radius of the sphere having the same volume as the grain) (m) and D_{ef} is the effective diffusivity (m^2/s).The moisture absorption curve is fitted by adjusting the value of D_{ef}.

In the study reported above, and other work done on hydration analysis using Fick's law, the limitation of determining saturation moisture content is reported for rice. That is due to variation in moisture content of seeds with gelatinization depending on starch content. Bello and group studied the impact of temperature on diffusivity rate of rice while soaking for 24 hours using the diffusion model derived from Fick's second law. Figure.1 shows the equilibrium moisture content of rice remained relatively constant after 45^{o} C, with slight increase from 25^{o} C to 35^{o} C, while the diffusivity increased with increase in temperature. The moisture content was determined using the Fick's law with less than 3% error.

Capillary flow model

Studies are carried out to look at water uptake through foods as a capillary flow in the porous media driven by energy potential, rather than diffusion, accounting for anisotropy effects (Weerts, Lian, & Martin, 2003). One of the main advantages proposed for capillary model is that it can be derived through capillary relationships from physical-based water activity curves commonly used in food science and engineering. Different models for rehydration of dry food particles based on porous media are summarized elsewhere (Sam Saguy, Marabi, & Wallach, 2005). The porous media models are derived from Lucas-Washburn equation with following main assumptions: 1. Food is in form of microscopically uniform porous media, 2. Pore size is average throughout. The equation for equilibrium liquid rise in porous foods is given below:

$$y(t) = \frac{k_1}{k_2}\left[1 - e^{\left(-\frac{k_2 y(t)}{k_2}\right)} e^{\left(-\frac{{k_2}^2 t}{k_1}\right)}\right]; k_1 = \frac{r\gamma\cos(\delta)}{2\mu} \; and \; k_2 = \frac{r^2 g\rho}{8\mu}$$

Where, k_1 and k_2 are permeability parameters; y is distance travelled by the liquid at time t (m); r is pore radius (m); t is time (s); γ is surface tension (N/m); δ is contact angle (^{o}); μ is viscosity (N s/m^2); g is gravity (m/s^2).

Goula *et al* analyzed the application of capillary flow approach to describe water absorption through dried tomatoes, and found that the capillary model adequately describes rehydration behavior of dried foods (Athanasia M Goula & Konstantinos G Adamopoulos, 2009). Figure. 2 shows the capillary model predicted and measured moisture content values of dried tomato with no differences among ($P < 0.05$) slope and intercept. The biggest advantage of capillary model among both physical models is due its consideration of gravity, which is missing in diffusion model and it is comprehensive in its constitutive relationships required for capillary flow, its complexity might be a hindrance to utilization in industry, particularly in cereal and legume hydration.

Becker model:

Becker's model is derived from Fick's law of diffusion considering solids with arbitrary shape (Becker, 1960). The primary drawback of the equation is its basis using law of diffusion and difficulty in calculation of ΔM_0, which is initial moisture gain, caused by capillary forces and error in determining surface moisture content. Becker's model is given as:

$$M_t = M_0 + \Delta M_0 + \frac{2}{\sqrt{\pi}}(M_e - M_0)\left(\frac{S}{V}\right)\sqrt{(D_{eff})(t)}$$

Where, M_t is moisture content (% dry basis) at time, t (s); M_e is equilibrium moisture content (% dry basis); M_0 is initial moisture content (% dry basis); ΔM_0, is initial moisture gain (% dry

basis); M_s is surface moisture content (% dry basis); S is surface area (m^2); V is volume (m^3); D_{eff} is effective diffusion coefficient (m^2/s). Roy *et al*, used Becker model to predict hydration of four different rice cultivars with a correlation of r= 0.98 as shown in Figure 3 (Bandyopadhyay & Roy, 1978).

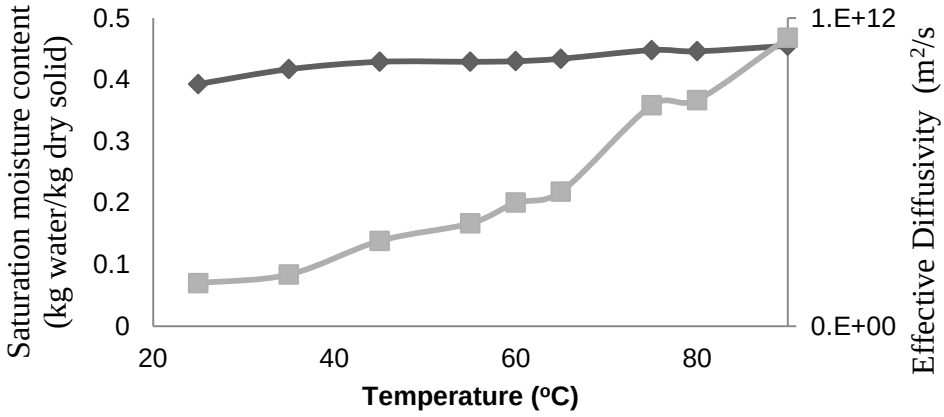

Figure 1.1. Effect of temperature on diffusivity (■) and saturation moisture content (◆) of rice

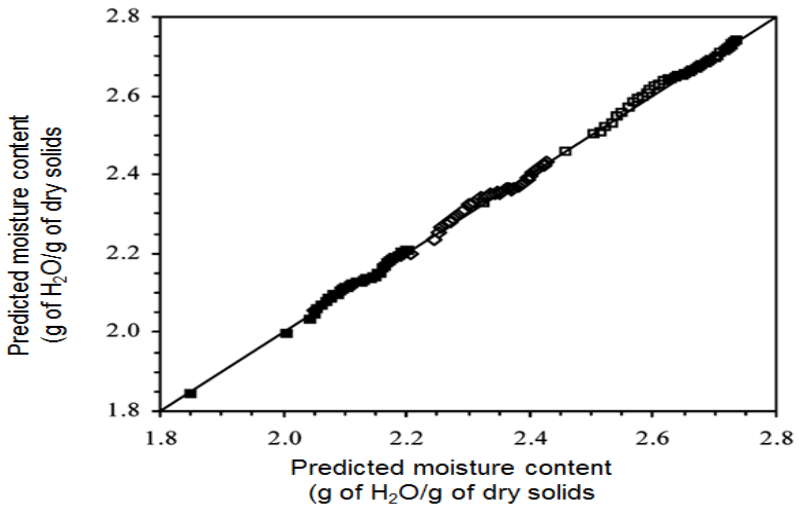

Figure 1.2. Experimental and capillary model predicted moisture content of rehydrated dry tomatoes

Exponential model:

The simplest empirical model described in literature is exponential model, which describes relationship of moisture content for particular food over time (Diamante & Munro, 1991).

$$\frac{M_t - M_e}{M_0 - M_e} = ae^{(-bt)}$$

Where M_t is moisture content (% dry basis) at time, t (s); M_e is equilibrium moisture content (% dry basis); M_0 is initial moisture content (% dry basis); a and b are empirical constants.

A modified version of above exponential equation is proposed by Maskan to model water uptake of wheat kernels during soaking considering temperature, T given as: $\frac{M_t - M_e}{M_0 - M_e} = 7259 \times exp^{(0.031t - 1470/T)}$ (Maskan, 2001).

Maskan reported that, model correlated with experimental value with r=0.925.

Peleg model:

The Peleg model is first introduced to disrobe moisture sorption curves for both granular foods and seeds (Peleg, 1988). It is one of the first non-exponential models to be used for understanding hydration. Ever since its conception, the model has been widely used to model hydration process of many food materials (Cunningham, McMinn, Magee, & Richardson, 2007; Athanasia M. Goula & Konstantinos G. Adamopoulos, 2009; P. Sopade & Obekpa, 1990; P. A. Sopade, Ajisegiri, & Badau, 1992; Turhan, Sayar, & Gunasekaran, 2002). The model is described as:

$$M_t = M_0 + \frac{t}{(k_1 + k_2 t)}$$

Where, M_t is moisture content (% dry basis) at time, t (s); M_0 is initial moisture content (% dry basis); k_1 is constant related to the initial rate of sorption (hour per % weight of dry solids to water); k_2 is constant related to equilibrium moisture content (% weight of dry solids to water).

Peleg model has been extensively used to study cereal and legume hydration. The model was also used as basis for testing new empirical and mechanistic models (Coutinho, Omoto, dos Santos Conceição, Andrade, & Jorge, 2010). Solomon used Peleg model to understand hydration kinetics of lupin seeds at temperatures of 20^o C, 30^o C, 40^o C and 50^o C for 9 to 12 hours. Figure 4 shows experimental moisture content of lupin seeds along with Peleg model curves. Solomon reported that Peleg model predicted the experimental results with co-efficient of determination, R^2= 0.96 to 0.99. Sopade *et al* used the Peleg equation to model water absorption of maize, millet and sorghum at temperatures of 10^o C, 30^o C and 50^o C. Figure. 5 shows the Peleg model predicted and experimental values of maize, millet and sorghum. The correlation between predicted and experimental values is reported as, r^2= 0.98 to 0.99. Sapode *et al* also reported that Peleg constant k_1 was temperature dependent, while k_2 was unaffected.

Similar high correlation (>.95) was reported for Peleg model used to study the hydration kinetics of cereals and legumes (Cunningham, McMinn, Magee, & Richardson, 2007; García-Pascual, Sanjuán, Melis, & Mulet, 2006; Athanasia M. Goula & Konstantinos G. Adamopoulos, 2009; Turhan, Sayar, & Gunasekaran, 2002).

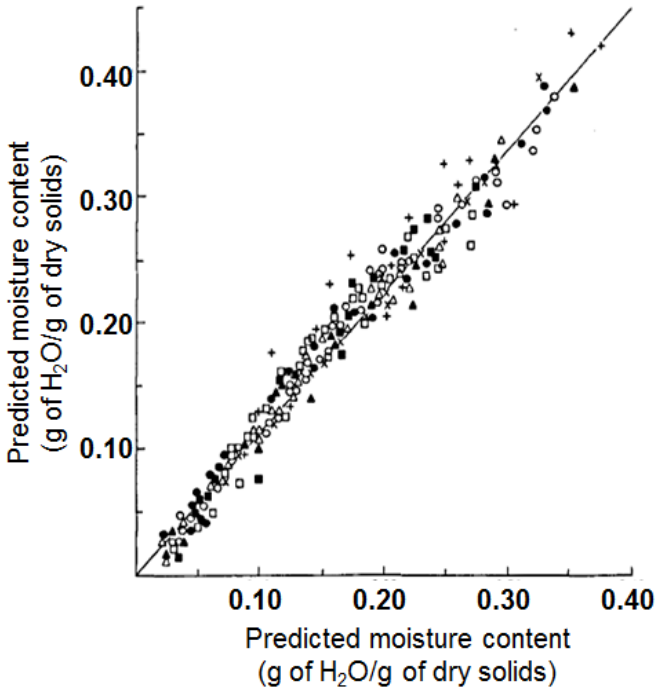

Figure 1.3. Correlation between predicted and measured moisture content of different rice varieties using Becker model (Bandyopadhyay & Roy, 1978).

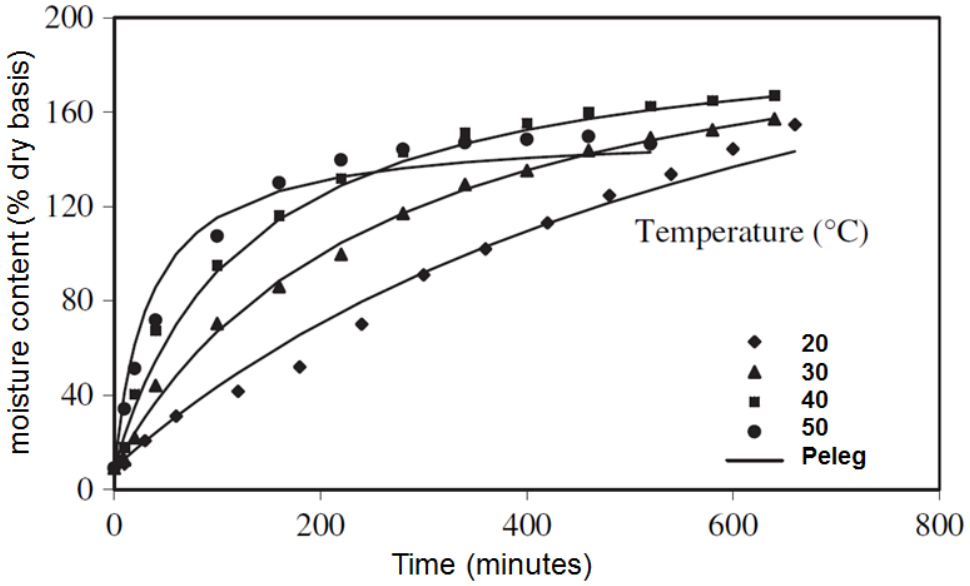

Figure 1.4. Measured and predicted (Peleg model) moisture content data for lupin seeds at different temperatures (Solomon, 2007).

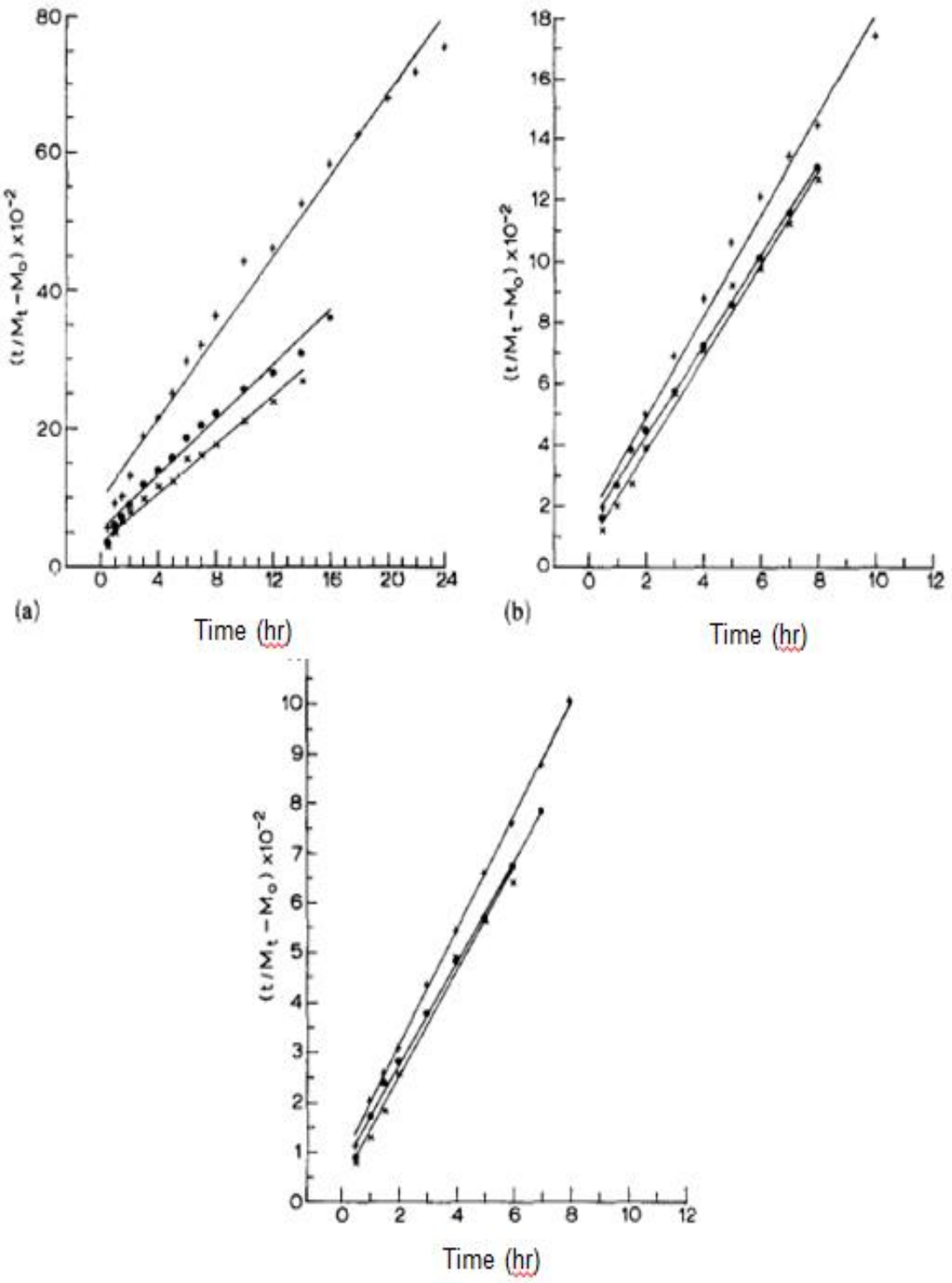

Figure 1.5. Application of Peleg model to experimental data on maize (a); millet (b); and sorghum (c) .

Weibull model:

An improved version of probabilistic Weibull distribution function has been utilized to describe rehydration of dried foods (Marabi & Saguy, 2009). The equation is:

$$\frac{M_t}{M_e} = 1 - e^{\left[-\left(\frac{t}{\alpha}\right)^{\beta}\right]}$$

Where, M_t is moisture content (% dry basis) at time, t (s); M_e is equilibrium moisture content (% dry basis); The model utilizes two empirical parameters; α, which is reciprocal of the process rate constant and is related rapid hydration; and β is shape parameter, related to the shape of initial lag phase (I Sam Saguy & Marabi). The equation is modified numerous times to fit different foods (Cunningham, McMinn, Magee, & Richardson, 2007; García-Pascual, Sanjuán, Melis, & Mulet, 2006; Athanasia M. Goula & Konstantinos G. Adamopoulos, 2009; I. Sam Saguy, Marabi, & Wallach, 2005). Prasad *et al* studied hydration kinetics of chick peas using different empirical and mechanistic models at temperatures of 40^o C, 50^o C and 60^o C. Figure 6 shows fitting of Peleg, Weibull and exponential models of measured moisture ratio (MR) values with time for chick peas. Table 1.4 shows estimated parameters for all three models reported in the study along with their goodness of fit. All three empirical models had correlation coefficient of r^2= 0.990 to 0.996 indicating that the models ability to describe hydration of chick peas. Table 1.5 is similar comparison fall three empirical models made with Harte *et al*'s unpublished data of black, pinto and navy beans with correlation coefficient of r^2= 0.985 to 0.996. Figure 7 shows the model fit for all measure volume readings taken while soaking the beans for 180 minutes at 55^o C.

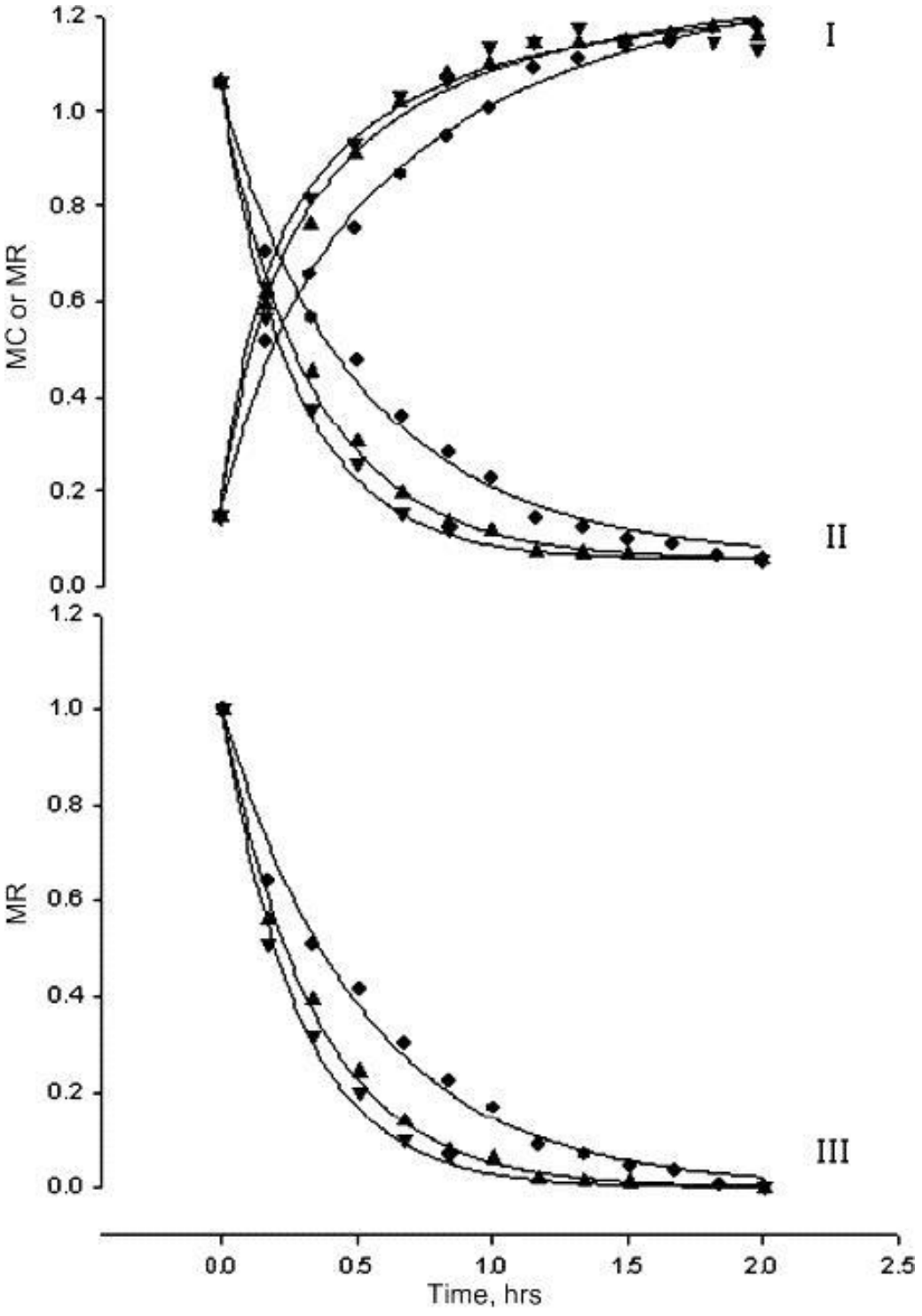

Figure 1.6. Fitting of Peleg (I), Weibull (II) and Exponential (III) models at 40^{o} C, 50^{o} C and 60^{o} C hydration temperatures of chick peas (Prasad, Vairagar, & Bera, 2010).

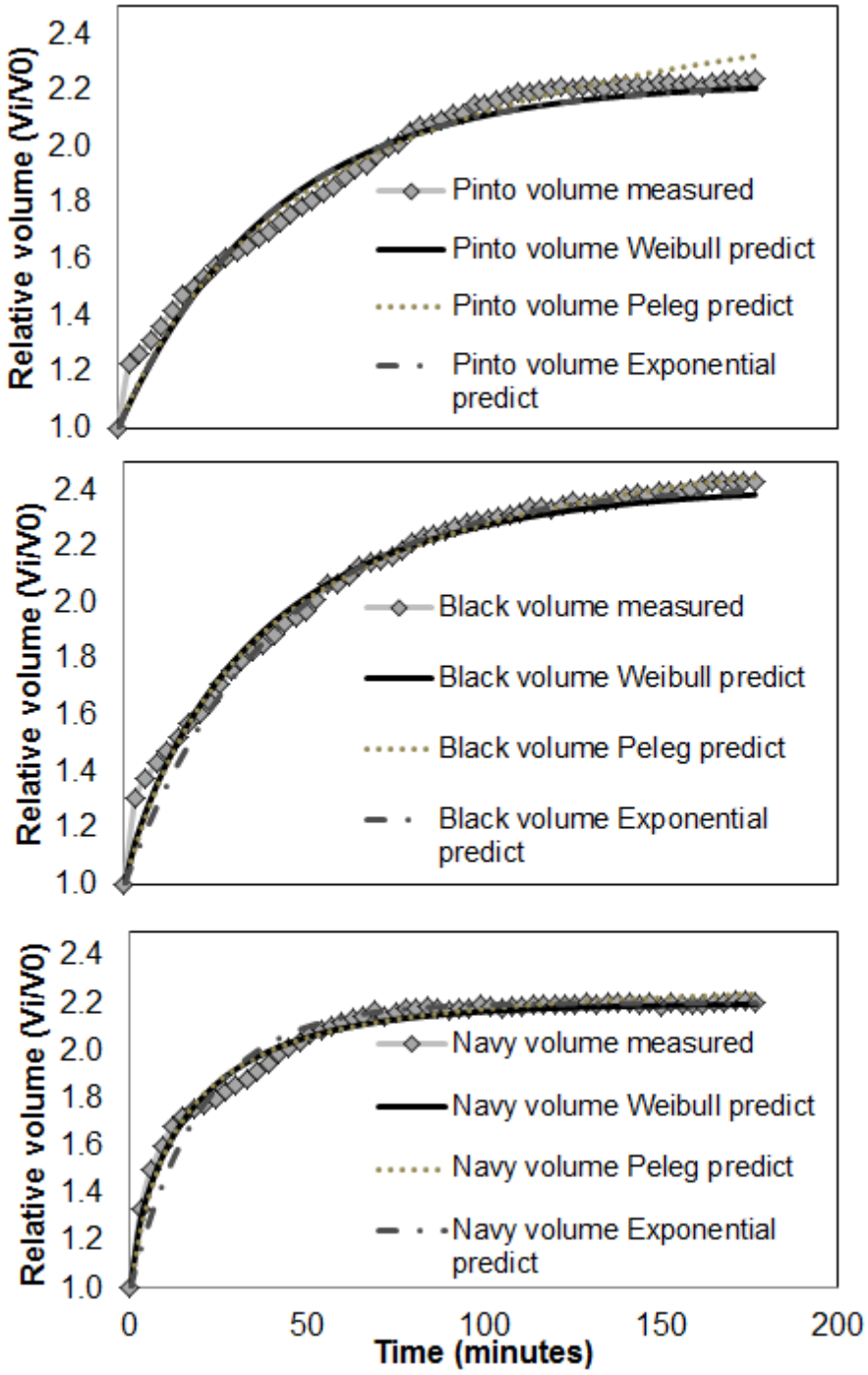

Figure 1.7. Fitting of Peleg (), Weibull () and Exponential ( ) models for measured volume readings of black, navy and pinto beans (Mannam, Harte unpublished data).

Table 1.4 Estimation of parameters and goodness of fit of Peleg, Weibull and Exponential models for chick pea hydration (Prasad, Vairagar, & Bera, 2010).

Temperature	Peleg model			Weibull model			Exponential model	
	K_1	K_2	R^2	α	□	R^2	K	R^2
(°C)								
40	0.399	0.756	0.995	0.931	0.506	0.991	1.936	0.99
50	0.232	0.835	0.994	0.951	0.329	0.996	2.996	0.996
60	0.191	0.866	0.991	0.964	0.273	0.991	3.615	0.991

Table 1.5 Estimation of parameters and goodness of fit of Peleg, Weibull and Exponential models for navy, black and pinto beans (Mannam, Harte unpublished data).

Variety of Legume	Peleg model			Weibull model			Exponential model	
	K_1	K_2	R^2	α	□	R^2	K	R^2
Black bean	21.36	0.569	0.995	38.75	0.862	0.994	40.35	0.994
Pinto bean	0.232	0.835	0.994	0.951	0.329	0.996	43.27	0.989
Navy bean	0.191	0.866	0.991	0.964	0.273	0.991	20.14	0.984

Current challenges in studies of cereal and legume hydration

Cereals and legumes are well studied and understood during process of hydration. As individual treatments hydration rates of seeds vary based on their properties, it is difficult to generalize hydration process in dry foods. When considering hydration as a processing step in preparation of functional foods, it is important to understand the factors affecting the process. Past and current research indicates a very good fundamental understanding of science behind hydration (Bamforth, 2003; Friedman, 1996; Rao & Lund, 1986; Reyes-Moreno, Paredes-López, & Gonzalez, 1993; Shridhar K. Sathe, Deshpande, Salunkhe, & Rackis, 1984; W., C., & R., 2012). Changes in nutritional value and ability to preserve important nutrients during hydration have

been an important focus (Friedman, 1996; Hornick, 1992; Kon, 1979). Extensive mathematical modeling has been done during hydration of seeds to provide an option due to the lack of fast and accurate experimental method (I. Sam Saguy, Marabi, & Wallach, 2005; W., C., & R., 2012). New technologies in imaging and microstructural analyses are used to support the established mathematical theories (Aguilera & Lillford, 1997).

The main challenge very seldom addressed when working with hydration of seeds is the lack of standard experimental techniques with a broad enough scope to study a wide range of factors impacting both soaking and steeping processes. Current modeling techniques fall short of usage in commercial environment due to the time required to obtain enough measurements to obtain the data required for rigorous statistical analyses. Better analytical tools to measure and record various seed parameters during hydration will both improve the quality of hydration studies, and also provide path to faster commercialization of important discoveries.

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CHAPTER II

Automatic Seed Hydration Analyzing System-Design and Evaluation

Abstract

Hydration characteristics of different cereals and legumes are important to understand in order to gain insights into their process-ability and to determine seed dormancy. Also, hydration kinetics provides valuable information the hydration rate and extent of volume and weight uptake. A seed hydration analyzing system is developed for application in industry and academia where there is a necessity to understand hydration rates of seeds. The system developed is able to simultaneously soak seeds and measure volume, weight and density. The system also records and presents data in real time via computer interface. The device was successfully evaluated to measure volume and weight with 97.87% and 99.08% accuracy respectively. Examples on scope of the developed hydration analyzing device are also presented.

Introduction

Cereals and legumes processing:

Cereals and legumes are processed primarily to remove indigestible components and make important nutrient available in foods. For cereals, common processing methods include milling, grinding, germination, fermentation and cooking. For legumes, processing methods can be categorized in to soaking and cooking. These different processing methods decrease the phytates, which cannot be absorbed in the human gastro-intestinal tract, and make nutrients and minerals such as proteins, iron, zinc and calcium bio-available (Hotz and Gibson, 2007). One or more methods mentioned are used in food industry to get the required protein retention and reduction of anti-nutrients, and water plays a prominent role during processing in preparation of cereals and legumes.

Hydration of cereals and legumes:

Cereals and legumes are dried after harvesting to preserve quality and prevent microbial growth during storage. Soaking as a simple step of hydration is used to rehydrate dried cereals and legumes, and in most cases, it is the preliminary step in preparation of functional foods using cereals and legumes (Muramatsu et al., 2006). In the case of rice, hydration is done prior to cooking, whereas for wheat and corn are either wet-milled or water is added before further processing after dry milling. In malt preparation, barley, wheat or maize are soaked for germination. And dry beans are soaked prior to cooking. Based on the final product, the extent of hydration of seeds varies with type of cereal and legume.

Importance of seed hydration analysis:

Apart from seed varieties and initial seed conditions, the process of soaking is affected by parameters including water temperature, water chemistry, pressure and soak time. These soak parameters are altered and controlled to achieve better and faster hydration for cereals and legumes (Resio et al., 2003). The hydration kinetics are determined by observing weight uptake of seeds (as moisture content) and more recently through advanced microscopic techniques (Muñoz et al., 2012). However, these tools are cumbersome (extensive labor is required) and accuracy is low, leading to the use of indirect methods that rely on modeling (Kashaninejad et al., 2009). Mathematical models became a norm for seed hydration research due to lack of

extensive experimental methods both in food and plant sciences (Bradford, 2002). Both empirical and physical models are proposed and used in academia and industry to understand hydration kinetics as affected by process conditions. There is a major need for better experimental tools to measure hydration kinetics accurately to optimize soaking and improve cereal and legume processing. The detailed study on how seeds hydrate provides valuable data on behavior in later stages of processing. Understanding water uptake of dry beans is essential to determine bean weight and volume throughout thermal processing in the canning industry. Also, hydration kinetics provides valuable data towards determining seed dormancy, which is a major hurdle in processing and malting industries (Muramatsu et al., 2006). Current methods to measure hydration kinetics rely on experience through observation and indirect methods – such as moisture content and empirical modeling. These analytical methods are seldom used due to lack of measurement accuracy for volume and weight. Hydration rates of seeds are previously determined by means of measuring moisture content. Measuring moisture content is time consuming, tedious process, and destroys the sample. The only previously reported direct volume measurement of seed while soaking was through imaging (Amin et al., 2004). Continuous monitoring of seeds while hydration is difficult due to quantity of seeds that are generally hydrated. Measurement of weight is simple but requires manual labor. Understanding and analyzing the hydration process is essential to determine the effectiveness of method employed and quality of the particular cereal or legume. A stand alone, easy-to-use time saving hydration analyzing system was developed to cater to food industry and research community. This article describes an automatic system that can be interfaced with a computer and water baths to obtain weight, volume and density of seeds while undergoing hydration. The system utilizes current available data acquisition technology and sensors to collectively run as an analytical tool to analyze seed hydration.

Seed Hydration Analyzing System

The system was developed to directly measure hydration profiles of seeds while soaking. The technology consists of a device which can hold seeds submerged in water and measures volume, weight and density in minimum of one minute intervals over a period of hours or days. The data acquisition system is controlled by a computer using LabView® software. The system designed to operate at temperatures ranging from 0^{o}C to 99^{o}C. The unit is designed to minimize time

required and eliminates manual labor to measure volume and weight of seeds while soaking. The automatic nature of the system will enable recording and storage of data with minimum human error. A patent application has been filed in United Stated Patent and Trademark Office (USPTO), with application number: 13871347.

System Overview:

The complete system is a configuration of a set of valves (220, 230, and 240) and pumps (215, 225, 245, and 325) that are used to control flow of water. For the system includes two external water baths (210, 310) and a computer with software (Figure 2.1). The seed hydration chambers (140) are enclosed along with measuring chamber (120) in a temperature controlled water jacket (310). The measuring chamber is designed to enable volume measurement of the amount of water that is delivered to the hydration chambers. The measuring chamber (120) is equipped with an ultrasonic sensor (125) distance mounted on the chamber. Figure 2.2 illustrates the complete assembly of the device including three hydration chambers and a measuring chamber enclosed in a water jacket along with box enclosure for valves and pumps along with electronics to provide power to valves, pumps and sensors and acquire data. The system runs stand alone with set of input parameters including soak time, measurement time intervals, and temperature.

Detailed Description:

Water Jacket: In the device set up, the water jacket sits on top of the enclosed box containing valves and pumps. Figure 2.3 shows water jacket that encloses the seed hydration chambers equally placed along with measuring chamber. The inlets for chambers are through water jacket's bottom plate (305). Water is circulated to the water jacket from water bath using inlet and outlet at the side, and temperature of water is maintained at the same temperature as feed water bath used for hydration of seeds. The water jacket is made of chlorinated polyvinyl chloride material. The main body is a standard cylinder CPVC pipe with top (315) and bottom plates adhered with plastic cement. The water jacket is designed to withstand temperatures of up to 99^{o}C.

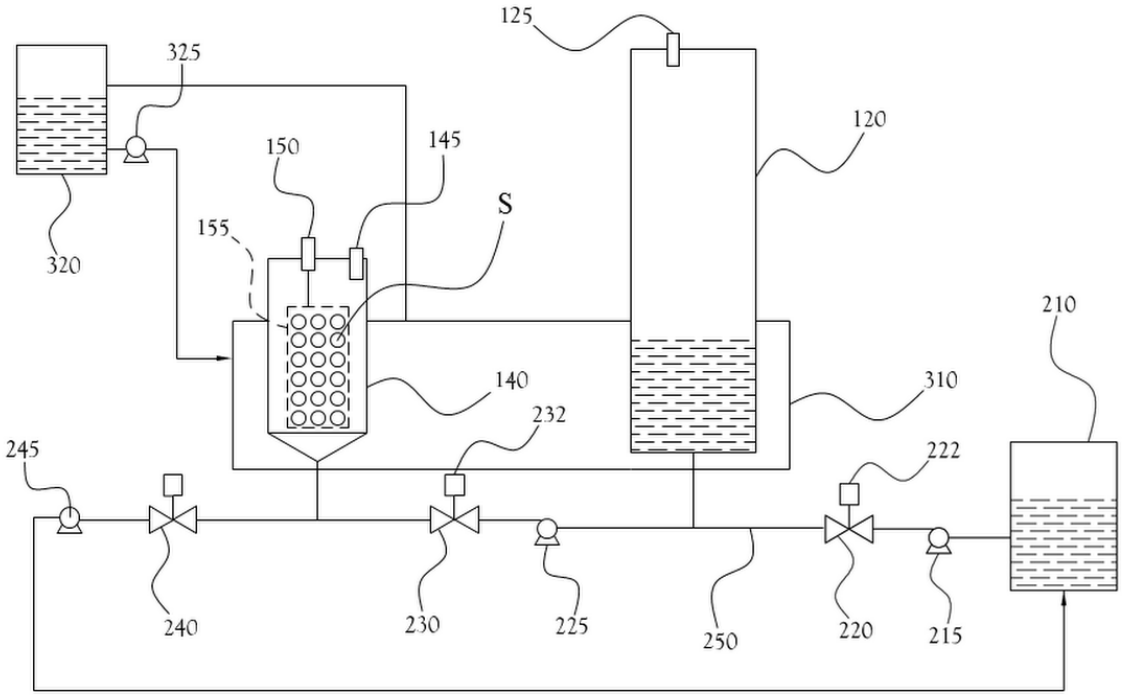

Figure 2.1. System set up showing water flow control and main sensors configuration.

Measuring chamber: The primary component of measuring chamber (120) is ultrasonic sensor (125) with range of 2-18 centimeters. Figure 2.4 shows measuring chamber containing orifices (135) surrounding ultrasonic sensor to prevent condensation, with a fan (115) installed in one of the orifices. The fan is turned on when ultrasonic sensor is not taking any measurements. The length of measuring chamber is limited by the range of ultrasonic sensor, which in turn limits volume measurement of seeds in soak chamber. For an ultrasonic sensor with range of 2-18 centimeters the maximum measureable volume is 550 milliliters.

Soak chambers: Three soak chambers capable of holding seeds (S) with a seed basket (155) attached to load cell (150) for hydration are enclosed with water jacket above the box enclosure. The lid (165) of soak chambers contains a level switch (145), which is used to keep the water in soak chamber at a constant level. The total volume of seeds that can be held in soak chambers can be changed by placing spacers (175) in between the lids and respective soak chambers, they are held together by flat head screws through holes. Figure 2.5 shows cross-sectional view of soak chamber, lid and spacer. Water to soak chambers is fed from measuring chamber controlled

by one valve in between each of soak chambers and measuring chamber. A self-priming centrifugal pump is used to draw water from measuring chamber to each soak chamber. The soak chambers are filled separately and at regular intervals from the bottom inlet. A valve and diaphragm pump between the soak chambers and soak water bath helps to drain water.

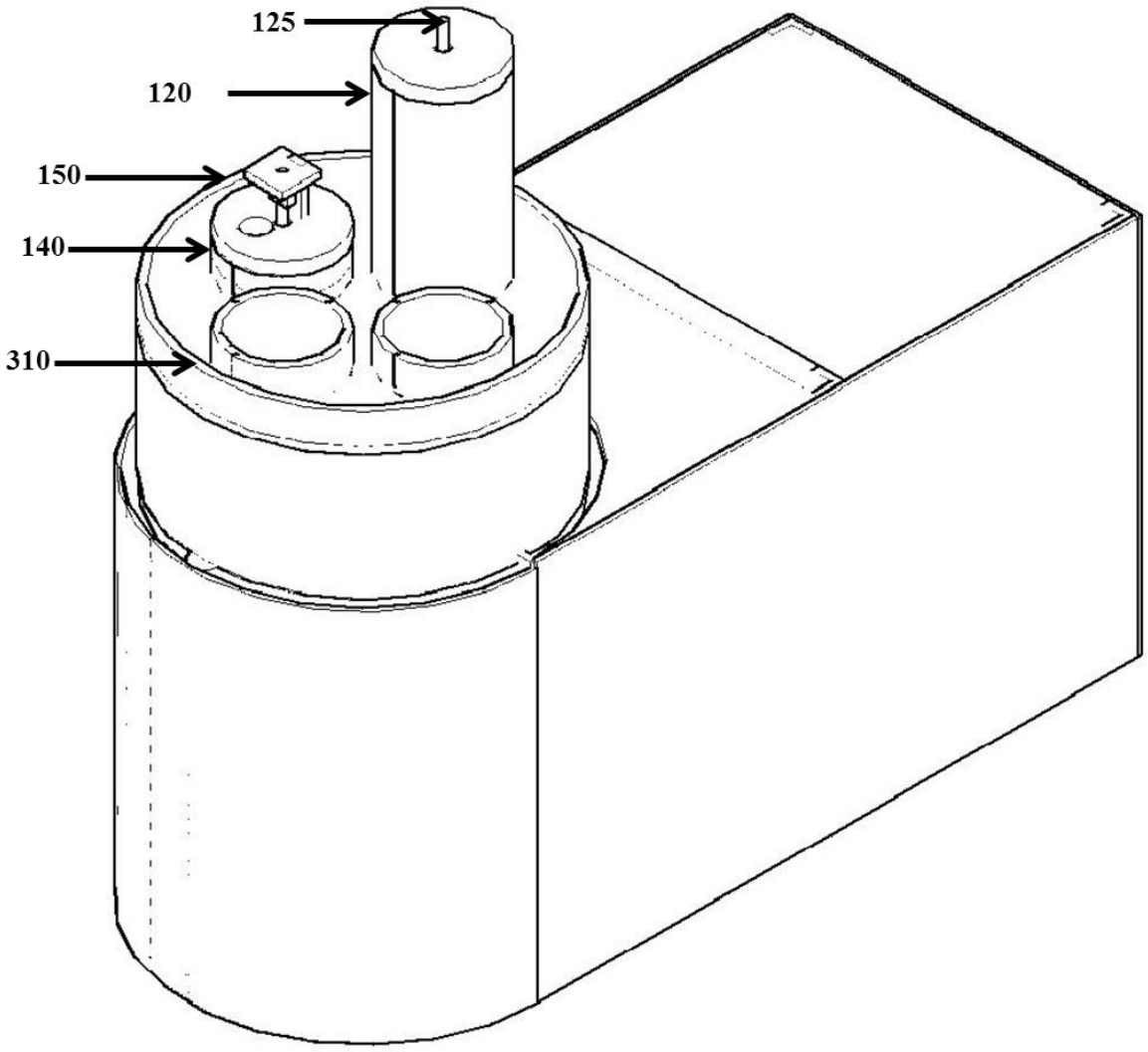

Figure 2.2. Isometric view of the seed hydration analyzing device set up with different hardware.

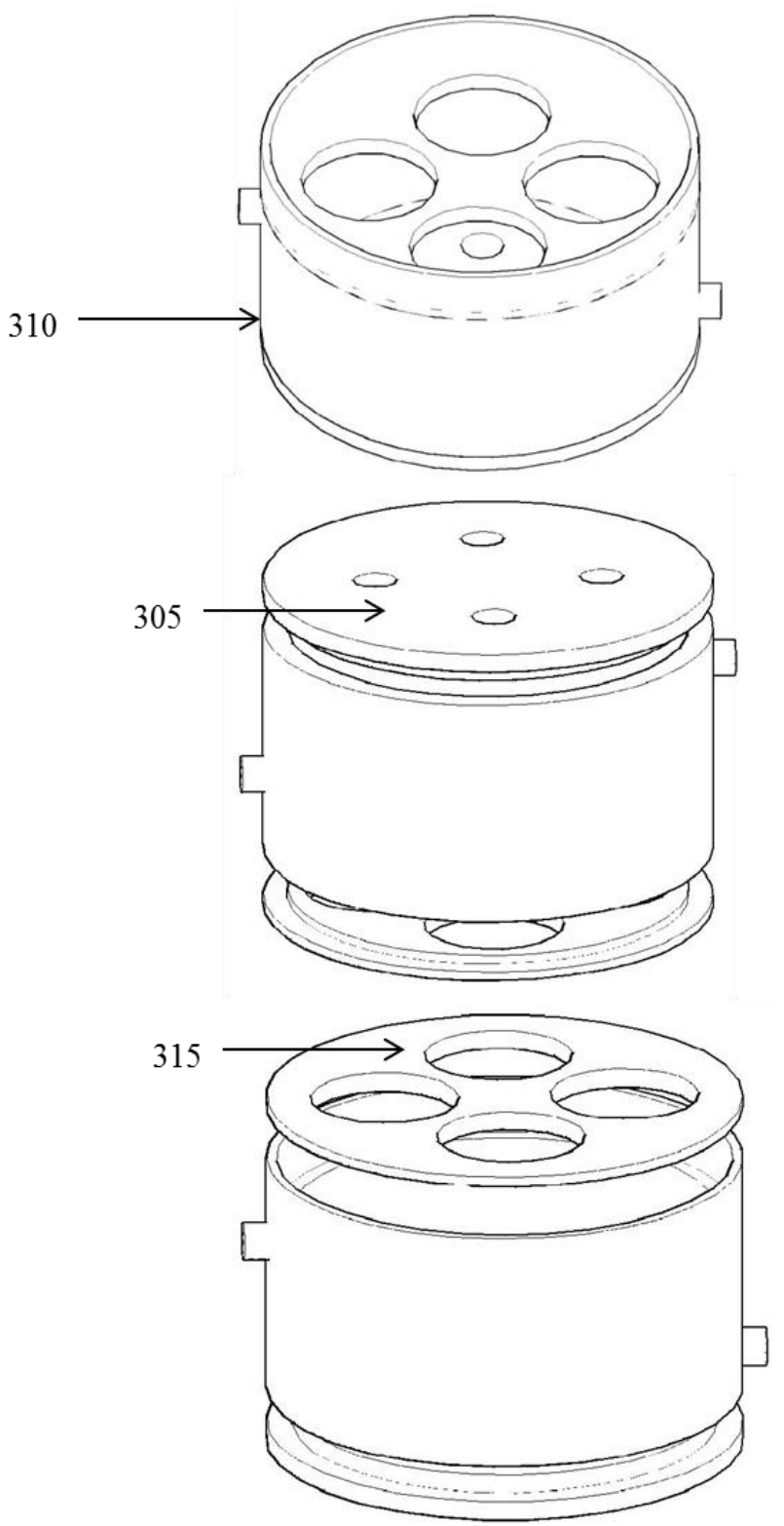

Figure 2.3. Isometric view of the water jacket with top and bottom plate views.

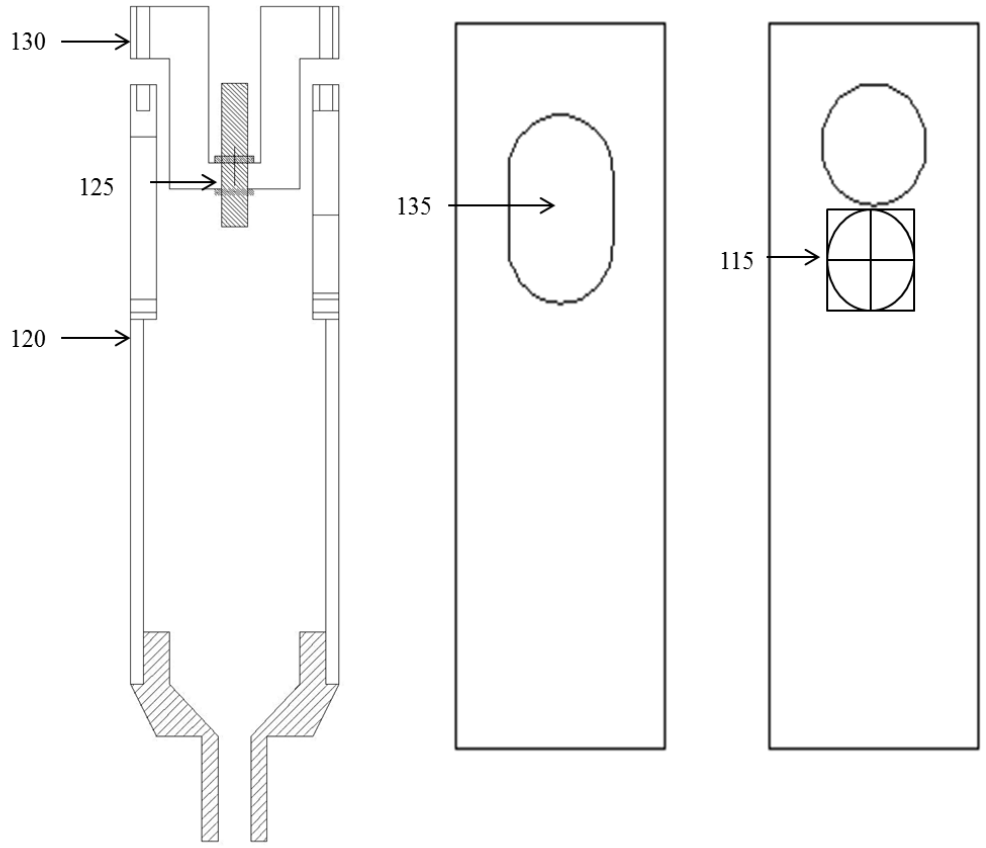

Figure 2.4. Cross-sectional view of measuring chamber.

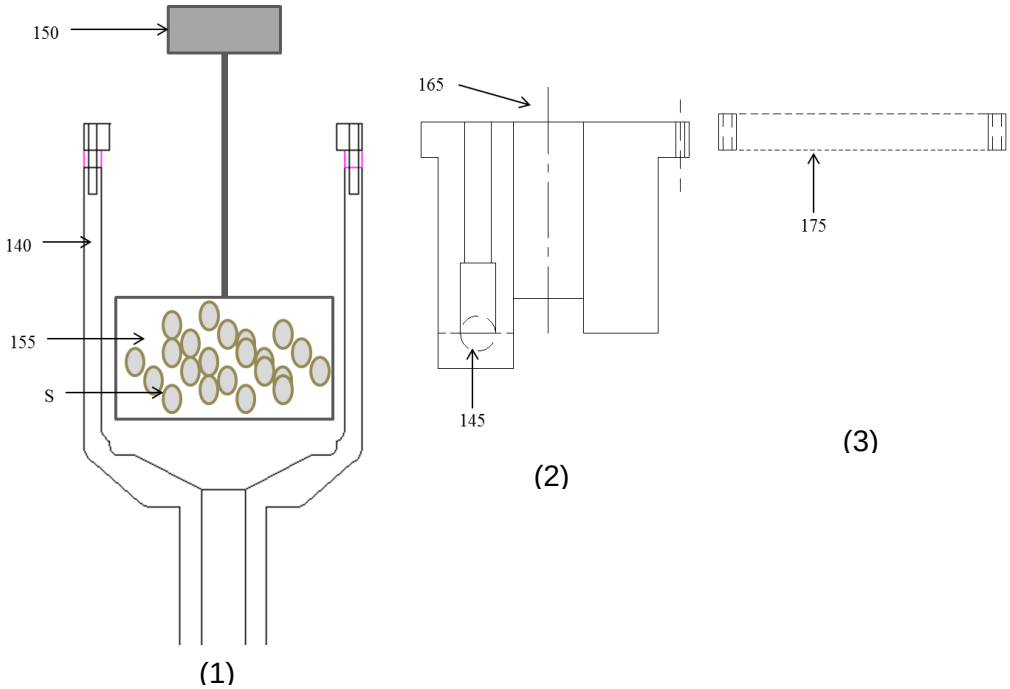

Figure 2.5. Cross-sectional view of soak chamber (1), lid (2) and spacer (3).

Working Principles and Flow of Operation

The developed system functions using LabVIEW® to measure and record volume and weight of seeds during hydration. Figure 2.6 shows the working flow of automatic system. The initial parameters such as time of hydration, measurement interval are set in software and system follow pre-programmed steps as described in flow chart (Figure 2.6) and both volume and weight measurements are taken simultaneously at predetermined intervals. A detailed step by step standard operating procedure, including maintenance, part specifications and safety procedures is included in appendix II.

Volume measurement: Volume of seeds in the hydration chamber is measured by means of a separate chamber described as measuring chamber in section 2.2.2. The chamber is a long pipe with water fed from bottom. The steps for measuring volume are as follows:

a. The water flows first into measuring chamber from where it is fed to each of soak chambers with a network of valves and pumps.
b. Water enters measuring chamber via a valve and utilizing pump from water bath.
c. Ultrasonic sensor installed at center on top of the lid measures water level continuously and valve is closed when it reaches the predetermined level.
d. Level of water in measuring chamber is recorded using ultrasonic sensor.
e. Water is then pumped to soak chambers until the level switch and the level of water is measured again.
f. The volume of empty soak chamber is calculated by the difference of initial water level and final water level of measuring chamber [volume of soak chamber = pi*(diameter^2/4)*water level difference].
g. The cycle is repeated for each of the three soak chambers to determine their initial volumes (V_{i1}, V_{i2}, and V_{i3}).
h. The volume is again measured with seeds in the soak chambers (V_{b1}, V_{b2}, and V_{b3})as described before.
i. The final volume of seeds in each soak chamber is calculated by difference of initial volume and volume of soak chamber with seeds ($V_1=V_{i1}-V_{b1}$).
j. The cycle (steps a-i) is repeated at predetermined time intervals and volume of seeds is recorded each time.

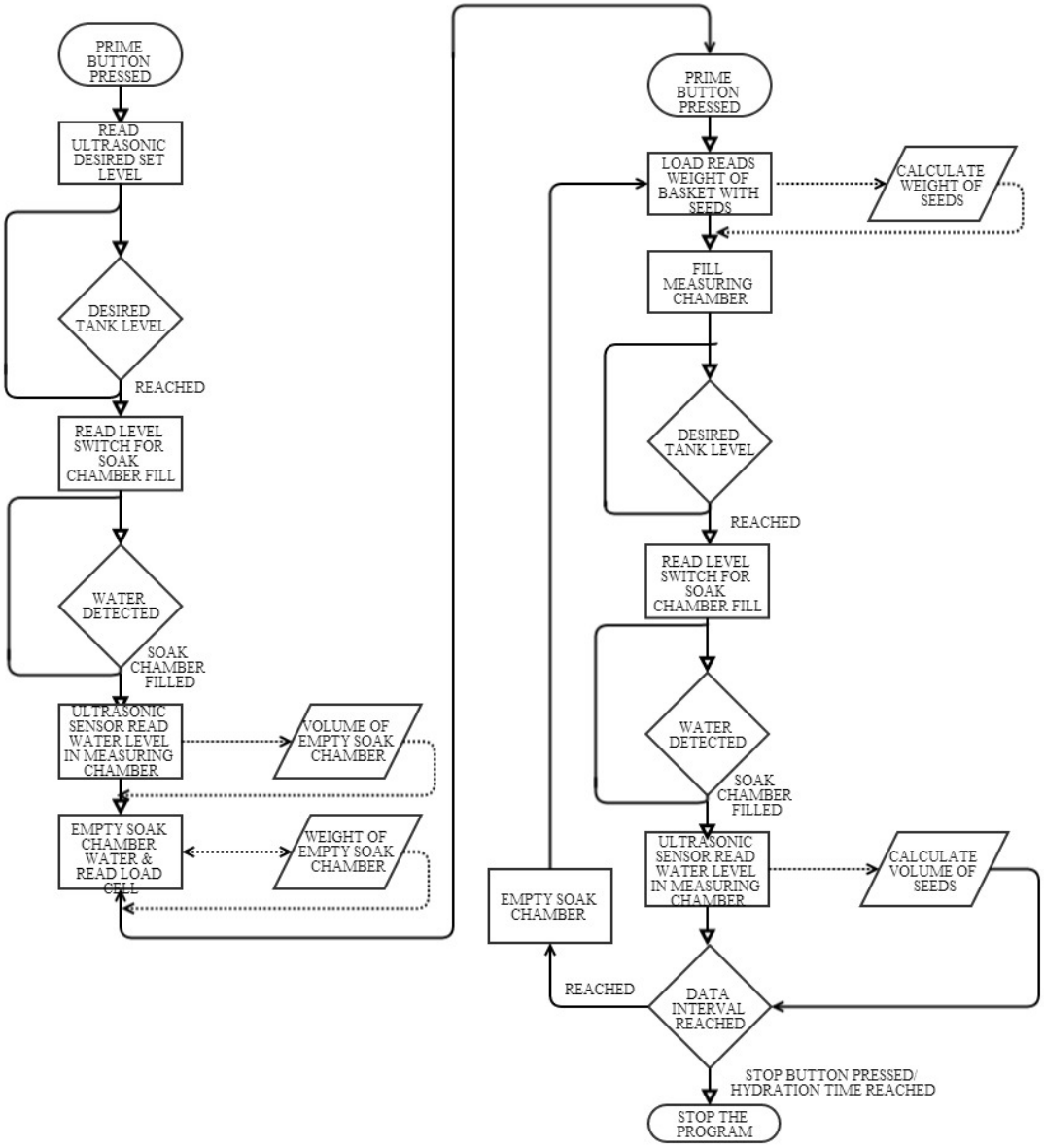

Figure 2.6. Flow chart showing the data measurement process for hydration analyzing system.

2.3.2 Weight measurement: Each of the soak chambers can hold seeds in a basket hanging from load cells mounted on top of the soak chamber lid. The steps for measuring weight are as follows:

a. The weight of empty basket without seeds is recorded initially (W_{i1}, W_{i2}, W_{i3}).
b. Once the seeds are loaded, weight is recorded again (W_{b1}, W_{b2}, W_{b3}).

c. Individual seed weights in each chamber is measured by difference of empty basket weight and weight of basket with seeds ($W_1=W_{b1}-W_{i1}$).

Data recording: Time, volume, weight and bulk density are recorded in a text file format. Hydration rate, time required to reach equilibrium volume and weight, along with fitting standard models can be performed during post-processing analyses of the data.

Evaluation and scope of seed hydration analyzing system

The system developed can obtain data with very little set up time and monitoring time. Operation of the system requires minimum training and guidance. With a set of initial parameters (e.g., length of hydration, time intervals to record data, and temperature) the system can work independently to provide hydration rates of seeds. As an analytical apparatus, the system is versatile and user friendly because: 1) requires minimum labor and once sample is loaded, data can be obtained in form of hydration curves or raw data file. 2) System will not destroy sample, so can be used for later studies. 3) System can be used to study hydration at different temperature of soak water along with varying water chemistry.

Uncertainty Evaluation:

The system performance is evaluated using constant volume (47.65cc, 47.12cc, 47.48cc in soak chamber one two and three respectively) and constant weight (100.06g, 100.75g, 100.35g in soak chamber one two and three respectively) of marble balls in each soak chamber. Apart from sensor resolution limitations, factors such as air bubbles, water adhering to walls of chambers, test material contributes to systematic measurement errors.

Accuracy: Accuracy of system is calculated by measuring a fixed volume and weight of marbles for 2 hours at two minute interval. For volume measurements, the average accuracy of the system over 2 hour period with three replicates is 97.87%., and for weight it is 99.08%.

System repeatability: The variability (covariance) of repeated measurements of system is calculated to be 1.6% for volume and 0.29% for weight.

Replicate Variation: Measurement variation among replicates varied from each replicates taken from three soak chambers by a maximum of 2.2% for volume and 0.2% for weight.

Scope of Seed Hydration Analyzing Device

The system can be used to obtain hydration rates of different seeds. Immediate applications include the soaking of Bean before canning, the stepping of barley before malting in the beer industry. As an analytical tool, it can used to analyze hydration and dehydration of any type of seed in industry and academic research and development. In Figure 2.7, an example data from the system with barley is presented. The data shows hydration rate of barley in form of relative volume (Fig 2.7a), relative weight (Fig 2.7b) and relative density (Fig 2.7c) in an experiment carried out for 75hours at 18^{o} C. Data is recorded every 15minutes for each chamber and is shown as replicates 1-3 in the graphs. The data can be useful for malting industry to understand malting qualities of barley in during beer manufacturing. Figure 2.8, shows application of the system using navy beans for determines effect of salt concentrations prominent for canned beans industry. The data shows hydration rate of navy beans in form of relative volume, relative weight and relative density in an experiment carried out for 3hours at 55^{o} C.

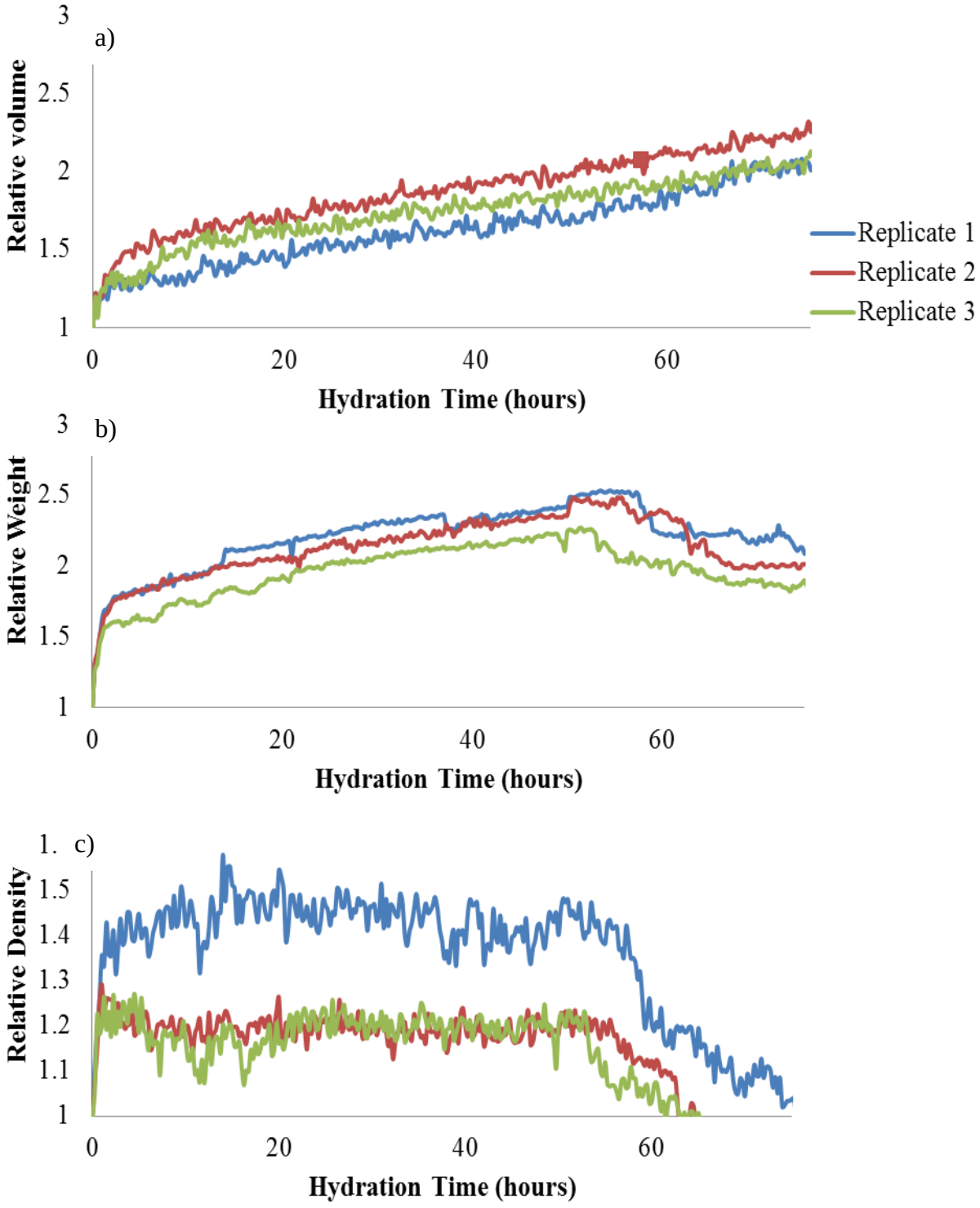

Figure 2.7. Data obtained from seed hydration analyzing device while steeping of barley for 75 hours at 18^{o}C.

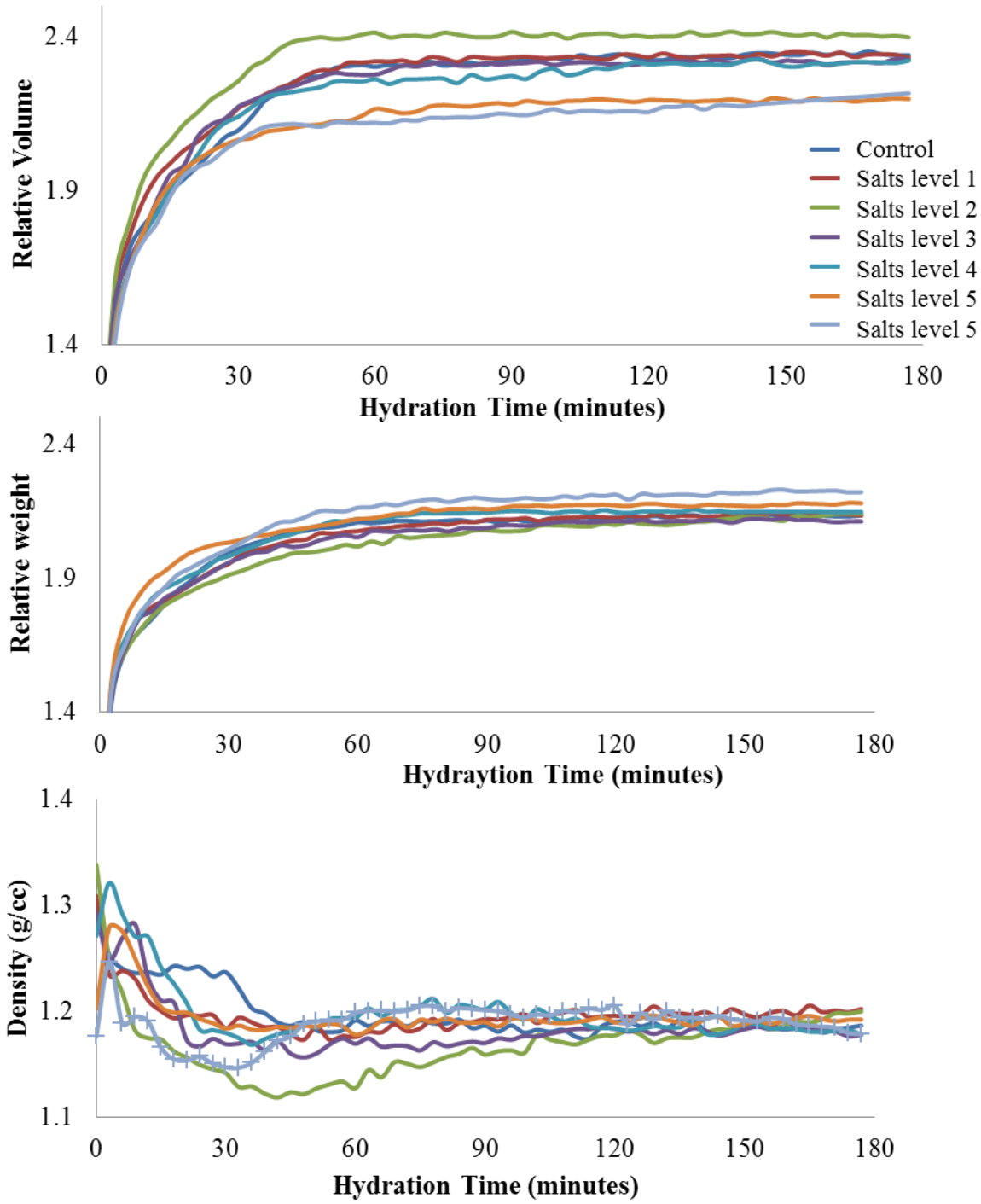

Figure 2.8. Data obtained from seed hydration analyzing device while soaking of navy beans for 3 hours at 55^{o}C to determine effect of salt concentrations.

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APPENDIX I

Standard Operating Procedure for Seed Hydration Analyzing Device

I. OBJECTIVE

This procedure establishes a uniform method to operate BVAT and obtain volume of weight data of beans during soaking. The technician is responsible for diligently applying and conforming to all instructions listed in this procedure.

II. SAFETY MEASURES (no specific safety measures)

a. Follow all GMP and safety polices in the Lab

b. Be aware of any spills and water present around the device

III. PRECAUTIONS

a. B-VAT utilizes a set of sensors, pumps and valves to take measurements and control water flow. Remember to check them all for proper functionality before running an experiment

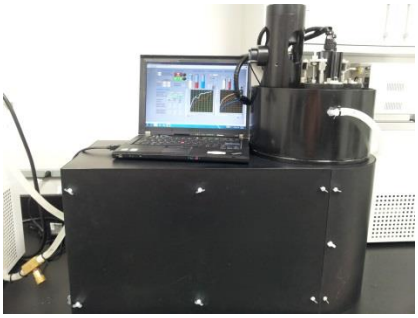
b. Make sure the basket is hanging from load cell without touching wall of soak chamber

c. For corrective actions, please refer to APPENDIX III in this document

d. For Emergency shut off, please refer to APPENDIX IV in this document

IV. MATERIALS

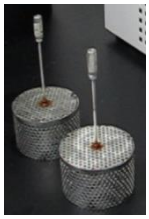
a. Bean Volume Automatic Tester (Main device)



b. One large water bath (20L) connected to soak chambers

c. One small water bath (6L) connected to water-jacket

d. Three Load cell baskets (Two pictured)



e. i. Three Plastic cups for measuring bean sample weight (left)

ii. Weighing machine to measure bean sample weight (right)

V. WATER BATHS SET UP

Large Water bath (20L) connected to soak chambers:

a. Make sure the safety valve between water bath and B-VAT is closed completely

b. Make sure there is no water present in water bath, if present drain the water and rinse it with clean water and dry it with clean cloth or paper towels

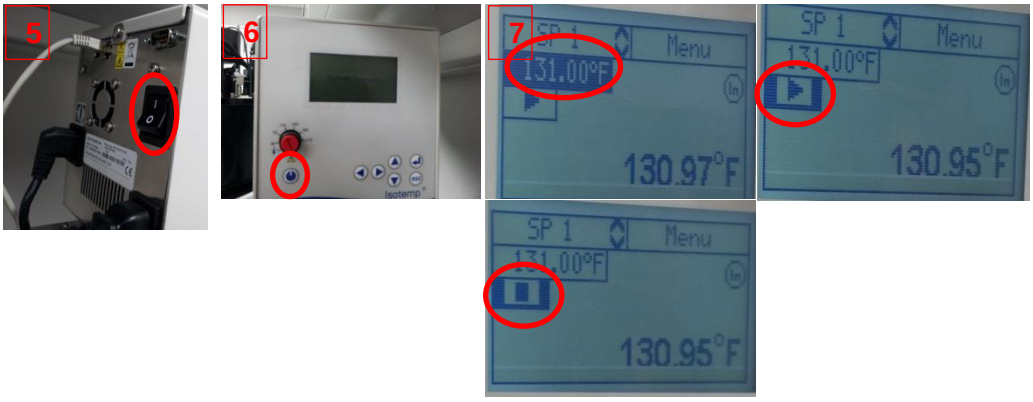
c. Collect water (soft or hard water, except for deionized water) in 5 gallon plastic bucket using water from pilot plant with temperature required for test (e.g: 131 F for regular bean soak)

d. Fill water bath with water to safe level

e. Turn on (power switch) water bath

f. Press start button on control panel

g. Set water temperature to required soak water temperature (e.g: 131 F for regular bean soak) and close the lid



Small Water bath (6L) connected to water jacket:

a. Check water-level in water bath. If water is less then safe level, fill it up to mark with water from pilot plant with temperature required for test (e.g: 131 F for regular bean soak)

b. Turn on (power switch) water bath and press start button on control panel (Similar to large water bath settings, pictured above)

c. Set water temperature to required soak water temperature (e.g: 131 F for regular bean soak) and close the lid

VI. SOFTWARE, EQUIPMENT AND SAMPLE PREPARATION AND DEVICE CHECKS

a. Turn on Laptop connected to B-VAT

b. Connect the 2 USB cables to the laptop

c. Open MAX® software and check if data cards are communicating with laptop (USB-6008, 6501 should be lit green, under >>My System >**devices and interfaces tab** on left navigation pane)

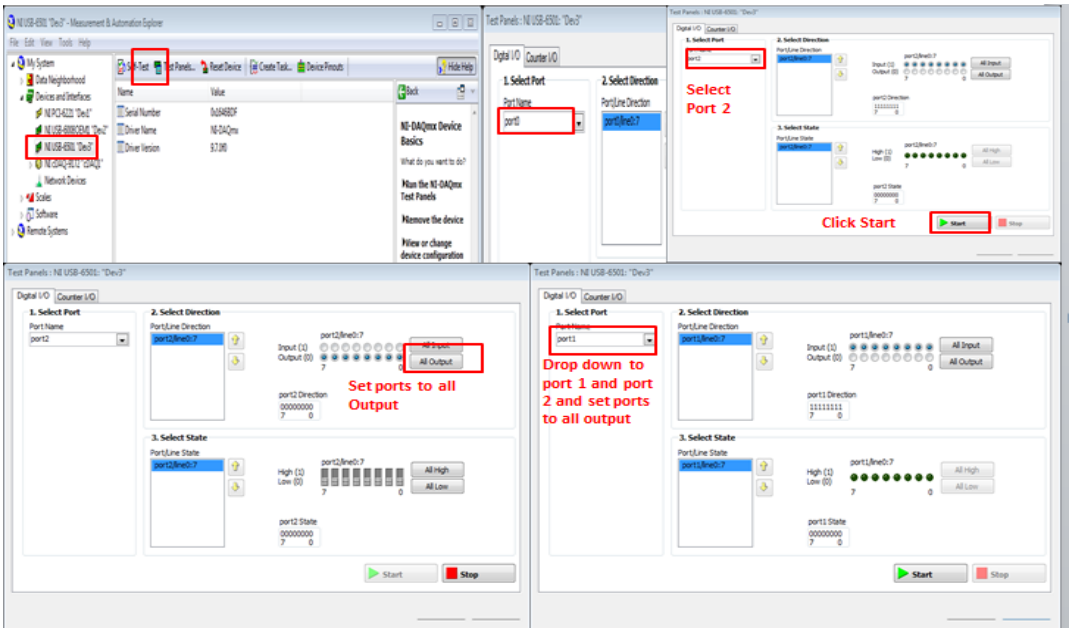
a. 

d. Test for device checks:

- **For Valves and Pumps** (Necessary before every experiment)
    i. Click on USB-6501 and click on test panels
    ii. Click Start button
    iii. Select Port 2 – and set all output

iv. Select Port 1 – and set all output

v. Select Port 0 – and set all output



- **Turn power to B-VAT ON (Switch located on Back of device**

  i. For pumps: Go to Port 1 (Fill pump= 0 ;Drain pump= 1)

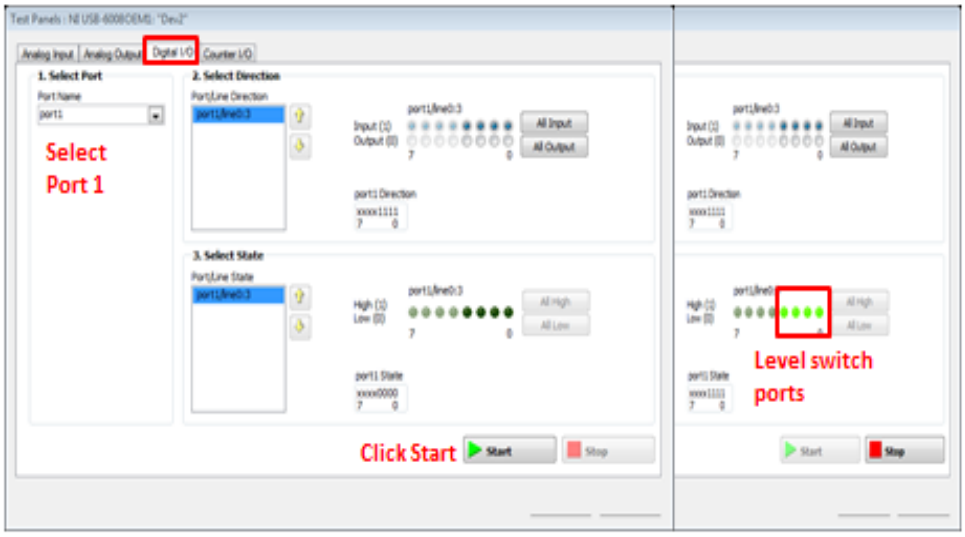
  ii. For Valves: Go to Port 2, for Chamber 1 (Fill = 0; Drain =1). For Chamber 2 (Fill = 2; Drain =3). For Chamber 3 (Fill = 4; Drain =5). For Device Fill Valve from water bath = 6

- **For Load cells, level switches and Ultrasonic sensors** (Necessary when operating after 4 weeks)

  i. Click on USB-6008 and click on test panels

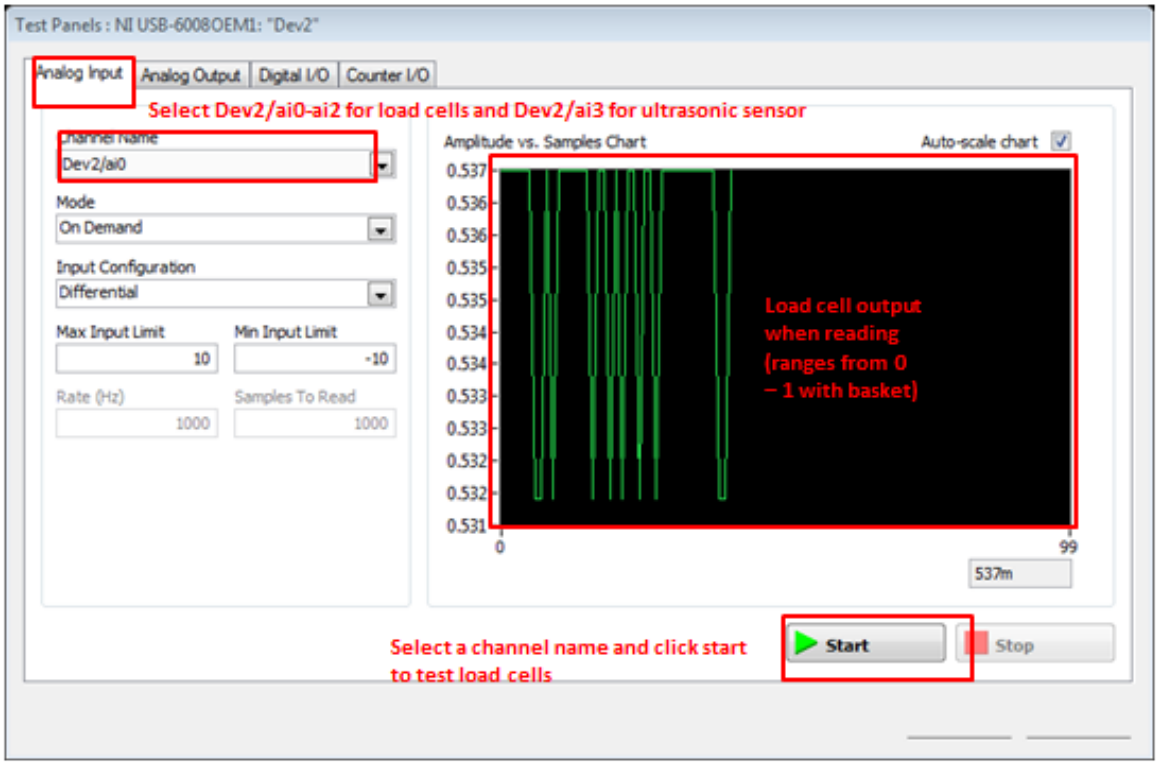
  ii. For level switches: Go to Digital I/O (port 1). Click Start. For chamber 1 switch = 0. For chamber 2 switch = 1 For chamber 3 switch = 2 (All switches are lit green, place a wet kim wipe to test if switch is working

well. With wet wipe, the switch indicator on designated port will turn dark from bright green)



i. For load cells: Goto Analog input. For chamber 1 load cell = ai0. For chamber 2 load cell = ai1 For chamber 3 load cell = ai2 (All load cells will be reading in range of 0-0.1 V without basket hanging for proper operation)

ii. For Ultrasonic sensor: Go to Analog output = a13 (With no water in measuring chamber, reading will be constant at 10 with proper operation)



i. After device checks, close MAX software

ii. Connect the bean baskets to load cell, if not connected or check connections for tightness and make sure they are not touching walls of chambers

iii. Using atleast one pin (maximum two), align all chamber lids with chambers to minimize vibrations on load cells

iv. In the B-VAT software user-interface screen provide the test conditions for beans soaking:

   a. Total time of run (in minutes, for example default is set to: 180 to run experiment for 3 hours)

   b. Reading intervals (in seconds, for example default is set to: 180 to collect data every 3 minutes)

c. Right click on each changed value and >go to data operations>>change current value to default

- Sample preparation:
    a. Using 3 scuffle cups and weighing balance, measure required amount of bean sample to test (sample weight can be between 25g to 75g for navy beans, 25g-60g for pinto, soy beans)

VII. EXPERIMENTAL PROCEDURE

a. Select B-VAT Lab View program from Software folder and open and run it

a. Priming:

b. Press ***PRIMING*** button on Lab View ® program

c. B-VAT starts priming step to collect empty chamber volume and tare bean basket weight readings

d. Once first cycle is done, check the volume and weight readings for consistency (volume range of 475-495 ml, tare weight of 85g-95g).

e. If all three chambers vary by more than 10ml in volume and 5g by weight repeat priming step by pressing ***PRIMING*** button until consistence readings are obtained/

a. Bean measurements:

f. Once consistence readings are obtained from priming step, remove baskets from load cells (shake off water hanging to basket into chambers)

g. Put pre-weighed beans in scuffle cups into them carefully, and close baskets

h. Reconnect the loaded baskets to load cells

i. Press ***BEANS LOADED*** button,

i. The program will ask for location to save the data: select the location and file name in this format: (BEANNAME_TIMEOFSOAK_TEMPERATUREOFSOAK_WEIGHTOFEACHSAMPLE_TESTDATE)

ii. Once file name is given – the green light adjacent to beans loaded button will turn on and BVAT will starts the soaking cycle

iii. During soaking cycle, BVAT will continuously measure volume and weight of beans at requested interval

iv. **Device can be left running without supervision but while BVAT is running, occasional checks on leaks, basket connections to load cells is recommended for better data collection

VIII. SHUTTING DOWN AND CLEANING

- **SHUTTING DOWN:**

The device is in idle condition once the experiment is finished and data is saved in designated file

a. Power off the water baths

b. Turn off the power to the device

c. Open MAX® software and check if data cards are communicating with laptop (USB-6008, 6501 should be lit green)

d. Initialize all valves and pumps:

    a. Click on USB-6501 and click on test device

    b. Click Run

    c. Select Port 2 – and set all output

d. Select Port 1 – and set all output

e. Select Port 0 – and set all output

f. Turn power to B-VAT ON

e. Power up the device

f. Drain the water from soak chambers using Lab VIEW drain programs (Separate programs for each chamber)

a. Drain Chamber 1: Run the program, and click on empty soak chamber and fill buttons together to drain water from measuring chamber and first soak chamber.

b. Drain Chamber 2: Run the program, and click on empty soak chamber to drain water from second soak chamber

c. Drain Chamber 3: Run the program, and click on empty soak chamber to drain water from third soak chamber

g. Carefully remove bean baskets from load cells and empty soaked beans (Can be used for moisture analysis)

h. Clean the level switches on each chamber lids

- **CLEANING:**

a. Empty water from big water bath (20L)

b. Fill water bath with water to safe level with clean soft water at room temperature

c. Turn on (power switch) water bath and press start button on control panel

d. Do not set water temperature (can leave to last setting)

e. Press Start button

- **Flushing BVAT with clean water:**
    a. Open BVAT Lab VIEW Program
    b. Press ***PRIMING*** button on Lab View program
    c. B-VAT starts priming step to collect empty chamber volume and tare bean basket weight readings and flushes the system with clean water
    d. Close the BVAT Program
    e. Drain the water from measuring chamber using soak chamber 1 drain programs
        a. Drain Chamber 1: Run the program, and click on empty soak chamber and fill buttons together to drain water from measuring chamber and first soak chamber.
    f. Power off the device (Turn power switch to off position)
    g. Turn off big water bath
    h. Empty flushed water from big water bath (20L)

IX. RESULTS ACQUISITION AND ANALYSIS

- BVAT saves data in text format (.txt) at specified file location, it can transferred to excel format using Microsoft excel
    - Open Microsoft excel program
    - Open text file using excel
    - Weibull distribution fit for volume and weight

X. PARTS AND SPECIFICATIONS in links

a. WATER BATHS

i. Large water bath (20 L) from Fishersci

ii. Small water bath (6L) from Fishersci

b. ULTRASONIC SENSOR from Baumer

i. Range 2-20 cm (maximum volume: 550 ml)

ii. Temperature (non-contact) not a concern)

iii. Voltage 24 V

c. DEHUMIDIFIER FAN from Digikey

i. To remove condensation from ultrasonic sensor

ii. Operates when ultrasonic sensor is not measuring – controlled by Lab VIEW program

d. LOAD CELL from Cooper instruments

i. 1lb capacity, incl. seed basket

ii. Need regular calibration and maintenance (every two months): Procedure in Appendix II

e. LEVEL SWITCH from Honeywell

i. Maximum rated operating temperature 125^{o} C (257^{o} F)

ii. Need regular cleaning

iii. Appendix III for troubleshooting

f. SOLENOID VALVES from Jefferson Valves

i. Maximum rated operating temperature 100^{o} C (212 ^{o} F)

ii. Need regular cleaning (every six months): Procedure in Section XII

g. DRAIN PUMP from Grainger

i. Maximum rated operating temperature 125^{o} C (257 ^{o} F)

ii. Self cleaning

iii. Section XII for troubleshooting

h. FILL PUMP from Amazon

i. Maximum rated operating temperature 80^{o} C (177 ^{o} F)

ii. Can operate higher temperature (due to meager operation)

iii. Section XII for troubleshooting

i. DATA COMMUNICATIONS from National Instruments

i. No maintenance required. Contact NI customer care for troubleshooting

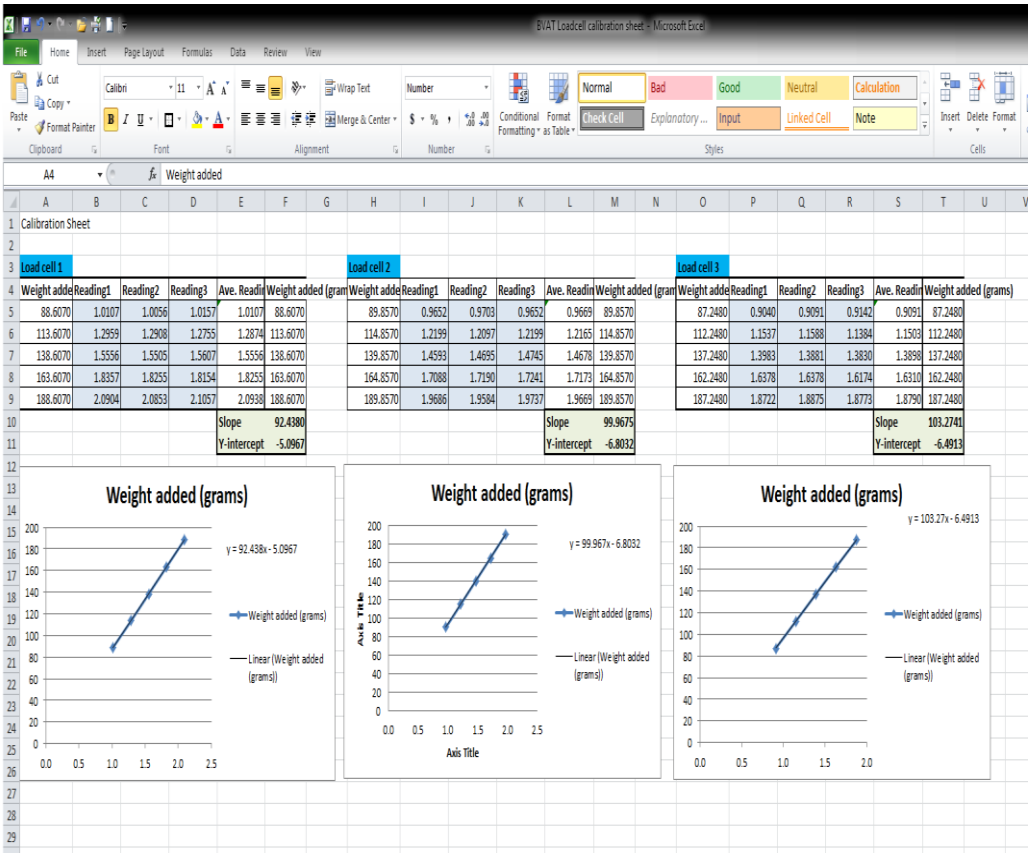
ii. NI USB 6501 for valves and pumps

iii. NI USB 6008 for sensor and load cells

XI. BVAT LOAD CELL CALIBRATION

- Materials Required for calibration:
    - Standard weights: 5g, 10g, 20g, 20g, 50g

- Turn on Laptop connected to B-VAT
- Open BVAT Calibration excel spreadsheet in ‘BVAT folder’ present in RD&I Group folder in Shared Network Drive



- Connect the 2 USB cables to the laptop
- Open MAX® software and check if data cards are communicating with laptop (USB-6008, 6501 should be lit green, under >>My System >*devices and interfaces tab on left navigation pane)*
- Click on USB-6501 and click on test panels
- Click Start button
- Select Port 2 – and set all output
- Select Port 1 – and set all output
- Select Port 0 – and set all output
- Turn power to B-VAT ON (Switch located on Back of device
- Selecting Load cell tasks to calibrate

- In MAX® software select NI Tasks under My System (MySystem >Data Neighborhood >> NI-DAQmx Tasks)

Recording calibration data in spreadsheet steps:

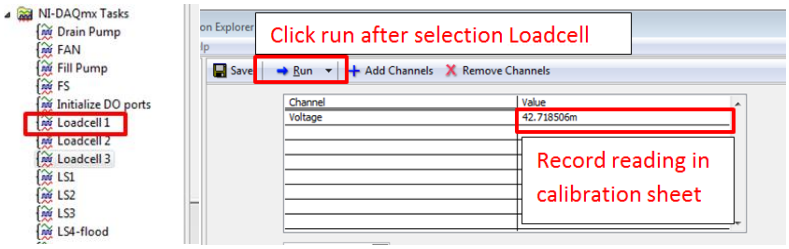
<u>Chamber 1, load cell calibration</u>

Empty basket reading

j. Connect specific basket to respective chamber load cell securely

k. For first chamber: click on load cell in NI DAXmx Tasks and click Run

l. Take two more by pressing run twice



B**asket +25g**

- Disconnect the basket from load cell, Add 25g (20g+5g standard weights)
- Reconnect the basket to respective chamber load cell securely
- Go back NI DAXmx Tasks and click Run to collect reading 2 for basket +25g reading
- Take two more by pressing run twice

Basket +50g

- Disconnect the basket from load cell, Add 50g (20g+20g+ 10g standard weights)
- Reconnect the basket to respective chamber load cell securely
- Go back NI DAXmx Tasks and click Run to collect reading 3 for basket +50g reading

- Take two more by pressing run twice

Basket +75g

- Disconnect the basket from load cell, Add 75g (50g+20g+5g standard weights)
- Reconnect the basket to respective chamber load cell securely
- Go back NI DAXmx Tasks and click Run to collect reading 2 for basket +75g reading
- Take two more by pressing run twice

Basket +100g

- Disconnect the basket from load cell, Add 25g (20g+5g standard weights)
- Reconnect the basket to respective chamber load cell securely
- Go back NI DAXmx Tasks and click Run to collect reading 2 for basket +25g reading
- Take two more by pressing run twice

Chamber 2& 3 , load cell calibration: Repeat the same steps as for chamber 1

Inputting calibrated load cell data to Lab VIEW program**:**

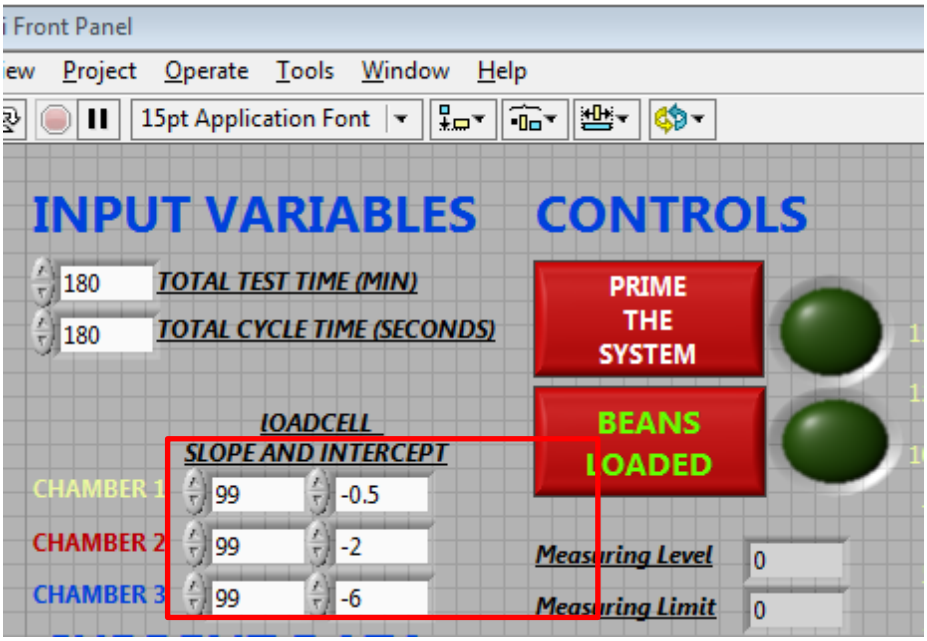
- Note the load cell calibration (slope and intercept) from excel spreadsheet. The values are calculated with pre-fixed formulas, and change with new data for each calibration.

Calibration Sheet

Load cell 1

Weight added	Reading1	Reading2	Reading3	Ave. Reading	Weight added (grams)
88.6070	1.0107	1.0056	1.0157	1.0107	88.6070
113.6070	1.2959	1.2908	1.2755	1.2874	113.6070
138.6070	1.5556	1.5505	1.5607	1.5556	138.6070
163.6070	1.8357	1.8255	1.8154	1.8255	163.6070
188.6070	2.0904	2.0853	2.1057	2.0938	188.6070
				Slope	**92.4380**
				Y-intercept	**-5.0967**

- Input the slope and intercept values of each loadcell in main BVAT Program



- Once values are added to load cell slope and intercept boxes, right click on value box >select data operations >> select make current value default
- Save the program after making all values default

XII. EQUIPMENT CHECKS AND CORRECTIVE ACTIONS:

The following checks are made while closing and powering up the device: Using MAX, check the functionality of all pump, valves, load cell and sensors as indicated in SOFTWARE, EQUIPMENT AND SAMPLE PREPARATION AND DEVICE CHECKS section.

a. Valves and Pumps:
    i. Mal-functioning: replace immediately
    ii. Not-working: Check fuse and replace blown fuses
    iii. Not-working with correct fuse: Replace part
b. Sensors:
    i. Mal-functioning: check them separately, at room temperature
    ii. Not-working: Check for power source in electronics box
    iii. Not-working with power: Replace part
c. Load cell:
    i. Not-measuring accurately: Check power source and calibrate with standard weights and change slope and intercept for load cell calculations accordingly in the program
    ii. Not-working with power: Replace part
d. Lab View ®:
    i. Lab View ® not communicating with device:
        1. Check USB connections
        2. Check if the DAQ cards are powered up (green light blinking)
        3. If reconnecting USB doesn't correct, contact National Instruments

The following checks are made before and after the device are used:

1. Check for leaks in valves and pumps enclosure
2. Check for leaks in water jacket

XIII. EMERGENCY SHUT OFF PROCEDURE

In case of sensor, valve or pump mal-function or a leak in water jacket develops while device is running; the following shut down procedure is recommended:

1. Power off the water baths
2. Power off the device
3. Equipment check should be carried out and corrected as indicated in Appendix III
4. If it's a hardware failure, follow the corrective actions in Appendix III

XIV. REFERENCES

Lab VIEW® and National Instruments contact:

- Website: National Instruments for support
- Knoxville Representative: Fred Milling and Brian Burress

CHAPTER III

Studies on Impact of Seed Varieties and Soak Water Salt (Na^{2+} and Ca^{2+} ions) Concentration on Hydration of Common Beans (*Phaseolus Vulgaris)* using Seed Hydration Analyzing Device

ABSTRACT

Common dry beans are most consumed legumes in the world, and highly recommended in healthy diet for their nutrient content and low cost. Dry beans are favored ingredients for many native dishes in United States with many popular products in commercial market. Soaking is a primary step in preparation of both commercial and house-hold dishes using common beans. In industry, beans are soaked traditionally for 3-4 hours at temperature reaching up to 55^{o} C. Knowledge of different extrinsic and intrinsic parameters on beans during soaking is essential for better quality product. Different identity preserved navy, black and pinto bean cultivars on water uptake and weight retention during soaking were studied along with impact of salt (Na^{+} and Ca^{2+} ions) concentration on hydration of regular navy beans and stored navy beans are studies. Beans are soaked in a seed hydration analyzing device which simultaneously records volume and weight readings during soaking. Weibull distribution fit is applied to data and its parameters are utilized to understand variations among different cultivars and impact of salt concentrations on navy beans hydration. Results from statistical analysis indicated little or no significant difference among regional cultivars, while significant differences were found among international navy bean cultivars. Also Na^{+} and Ca^{2+}ions concentrations in soak water showed significant influence on hydration profiles of regular and stored navy beans.

Introduction

Common beans (*Phaseolus vulgaris*- referred to as beans in this chapter*)* are popular in diets of many parts of the world. They are more popular in under-developed and developing nations due to their low-cost and nutrient rich quality (Carlos Popelka, Terryn, & Higgins, 2004). In recent years, beans have found high recommendation in United States for their high nutritional quality and low calorie content. The unpopularity of beans in United States is attributed to lack of familiarity and longer preparation time (Zanovec, O'Neil, & Nicklas, 2011). In United States pinto, black and white (navy) beans are most consumed varieties. Beans are mainly available in United States in form of pre-cooked canned or dry products (Geil & Anderson, 1994; Siddiq & Uebersax, 2012). Beans are termed as nutrient dense food, which provide high protein, minerals, dietary fiber and vitamin content for relatively few calories and low in solids fats, sugars, starches and sodium content (Services, 2010). There are many health and dietary benefits that are attributed to consumption of beans due to presence of bioactive proteins and peptides, phenolic compounds with antioxidant properties and indigestible starches. Studies have shown that inclusion of beans in diet has significant impact on prevention of heart diseases and cancer cell growth due to their high saponin content (Sathe, 2012; Shi, Arunasalam, Yeung, Kakuda, Mittal, & Jiang, 2004; Siddiq & Uebersax, 2012). Although cooking reduces the nutritional quality of beans, consumption of recommended amounts of low sodium canned beans have shown positive effect in dietary quality of consumers (Mitchell, Lawrence, Hartman, & Curran, 2009; Sathe, 2012).

Beans are primarily processed for removal of their phytate content, which cannot be absorbed in human gastro-intestinal tract (Lestienne, Icard-Vernière, Mouquet, Picq, & Trèche, 2005; Oberleas, 1983). In canning industry, beans are cooked in three main steps: initially soaking is

carried out to slowly increase water content and remove phytate, while improving bio-availability of nutrients. Second step is blanching during which the beans obtain uniform texture by quickly increasing the moisture content without loss of nutrients. Finally, beans are thermally processed at high temperature to reduce harmful bacteria such as *Clostridium botulinum* to safe levels. Based on variety one or more of the above mentioned processing techniques are recommended for cooking of beans. Most of the physiological and chemical changes in beans occur during soaking with moisture content increasing from around 15% to roughly 55%, while anti-nutrients such as Na^{+}, Mg^{2+} and K^{+} phytates diffuse passively into water (Perlas & Gibson, 2002). Soaking of beans also reduces the time and energy requirements of downstream processes in industry. For this reason, soaking has been extensively studied to understand different parameters such as water temperature, soak water chemistry and length of soaking along with impact of seed variety and initial moisture content (Siddiq & Uebersax, 2012).

Traditional soaking is very time consuming and can take up to 8 to 16 hours at room temperature. Temperatures above 60^{o} C have shown reduced water holding capacity in beans with leaching out of soluble solids into soak water. Thus an optimum temperature and time ranges of temperature above 50^{o} C for short duration are adopted in production of processed beans for effective time utilization and minimum nutritional loses. Apart from temperature and hydration time, many extrinsic and intrinsic factors of seeds and soak water are known to impact hydration rates of beans (Resio, Aguerre, & Suárez, 2003; Shan Xu, 2010; S. Xu, 2010). Characteristics such as variety, initial moisture content and age of beans cause irregularities in hydration rates while soaking. Also soak water parameters such as water chemistry and additives have proven to proven to impact hydration rates of beans (Haladjian, Fayad, Toufeili, Shadarevian, Sidahmed, Baydoun, et al., 2003).

Materials and Methods

The current study is research done to analyze the differences in hydration rates of different navy, black and pinto bean cultivars along with effect of Na^+ and Ca^{2+} ions on aged and regular navy beans. Differences among different navy bean cultivars grown in Unites States along with cultivars from China, Ethiopia, and Argentina are studied. Prior studies have shown the differences in hydration rates among different varieties and current work further tries to understand differences among cultivars and growing regions (Bressani, 1993). Also, different salt additives were positively employed to achieve higher hydration rates in common beans (De León, Elias, & Bressani, 1992). The current study is focused on impact of Na^+ and Ca^{2+} ions, without further additives on hydration rates. The studies are carried out using seed hydration analyzing device, which uses a load cell and an ultrasonic sensor to record volume and weight of beans respectively over time during soaking. Each soaking experiment in the device is carried out at 55^o C using a circulating water bath with soak water for 3 hours. Three replicates with 60 g samples of each bean cultivar and Na^+ and Ca^{2+} ion concentration are carried out to obtain an average hydration rate curve for each experiment. The device is connected to a computer installed with LabView® software in which measurements are recorded at 3 minutes intervals for each of the replicates and are stored in a text file for analysis.

Studies on regional and international cultivars:

Materials: Seven cultivars of navy beans, three black bean cultivars and three pinto bean cultivars are used to analyze differences among cultivars. Six international navy bean cultivars namely: Ethiopia, Argentina 1, Argentina 2, China 1, China 2, and control cultivar from United States are used to analyze differences among international cultivars. All the cultivars are identity

preserved and are obtained from Archer Daniels Midland Company (Decatur, IL). All the samples are stored using hermetically sealed containers at a dry place in ambient conditions. The test is carried out with regular tap water and same source is used for all cultivars. Initial moisture content of beans is recorded prior to soaking.

Effect of Na^+ and Ca^{2+} ions concentrations:

Materials: Navy beans with mix of cultivars across United States are acquired from Bush Brothers and Company, Knoxville for testing effect of Na^+ and Ca^{2+} ion concentrations soaking. Sodium and calcium ions are added in form of sodium chloride and Calcium chloride salts. Literature indicates a strong correlation between Na^+ ion concentration in soak water and volume of beans, while Ca^{2+} concentration improved weight retention (Siddiq & Uebersax, 2012). Processed soft water and hard water are used for all the experiments with constant salt ion concentrations. Initial experiments are conducted with addition of separate sodium chloride and calcium chloride concentrations to soft water with low Na^+ and medium Ca^{2+} ion concentrations. The final range of concentrations are five levels ranging from very low to very high for Na^+ ion concentrations and two levels ranging from low to high for , Ca^{2+}. The salt concentrations are compared with soft water control. Experimental data is processed for final weight and volume of beans to understand the optimum Na^+ and Ca^{2+} ion concentrations required to obtain maximum weight and volume. The optimum salt concentrations are later employed to stored beans which have low initial moisture content to analyze the impact of Na^+ and Ca^{2+} ion concentrations (Matella, Mishra, & Dolan, 2012). A set of experiments are conducted with both NaCl and $CaCl_2$ are added together to understand combined effect. Stored navy beans are used to see the impact these salts on low initial moisture content navy beans compared to regular navy beans.

Hard water with higher calcium content is also used to achieve the optimum salt concentrations by mixing with soft water with lower calcium content and studied using stored navy beans with low Na^+ and high Ca^{2+} final concentrations.

Data Analysis:

The experimental data from seed hydration analyzing device is used to calculate relative volume and is fitted with Weibull distribution model (Cunningham, McMinn, Magee, & Richardson, 2007).

$$V_t = V_{eq} + (1 - V_{eq})e^{\left(-\left[\frac{t}{\alpha}\right]\right)^{\beta}}$$ – Weibull distribution equation for volume of beans.

V_t = Volume of beans at time, t;

V_{eq} = equilibrium volume of beans at end of soaking.

A (scale factor), β (shape factor) are Weibull parameters.

Weibull parameters such as α (scale factor), β (shape factor) and equilibrium volume (average of last 5 data points of soaking) are determined from the model fit for each replicate. Also time required to reach 95% of equilibrium volume is determined from hydration profiles of all experiments. PROC ANOVA and PROC MIXED methods of SAS (SAS Institute Inc., Cary, NC; 2009) are used to perform statistical analysis on three replicates of different experiments (cultivars and salt treatments) with dependent parameters such as as α (scale factor), β (shape factor) and V_{eq} (equilibrium volume). Differences in all experiments were considered significant at $P \leq 0.05$. Tukey grouping was performed to understand similarities and differences in set of experiments for same varieties, and treatments.

Results and Discussion

Studies on regional and international cultivars:

Regional navy bean cultivars: Hydration curves of soaking of 7 different regional navy bean cultivars grown in the United States are shown in Figure 3. 1 and Figure 3.2 for relative weight and relative volume respectively. The graphs are plotted from data obtained from seed hydration analyzing device, with relative units on y-axis and hydration time in minutes along x-axis, and indicate an increase in volume in the range of 2.16 to 2.21 times and weight of 2.12-2.16 times their initial volume and weight for all regional navy bean cultivars. Table 3.1 shows the Weibull parameters such as equilibrium volume, α (scale factor) and β (shape factor) of these cultivars along with Time required for each bean cultivar to achieve 95% of its final equilibrium volume is also calculated. From the statistical analysis on hydration data, equilibrium volumes ($P < 0.0186$) and shape factors, β ($P < 0.0194$) from Weibull distribution parameters indicated no significant difference. However, significant difference was observed among navy bean cultivars in time to reach 95% equilibrium volume and scale factors, α ($P < 0.0001$). Tukey grouping is designated for all the cultivars tested in table 3.1. The analysis shows that the cultivars are not significantly different in their ability to reach maximum hydration, except for the time taken to reach 95% of the equilibrium volume.

Regional black bean cultivars: Hydration curves of soaking of 3 different regional black bean cultivars grown in the United States are shown in Figure 3.3 and Figure 3.4 for relative weight and relative volume respectively. The data indicates an increase in volume in the range of 2.43 to 2.55 times and weight of 2.32-2.49 times their initial volume and weight for all regional black bean cultivars. Table 2 shows the Weibull parameters along with time required for each black

bean cultivar to achieve 95% of its final equilibrium volume is also calculated. From the statistical analysis on hydration data, equilibrium volumes ($P < 0.01713$) from Weibull distribution parameters indicated no significant difference. However, significant differences were observed among black bean cultivars in time to reach 95% equilibrium volume and scale factors, α and shape factors, β ($P < 0.0001$). Tukey grouping is designated for all the cultivars tested in table 2. The analysis shows that the cultivars are not significantly different in their ability to reach maximum hydration, except for the time taken to reach 95% of the equilibrium volume and rate of hydration as indicated through and shape and shape factors.

Table 3.1. Weibull parameters from hydration profiles of various navy bean cultivars (Cultivars with same letter subscript are similar. [1,2] parameters with significant difference)

Cultivars	Equilibrium Volume (Veq) (P < 0.0186)	Time to reach 95% Veq[1] (P < 0.0001)	Alpha[2] (P < 0.0001)	Beta (P < 0.0194)
Navy 1[c, d]	1.55±0.016	47±1.732	23.65±0.263	0.99±0.026
Navy 2[d]	1.62±0.061	41±1.732	19.46±0.433	1.03±0.145
Navy 3[a, b]	1.66±0.018	54±3.000	25.17±1.771	0.90±0.069
Navy 4[b, c]	1.70±0.012	49±1.732	21.01±0.734	0.77±0.039
Navy 5[c, d]	1.60±0.016	46±1.732	20.57±0.965	0.90±0.053
Navy 6[a]	1.69±0.007	60±3.000	30.48±1.279	1.00±0.012
Navy 7[b, c]	1.55±0.010	51±3.000	21.52±1.396	0.83±0.010

Table 3.2. Weibull parameters from hydration profiles of various black bean (Cultivars with same letter subscript are similar. [1,2] parameters with significant difference).

Cultivars	Equilibrium Volume (Veq) (P < 0.1713)	Time to reach 95% Veq [1] (P < 0.0001)	Alpha [2] (P < 0.0001)	Beta [3] (P < 0.0001)
Black -1[b]	1.96±0.015	70±1.732	28.71±0.490	0.90±0.012
Black -2[a]	1.87±0.051	105±3.000	50.29±0.359	1.11±0.053
Black -3[c]	1.91±0.025	63±6.000	21.48±1.319	0.75±0.025

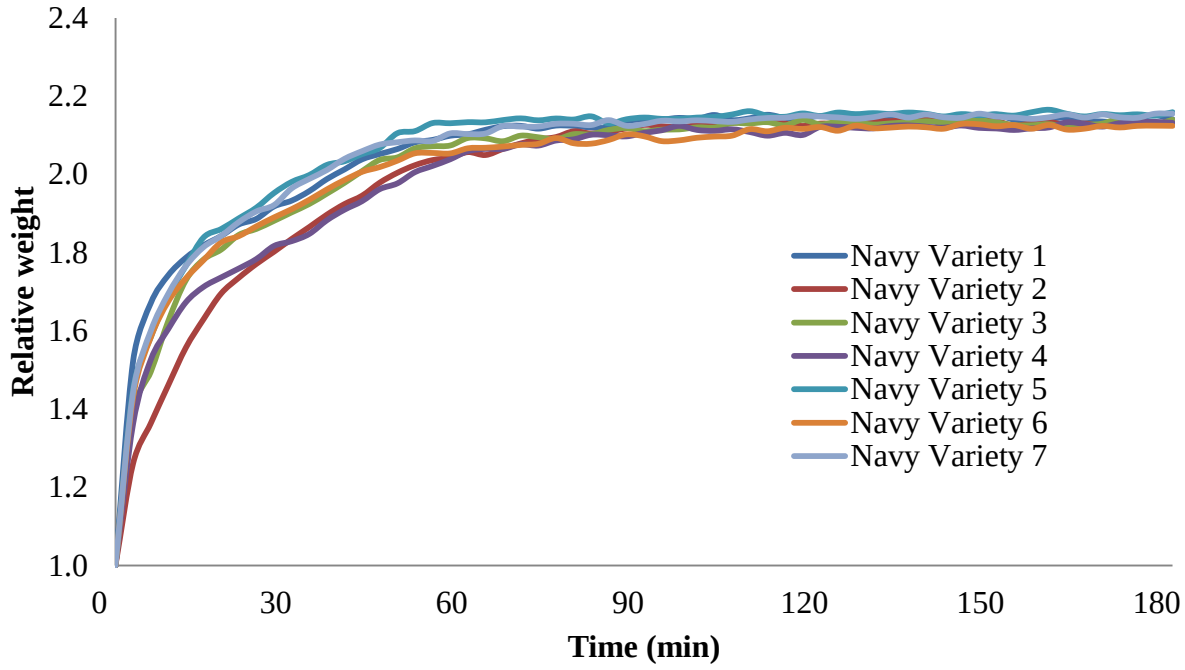


Figure 3.1. Relative weight of different cultivars of navy beans over time

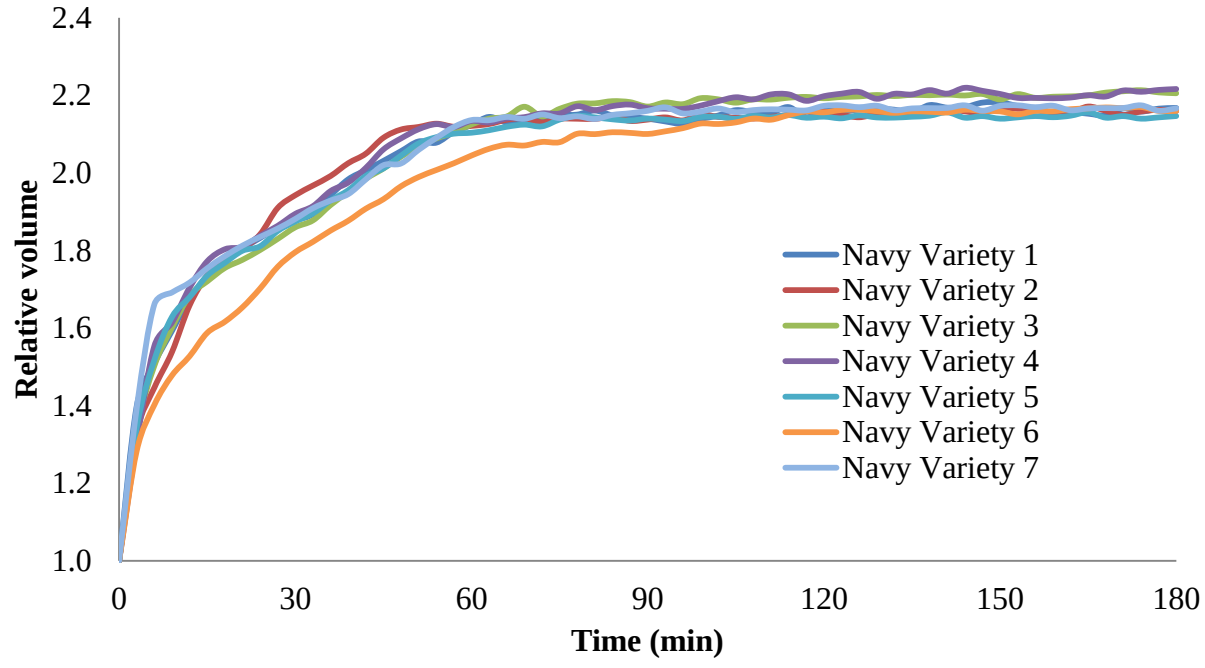


Figure 3.2. Relative volume of different cultivars of navy beans over time

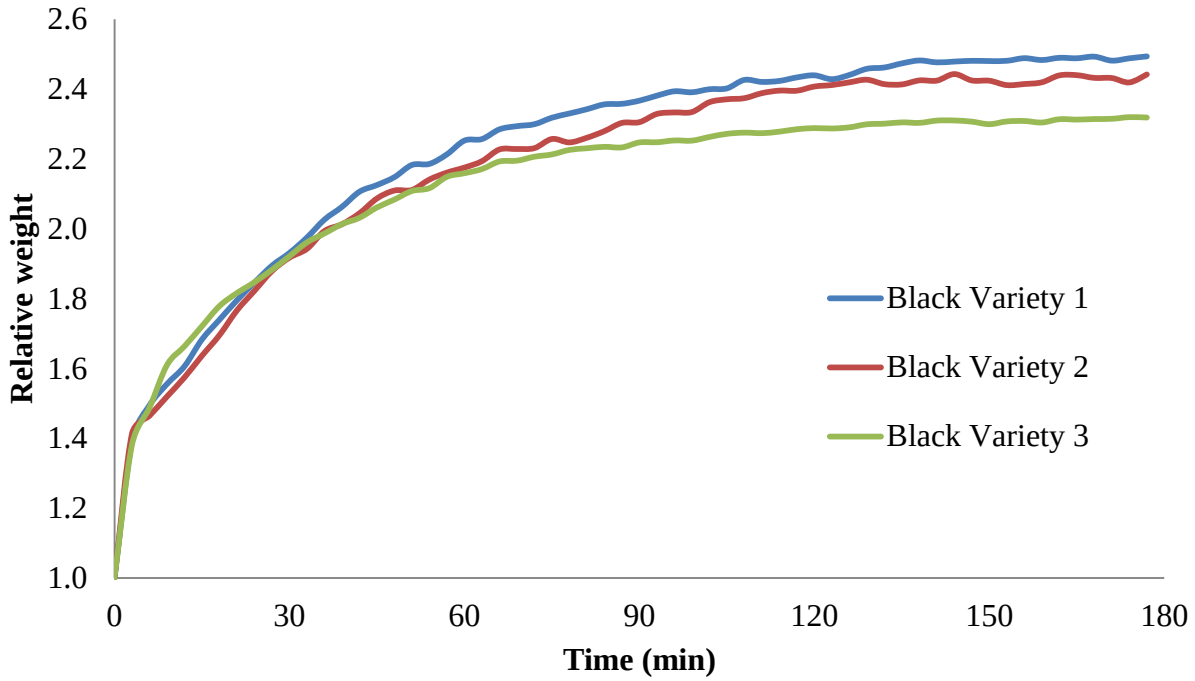


Figure 3.3. Relative weight of three different black bean cultivars

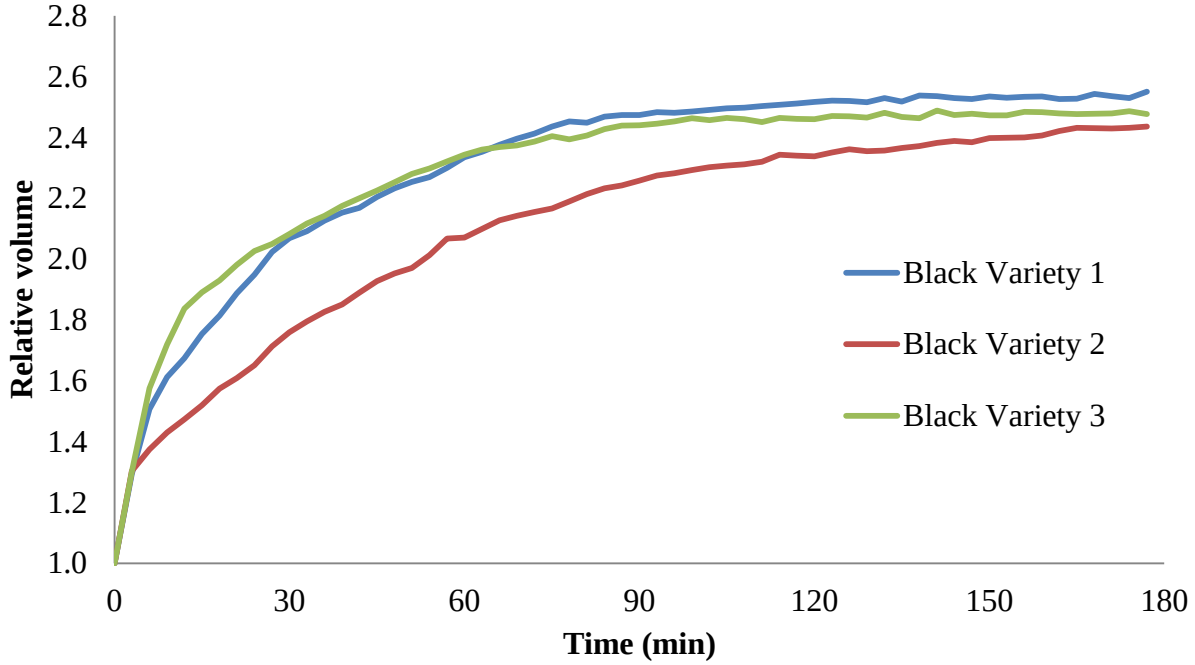


Figure 3.4. Relative volume of three different black bean cultivars

Regional pinto bean cultivars: Hydration curves of soaking of 3 different regional pinto bean cultivars grown in the United States are shown in Figure 3.5 and Figure 3.6 for relative weight and relative volume respectively. The data indicates an increase in volume in the range of 2.08 to 2.24 times and weight of 2.14-2.24 times their initial volume and weight for all regional black bean cultivars. Table 3.3 shows the Weibull parameters along with time required for each black bean cultivar to achieve 95% of its final equilibrium volume is also calculated. From the statistical analysis on hydration data, equilibrium volumes ($P < 0.0018$), time to reach 95% equilibrium volume ($P < 0.0031$) and scale factors, α ($P < 0.0031$)and shape factors, β ($P < 0.0177$) from Weibull distribution parameters indicated significant difference. Tukey grouping is designated for all the cultivars tested. The analysis shows that the cultivars are significantly different in their ability to reach maximum hydration time taken to reach 95% of the equilibrium volume and rate of hydration as indicated through and shape and shape factors.

International cultivars: The hydration profiles of soaking of 6 different international navy bean cultivars along with a regional navy bean cultivar grown in the United States as control are shown in figure 3.7 and figure 3.8 for relative weight and relative volume respectively.

Table 3.3. Weibull parameters from hydration profiles of various pinto bean cultivars produced in United States (Cultivars with same letter subscript are similar. [1,2] parameters with significant difference.)

Cultivars	Equilibrium Volume (Veq) (P < 0.0018)	Time to reach 95% Veq (P < 0.0031)	Alpha (P < 0.0031)	Beta (P < 0.0177)
Pinto -1 [c]	1.83 ±0.015	97.00 ±1.732	52.76 ±1.038	1.25 ±0.116
Pinto -2 [b]	1.97 ±0.029	101.00 ±4.582	62.78 ±2.736	1.52 ±0.0554
Pinto -3 [a]	2.05 ±0.014	117.00 ±3.000	73.81 ±2.152	1.47 ±0.169

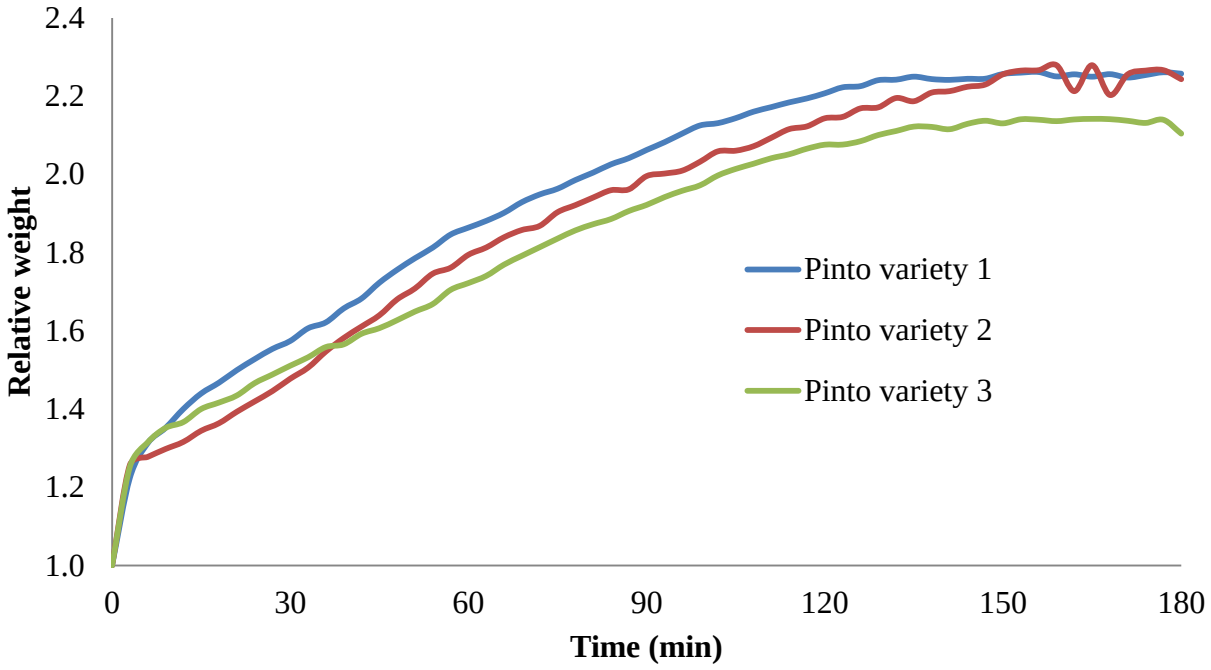


Figure 3.5. Relative weight of three different pinto bean cultivars

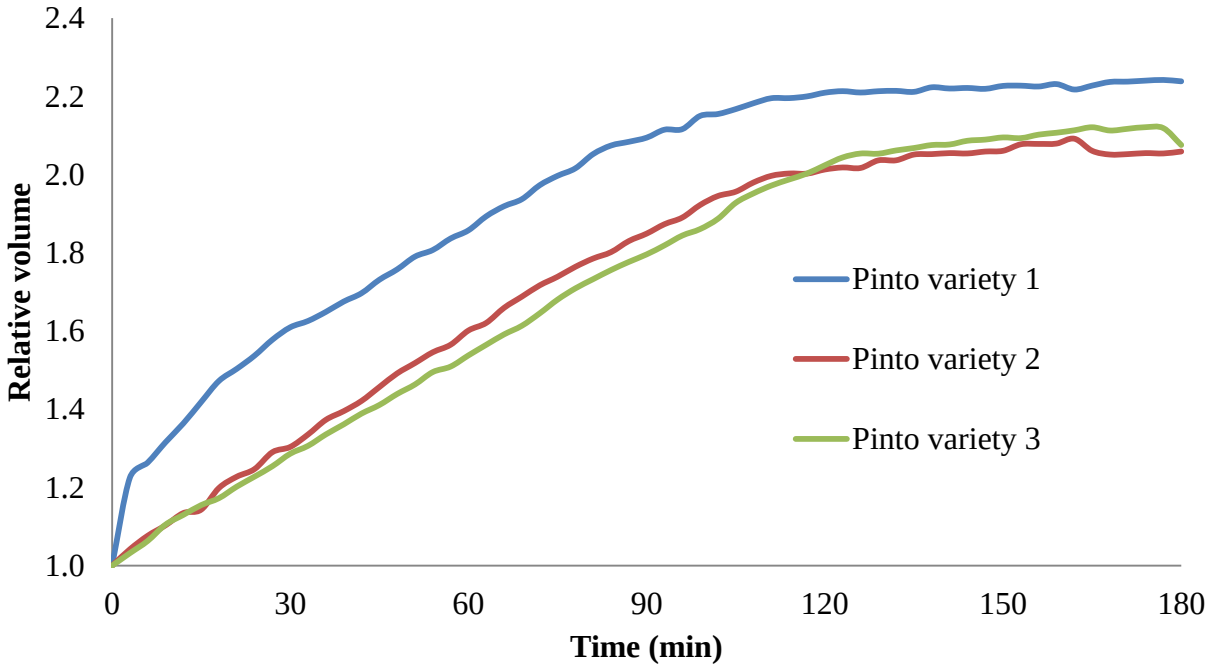


Figure 3.6. Relative volume of three different pinto bean cultivars

From the graphs, volume increase of all cultivars range from 1.77 to 2.59, while weight gain falls in the range of 2.11 to 2.55. Weibull parameters for these cultivars along with time taken to achieve 95% of equilibrium volume are presented in table 3.4. From the statistical analysis on hydration data, equilibrium volumes ($P < 0.0018$), time to reach 95% equilibrium volume ($P < 0.0031$) scale factors, α ($P < 0.0031$) and shape factors, β ($P < 0.0177$) from Weibull distribution parameters indicated significant difference among varieties. Tukey grouping is designated for all the cultivars tested in table 4. The analysis shows that the cultivars are significantly different in their ability to reach maximum hydration time taken to reach 95% of the equilibrium volume and rate of hydration as indicated through and shape and shape factors. When compared to the control navy bean cultivar grown in the United States (1.82) only Ethiopia, Argentina 1 and China 2 cultivars had closer or similar equilibrium volumes (1.78, 1.79, 1.77 respectively). Remaining cultivars which are Argentina 2 (2.02), and China1 (1.43) had higher and lower equilibrium volumes respectively then the control.

Table 3.4. Weibull parameters from hydration profiles of international navy bean cultivars (Cultivars with same letter subscript are similar. [1,2] parameters with significant difference. Control navy bean cultivar from United States is in bold).

Intl Cultivars of navy beans	Equilibrium Volume (Veq) (P < 0.0001)	Time to reach 95% Veq (P < 0.0001)	Alpha (P < 0.0001)	Beta (P < 0.0001)
Ethiopia [b]	1.78±0.034	86±6.245	30.05±1.449	0.73±0.005
Argentina-1 [b]	1.79±0.044	53±3.464	10.90±1.512	0.54±0.019
Argentina-2 [a]	2.02±0.037	104±9.165	47.86±4.349	1.07±0.021
China-1 [c]	1.43±0.031	66±3.000	31.92±1.925	0.86±0.056
China-2 [b]	1.77±0.036	57±3.000	17.51±0.084	0.67±0.009
Control [b]	**1.82±0.035**	**76±6.245**	**31.04±2.481**	**0.80±0.035**

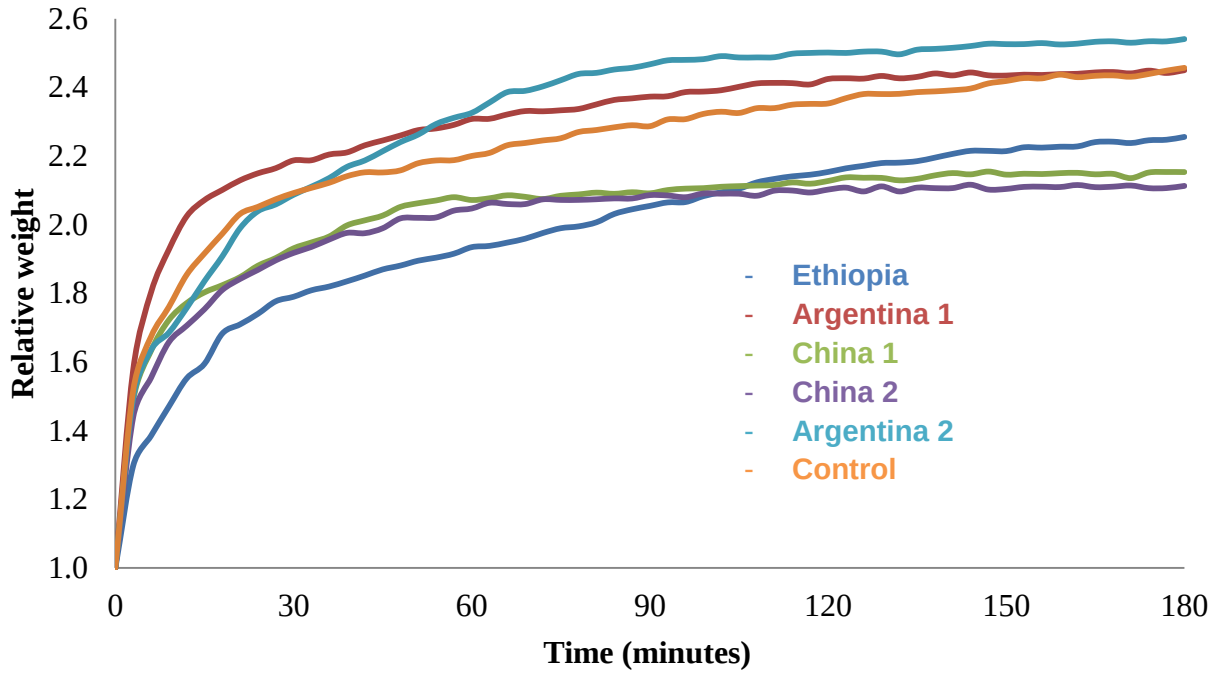


Figure 3.7. Relative weight of different cultivars of International navy beans over time

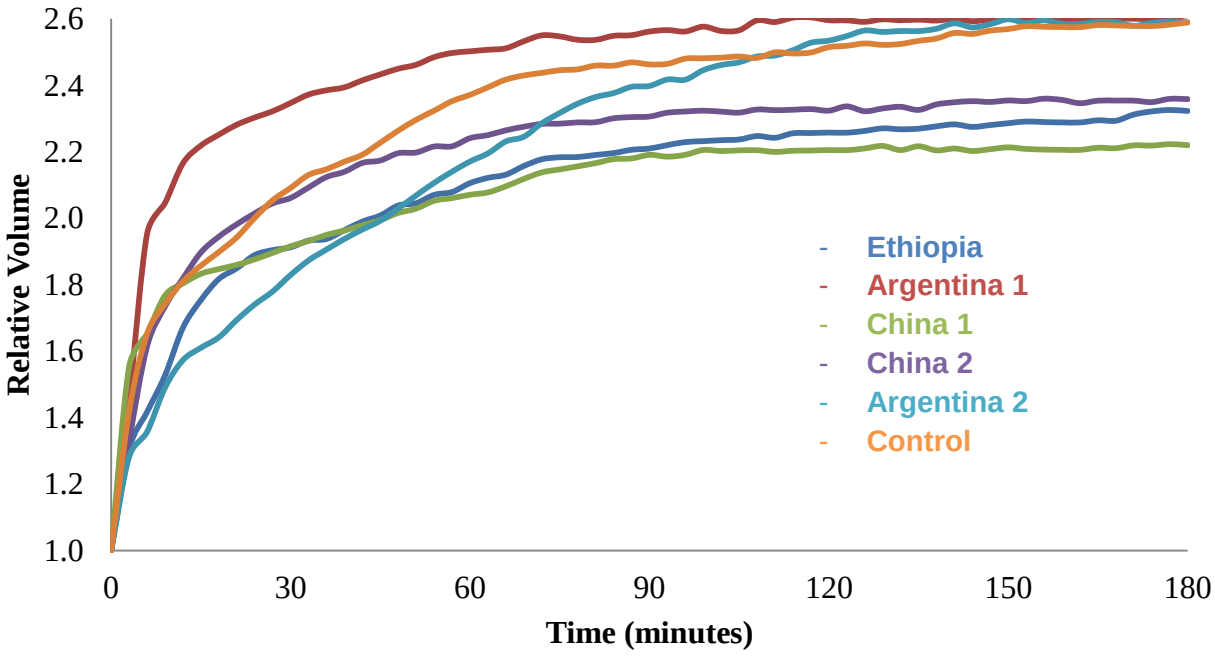


Figure 3.8. Relative volume of different cultivars of International navy beans over time

Effect of Na^+ and Ca^{2+} ions concentrations:

The data from studies on effect of Na^+ and Ca^{2+} ions concentrations in soak water during hydration of navy beans can be divided into three categories for discussion. First, is to determine the optimum Na^+ and Ca^{2+} ions concentrations in soak water that provides with maximum volume and weight gain in beans. Second, is to compare the effect of these salt treatments on stored navy beans along with ability to used soft and hard water ratio to achieve similar results.

Optimum Na^+ and Ca^{2+} ions concentrations: The hydration profiles of navy beans with different concentrations of Na^+ and Ca^{2+} ions are shown in Figure 3.9 and Figure 3.10 for relative weight and relative volume respectively. Soft water with very low Na^+ concentration and very low Ca^{2+} concentration are used as control for this study. Na^+ ions are added in form of NaCl at increments of 30 ppm to soft water providing 4 different treatments (very low - very high). Ca^{2+} ions are added in form of $CaCl_2$ at 2 different treatments (low and high). Volume increase of all treatments ranged from 2.48 to 2.58, while weight gain falls in the range of 2.00 to 2.10. Table 3.5 shows the Weibull parameters for all the treatments. Time taken to reach 95% of equilibrium volume is in the range of 60-96 minutes. From the statistical analysis on hydration data, equilibrium volumes ($P < 0.0001$), time to reach 95% equilibrium volume ($P < 0.0037$) and scale factors, α ($P < 0.0009$) from Weibull distribution parameters indicated significant difference among varieties. Tukey grouping is designated for all the salt treatments on navy beans tested in table 5. The analysis shows that the treatments are significantly different in their ability to reach maximum hydration time taken to reach 95% of the equilibrium volume and rate of hydration as indicated through shape factors. The maximum volume change (3.21%) was observed with very high Na^+ ion concentration in soak water.

Table 3.5. Weibull parameters from hydration profiles of navy beans soaked with water containing different Na^+ and Ca^{2+} ion concentrations (Cultivars with same letter subscript are similar. [1,2] parameters with significant difference. Bold treatment is control with soft water).

Salt Concentrations		Equilibrium Volume (Veq)	Time to reach 95% Veq	Alpha	Beta
Sodium ions (ppm)	Calcium ions (ppm)	(P < 0.0001)	(P < 0.0037)	(P < 0.0009)	(P < 0.0680)
N	**C [b, c, d]**	**1.74±0.079**	**69±3.000**	**25.06±1.193**	**0.73±0.029**
Very low	low [a]	1.87±0.032	94±1.732	29.99±1.138	0.68±0.018
Low	low [a, b]	1.84±0.044	94±9.165	26.89±1.634	0.65±0.059
High	low [a]	1.90±0.036	99±10.816	31.79±1.699	0.71±0.049
Very high	low [a, b, c]	1.79±0.028	82±4.583	25.08±1.771	0.70±0.042
Very low	high [d]	1.63±0.034	87±6.000	30.66±3.057	0.78±0.063
Very low	high [d, c]	1.68±0.023	66±10.817	23.99±1.695	0.78±0.040

Low and high Ca^{2+} ions showed significant increase in weight (3- 3.5%). Studies on Ca^{2+} ion concentration were limited due to prior knowledge available of harder bean texture with higher concentrations (Thanos, 1998).

Effect of salt treatments on stored and regular navy beans: Table 6 shows the combined effect of Na^+ and Ca^{2+} ions concentrations on regular navy beans and stored navy beans. Soak water concentration is adjusted to very high and high Na^+ and Ca^{2+} ions to understand combined effect of salts on navy beans and stored navy beans. Also, the table provides impact of soft and hard water mix. Figure 3.11-3.12, and Figure 3.13-3.14 show hydration profiles of regular and rail car stored (aged) navy beans with relative weight and relative volume respectively. From the statistical analysis on hydration data, equilibrium volumes ($P < 0.4898$), time to reach 95% equilibrium volume ($P < 0.1616$) scale factors, α ($P < 0.9539$) and shape factors, β ($P < 0.8600$) from Weibull distribution parameters indicated no significant difference among varieties.

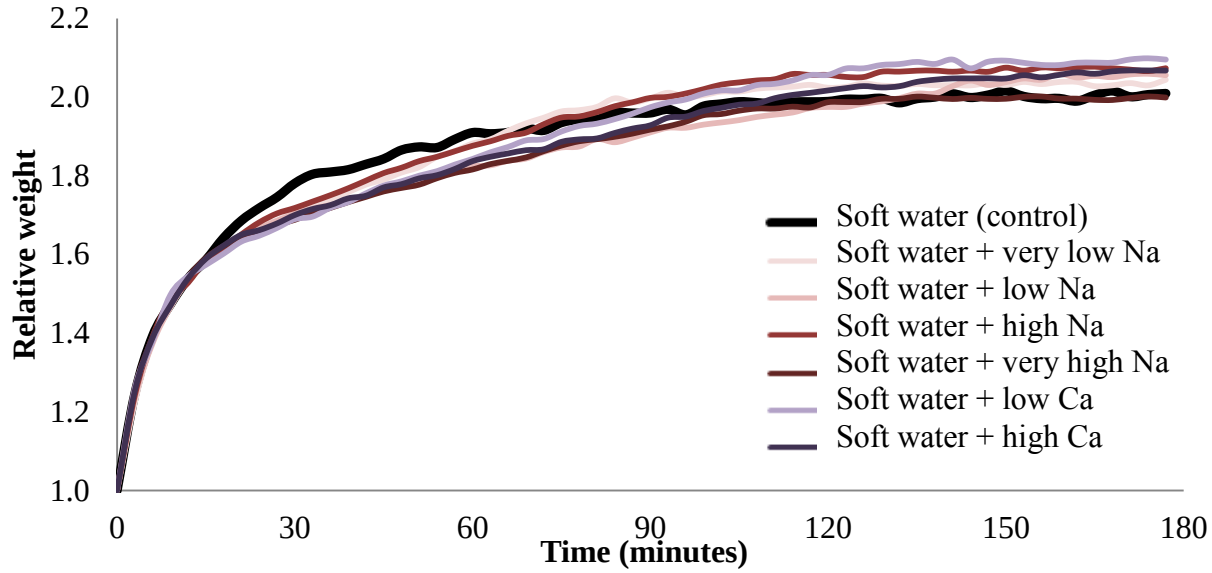


Figure 3.9. Relative weight of different Na^{2+} and Ca^{2+} ion concentrations treatments

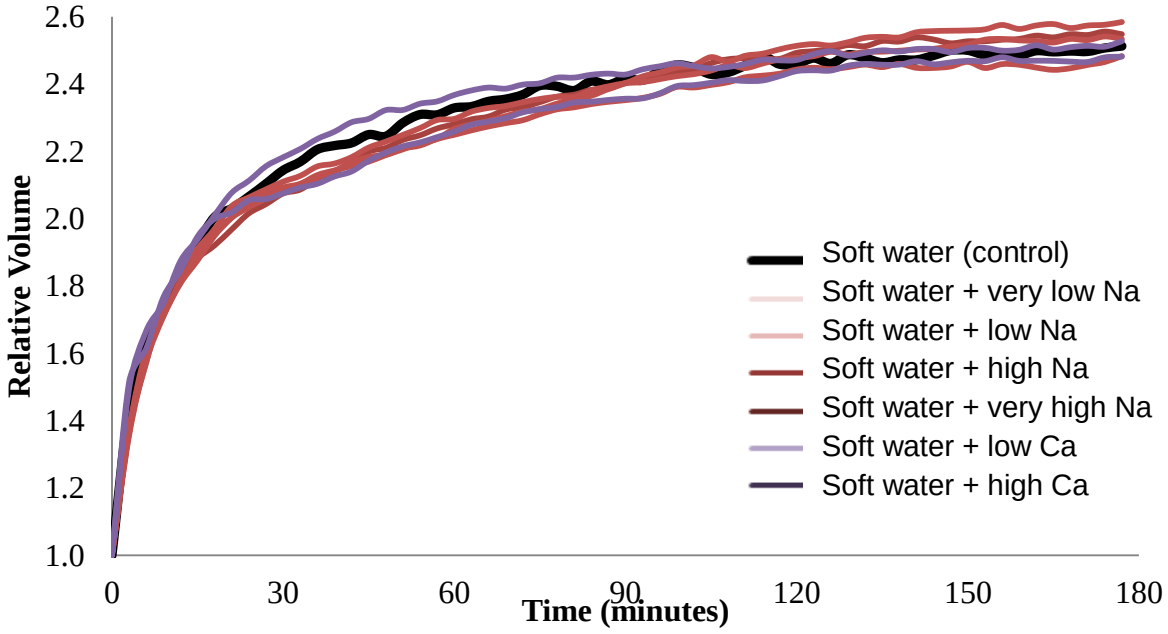


Figure 3.10. Relative volume of different Na^{+} and Ca^{2+} ion concentrations treatments

Tukey grouping is designated for all the salt treatments on navy beans tested in table 3.5. The analysis shows that the treatments are significantly different in their ability to reach maximum hydration time taken to reach 95% of the equilibrium volume and rate of hydration as indicated through shape factors for rail car stored navy beans. Although the combined effect of Na^+ and Ca^{2+} ions is very less for regular navy beans in both volume and weight, added salts improved stored beans weight and volume by 3.61% and 6.03 % respectively. Hard and soft water ratio had increase weight and volume of regular beans by 1% each, while the stored beans had no increase of volume but, a 4.52% increase in weight. From the data, the impact of Na^+ and Ca^{2+} ions is profound on stored beans as indicated by Tukey grouping for time to reach 95% of equilibrium volume.

Table 3.5. Weibull parameters from hydration profiles of navy beans soaked with water containing different Na^+ and Ca^{2+} ion concentrations added to hard water and a 6:1 soft and hard water ratio (Cultivars with same letter subscript are similar. Bold treatments are control with soft water).

Bean type	Water Type (Ion concentration in ppm)	Equilibrium Volume (Veq) (P < 0.4898)	Time to reach 95% Veq (P < 0.1616)	Alpha (P < 0.9539)	Beta (P < 0.8600)
Regular Navy	**Soft water**	**1.74±0.079**	**70±1.732**	**25.06±1.193**	**0.73±0.029**
	Soft and hard water mix	1.79±0.056	69±3.000	26.86±1.580	0.75±0.047
	Added salts to soft water	1.65±0.057	64±1.732	25.29±0.897	0.72±0.035
Stored Navy	**Soft water**	**1.88±0.075**	**100±1.732** [a]	**33.62±1.436**	**0.72±0.021**
	Soft and hard water mix	1.83±0.047	73±1.732 [b]	25.74±0.978	0.73±0.039
	Added salts to soft water	1.73±0.031	77±1.732 [b]	21.94±0.699	0.75±0.035

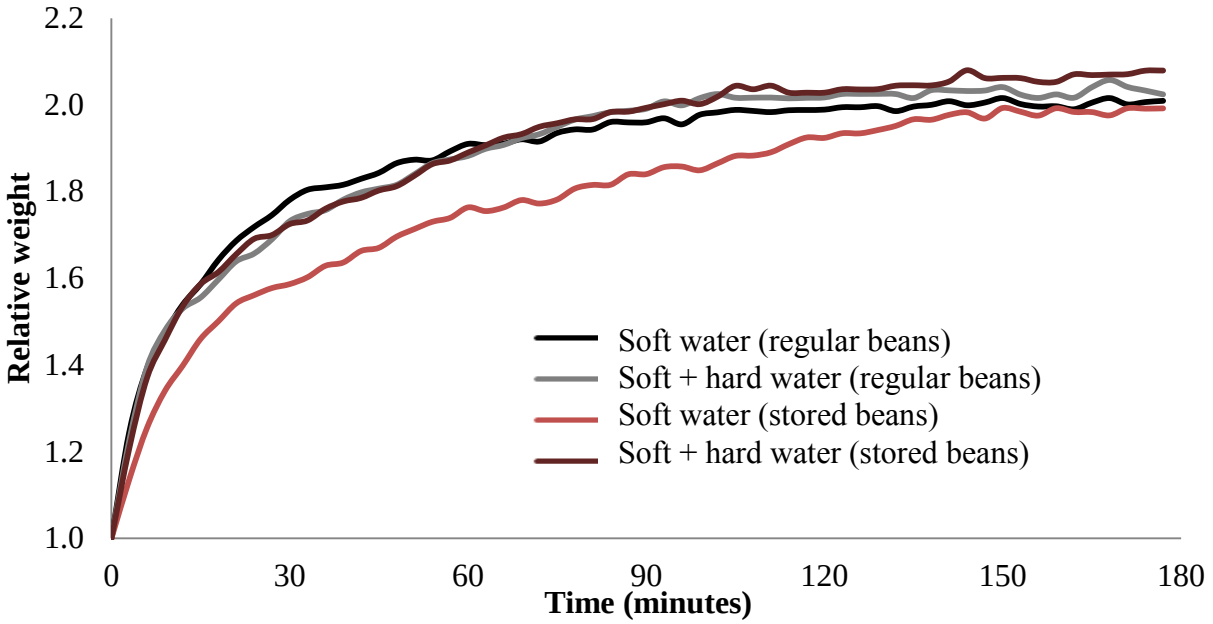


Figure 3.11. Relative weight of soft water and hard water ratio treatments compared with normal soft water regular and stored navy beans

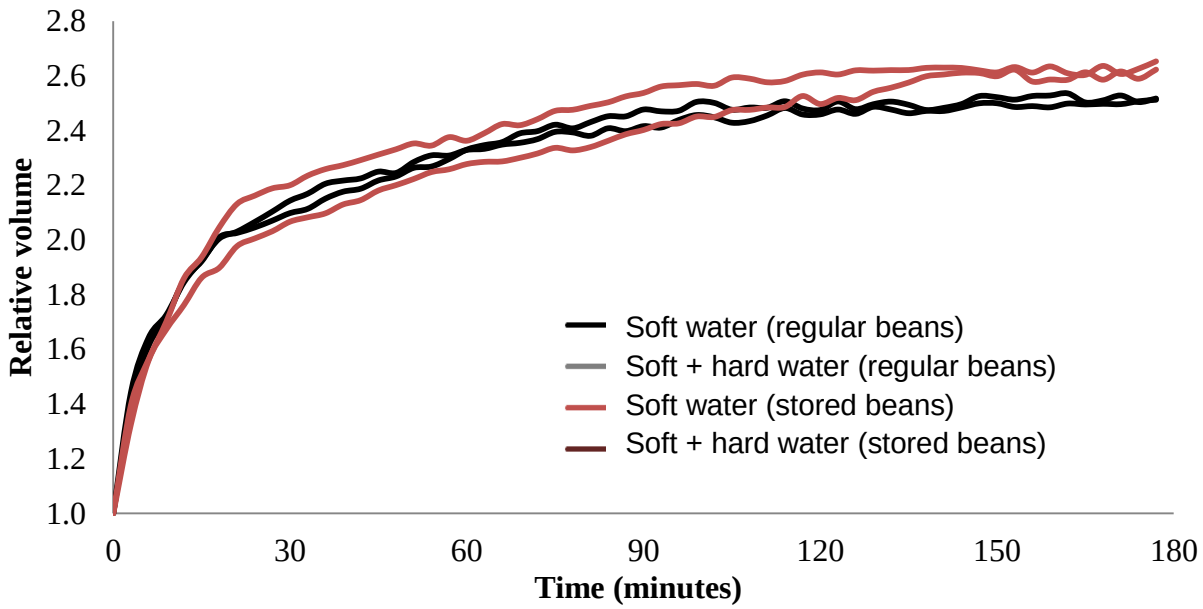


Figure 3.12. Relative volume of soft water and hard water ratio treatments compared with normal soft water regular and stored navy beans

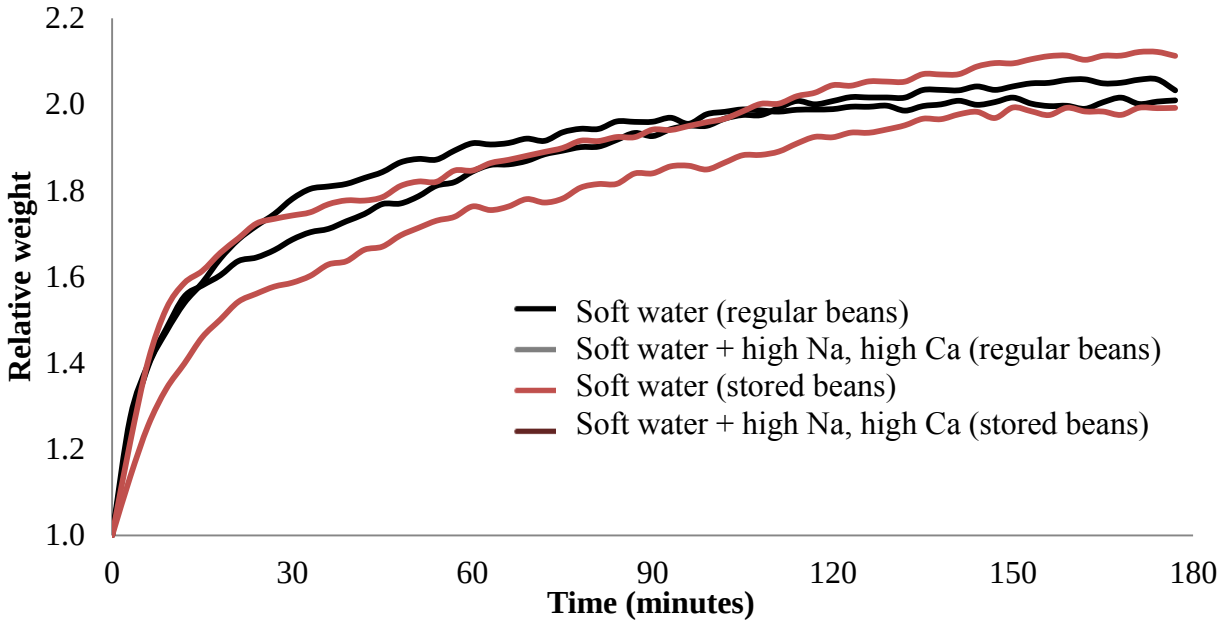


Figure 3.13. Relative weight of optimum Na^{+} and Ca^{2+} ion concentrations, compared to hard water treatments with regular and stored navy beans

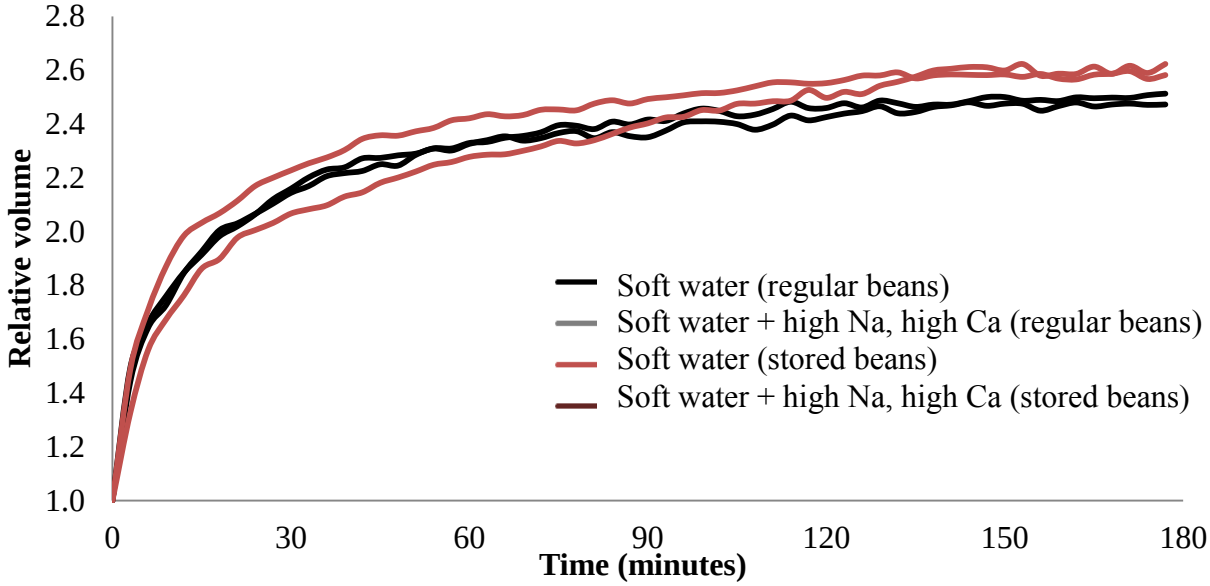


Figure 3.14. Relative volume of optimum Na^{+} and Ca^{2+} ion concentrations, compared to hard water treatments with regular and stored navy beans

Mix of soft and hard water provide weight increase for both stored and regular navy beans. Studies have shown that weight increase in beans due to calcium ions is attributed to cross-linkage of pectin in beans, due to which leakage of absorbed water is found to reduced (Balasubramanian, Slinkard, Tyler, & Vandenberg, 2000). Sodium ions impact on bean soaking is less studies for physiological changes, but proved to impact protein retention and bio-availability (Prodanov, Sierra, & Vidal-Valverde, 2004).

Conclusion

Navy bean, black bean and pinto bean cultivars grown in the United States have shown little or no difference in hydration profiles as determined by Weibull parameters and time required to reach 95% of equilibrium volume. International navy bean cultivars from Ethiopia, Argentina and China showed differences in hydration profiles when compared to a control navy bean cultivar. Effects of salt treatments on physiological characteristics of navy beans that are both stored and regular are studied with addition of NaCl, $CaCl_2$ to soft water. Initial experiments with addition of Na^+ and Ca^{2+} ions concentrations separately showed maximum volume at very high NaCl, and maximum weight for high $CaCl_2$ for regular navy beans. Combined treatments of very high NaCl and high $CaCl_2$ have improved weight and volume of stored beans, indicating possible application of these salts in improving bean yield for low initial moisture content beans. Further, studies on nutrient retention, sensory attributes have to be carried to understand comprehensive effect of these salts on bean hydration.

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CHAPTER IV

Theil Error Splitting Method for Analyzing Different Empirical Models in Hydration Studies of Cereals and Legumes

ABSTRACT

Dry beans such as navy, black and pinto are soaked in 55^{o} C of soak water temperature for 3 hours with volume measurements recorded every three minutes. Oil seeds such as black and yellow soybeans; and cereal grain barley are soaked in 24^{o} C of soak water temperature for 16 hours and 4 hours respectively with measurements recorded every 10 minutes and 5 minutes respectively. The volume versus time data generated from these soak studies were used to analyze five different empirical models (one non-exponential, and four exponential models) for prediction of measured volume data. Theil error splitting method is utilized to split the error in predicted data in to fixed and random error, and the analyses is applied to select satisfactory models for each seed soak study. From the analysis, no one model predicted the measured volume readings for all tested seeds satisfactorily. Weibull model worked better for all soak studies with certain limitations. All other models were better models in specific studies. The error splitting methods application was demonstrated in its robustness for analyzing mathematical models and is recommended when utilizing new model to understand hydration kinetics (volume, weight and moisture content) over time.

Introduction

Cereal and legumes constitute an important plant based food source. Seeds from leguminous crops (dry beans, peas and oil seeds e.g., navy, pinto, lima, chickpea, faba) and from grass crops (cereals, e.g., wheat, barely, rice, maize and sorghum) are processed to create a variety of foods and beverages for consumption (Venn & Mann, 2004; Williams, Grafenauer, & O'Shea, 2008). Hydration is an important step in preparation of cereals and legumes, which eliminates anti-nutrients and enables faster downstream processing (Sandberg, 2002). Optimization and process improvement during soaking has been extensively studied with experimental and modeling techniques to evaluate hydration kinetics such as moisture content, volume and weight increase and density changes of seeds (Gibson, Perlas, & Hotz, 2006; Han, Swanson, & Baik, 2007; Hotz & Gibson, 2007). Seed hydration studies are often time consuming and laborious process. Experimental determination of hydration kinetics has proven to be error prone due to presence of many variables, which led to popularity of both empirical models (Coutinho, Omoto, dos Santos Conceição, Andrade, & Jorge, 2010; Goula & Adamopoulos, 2009). Simplicity in application of these models has resulted in generation of new empirical and semi-empirical models to describe different hydration techniques. New models described are compared to a well-established model by means visual fit to arbitrate their suitability in predicting hydration (Kashaninejad, Dehghani, & Kashiri, 2009; Sam Saguy, Marabi, & Wallach, 2005). A better way to analyze and compare different models was proposed by Henri Theil through splitting of residual error into random and fixed sources and was earlier utilized in determining the best model for microbial inactivation studies (Harte, Black, & Davidson, 2009; Theil, Beerens, Tilanus, & De Leeuw, 1966). The current study is aimed at utilizing the Theil error splitting method as a tool towards analyzing

different models that are currently used along with different exponential models to determine best model to describe hydration studies of dry beans, oil seeds and cereals.

Materials and Methods

Hydration Studies: Volume measurements of dry beans, soybeans and barley are taken using seed hydration analyzing device. Specific hydration protocol for specific cereal or legume seed is selected.

Hydration of dry beans: Dry beans are rehydrated in preparation of canned beans (Thanos, 1998; Zanovec, O'Neil, & Nicklas, 2011). Soaking enables uniform cooking and prevention of leaching out of valuable nutrients from dry beans. For this study, samples of navy, pinto and black beans are procured from Archer Daniels Midland Company (Decatur, IL) from year 2012 crop. The beans are soaked in seed hydration analyzing device for 3 hours at 55^{o} C of soak water temperature. Volume of beans is measured and recorded every 3 minutes during hydration

Hydration of oil seeds: Soybeans are mainly hydrated in processing of snack foods such as roasted beans (Ingbian & Adegoke, 2007; Raghuvanshi & Bisht, 2010). Yellow and black soy beans are obtained from SK Foods (Fargo, ND). Soybeans are soaked in seed hydration analyzing device for 16 hours at 24^{o} C of soak water temperature. Volume of beans is measured and recorded every 10 minutes during hydration.

Hydration of cereals: Soaking of barley, wheat and sorghum is an important step in process of steeping during malt preparation (Williams et al., 2008; Yu, Cao, & Shahidi, 2012). Cereals such as millet, rice and sorghum are soaked prior to further processing such as sprouting or cooking. Sample of nomini barley variety is obtained from Virginia Tech Experimentation Station (Blacksburg, VA). Barley seeds are soaked in seed hydration analyzing for 4 hours at 24^{o} C of

soak water temperature. Volume of seeds is measured and recorded every 5 minutes during hydration.

Mathematical models and fitting methods: Five models are selected to fit the measured volume of dry beans, oil seeds and barley while soaking. The first model is a modified Weibull distribution function has been utilized to describe seed and dried food rehydration (Cunningham, McMinn, Magee, & Richardson, 2007; García-Pascual, Sanjuán, Melis, & Mulet, 2006).

$$V_t = V_e - V_e\left(e^{-\left(\frac{t}{\alpha}\right)^{\beta}}\right) \quad \text{--(1)}$$

Where V_t is volume of seeds at time *t* during hydration. V_e is equilibrium volume of seeds at end of hydration calculated by taking average of last five volume readings. A and β are Weibull distribution parameters.

The second model is proposed by Peleg to describe moisture sorption curves for seeds and granular foods (Peleg, 1988; Sopade, Ajisegiri, & Badau, 1992). It is one of the first non-exponential models proposed to describe hydration kinetics.

$$V_t = V_0 + \left(\frac{t}{k_1 + k_2 t}\right) \quad \text{--(2)}$$

Where V_t is volume of seeds at time *t* during hydration. V_0 is initial volume of seeds before hydration. $K_1$and k_2 are Peleg model parameters.

The third, fourth and fifth models are various exponential models selected to describe measured volume readings. Many similar exponential models are proposed before to describe hydration kinetics of cereals and legumes, and they are preferred for their least number of variables (Diamante & Munro, 1991; Maskan, 2001).

$$V_t = V_e + \left[(1 - V_e)\left(e^{-\left(\frac{t}{\alpha}\right)}\right)\right] \quad \text{--(3)}$$

Where V_t is volume of seeds at time *t* during hydration. V_e is equilibrium volume of seeds at end of hydration calculated by taking average of last five volume readings. A is model parameter.

$$V_t = \left(1 + \frac{t}{k_1}\right) + \mathrm{K}\left(1 - e^{-\left(\frac{t}{\alpha}\right)}\right) \quad \text{--(4)}$$

Where V_t is volume of seeds at time *t* during hydration. K is constant. A is a model parameter

$$V_t = \frac{(k_1+1)}{[k_1+(e^{-k_2 t})]} \quad \text{--(5)}$$

Where V_t is volume of seeds at time *t* during hydration. $k_1$and k_2 are model parameters.

The last model is similar to exponential model used to describe the exponential decay in viscosity during high pressure homogenization of polysaccharides (Harte & Venegas, 2010).

4.2.2 Error splitting method:

Theil's error splitting method for analysis of predicted data in comparison with observed data is established by means of calculating their difference into fixed and random error (Harte et al., 2009; Theil et al., 1966). The average error between predicted and observed results is sum of fixed and random error, while fixed error can be further split into bias and regression error. The bias fixed (B), regression fixed I and random errors (ε) are calculated for the predicted readings of volume of seeds during hydration using following equations.

$$B = \left(\widehat{N}_{obs} - \widehat{N}_{model}\right)^2 \quad \text{-- (6)}$$

$$R = \left(S_{N_{obs}} - \hat{\beta}_1 \cdot S_{N_{obs}}\right)^2 \quad \text{-- (7)}$$

$$\varepsilon = (1 - r^2) \cdot S^2{}_{N_{model}} \quad \text{-- (8)}$$

Total average error between q values of measured data points ($N_{obs,i}$) and predicted data points ($N_{model,i}$) is given as:

$$\frac{1}{q} \sum_{i=1}^{q} (N_{obs,i} - N_{model,i})^2 = B + R + \varepsilon \quad \text{-- (9)}$$

Where $\widehat{N}_{obs}$ and $\widehat{N}_{model}$ are the average experimental and modeled volume readings, calculated as:

$$\widehat{N}_{obs} = \frac{\sum_{i=1}^{q} N_{obs,i}}{q} \quad \text{-- (10)}$$

$$\widehat{N}_{model} = \frac{\sum_{i=1}^{q} N_{model,i}}{q} \quad \text{-- (11)}$$

Where $S_{N_{obs}}$ is the population standard deviation for experimental readings and $\hat{\beta}_1$is the experimental slope of the linear regression between predicted and observed volume readings calculated as follows:

$$S_{N_{obs}} = \sqrt{\frac{\sum_{i=1}^{q}(N_{obs,i} - \bar{N}_{obs})^2}{q}} \quad \text{-- (11)}$$

$$\hat{\beta}_1 = \frac{\sum_{i=1}^{q}[(N_{obs,i} - \bar{N}_{obs}) \cdot (N_{model,i} - \bar{N}_{model})]}{\sum_{i=1}^{q}(N_{obs,i} - \bar{N}_{obs})^2} \quad \text{-- (12)}$$

Where $S^2{}_{N_{model}}$the population variance for the predicted volume is values and r^2 is the coefficient of determination between experimental and predicted values, calculated as:

$$S^2{}_{N_{model}} = \frac{\sum_{i=1}^{q}(N_{model,i} - \bar{N}_{model})}{q} \quad \text{-- (13)}$$

$$r^2 = \frac{\sum_{i=1}^{q}[(N_{obs,i} - \bar{N}_{obs}) \cdot (N_{model,i} - \bar{N}_{model})]}{\sum_{i=1}^{q}(N_{obs,i} - \bar{N}_{obs})^2 \cdot \sum_{i=1}^{q}(N_{model,i} - \bar{N}_{model})^2} \quad \text{-- (14)}$$

The significance of each error is calculated as follows (Harte et al., 2009): The bias fixed error is tested using Student's pairwise comparison for mean, with the null hypothesis that $\mu_{N_{model}}$- $\mu_{N_{obs}} = 0$. The defined hypothesis is not rejected if $\bar{N}_{obs} - \bar{N}_{model} \in \mp S_D \cdot t_{(q-1;\alpha/2)}$, where is the Student's distribution value for q-1 degrees of freedom for a pairwise comparison set, α is the probability of type I error, and S_D is the standard deviation for the means, calculated as:

$$S_D = \sqrt{\frac{\sum_{i=1}^{q}(N_{obs,i} - \bar{N}_{model,i})^2 - [\sum_{i=1}^{q}(N_{obs,i} - \bar{N}_{model,i})^2 / q]}{(q-1) \cdot q}} \quad \text{-- (15)}$$

The regression fixed error is tested using Student's distribution test, with the null hypothesis that $\hat{\beta}_1 = 1$. The defined hypothesis is not rejected if $\hat{\beta}_1 \in \mp S_{\hat{\beta}_1} \cdot t_{(q-2;\alpha/2)}$, where is the Student's distribution value for q-1 degrees of freedom for a pairwise comparison set, α is the probability of type I error, and $S_{\hat{\beta}_1}$ calculated as:

$$S_D = \sqrt{\frac{\sum_{i=1}^{q}(N_{model,i} - \bar{N}_{model})^2 - \left[\sum_{i=1}^{q}\left[(N_{obs,i} - \bar{N}_{obs})\cdot(N_{model,i} - \bar{N}_{model})\right]^2 / \sum_{i=1}^{q}(N_{obs,i} - \bar{N}_{obs})^2\right]}{(q-2)\cdot\sum_{i=1}^{q}(N_{obs,i} - \bar{N}_{obs})^2}} \quad \text{-- (16)}$$

The random error is tested for normality with a 0 expected value and existing variance $\in \sim N(0, \sigma_s^2)$. Normality for random error term is tested using the Shapiro-Wilk test. The observed and predicted data are compared by means of correlation of coefficient I. The statistical analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC, 2009).

After error analysis the models were evaluated to select best model that can satisfactorily predict measured volume readings (Harte et al., 2009). The criteria for a model to be satisfying are: (i) minimizing the average squared difference between observed and predicted volume readings of seeds (fixed error which is combination of bias and regression errors), (ii) maximizing the contribution of fixed random error , (iii) maximize the coefficient of correlation between measured and predicted volume readings.

Results and Discussion:

Hydration of dry beans:

Navy, black and pinto beans are soaked at 55^{o}C of soak water temperature for 3 hours in seed hydration analyzing device. Volume of each bean variety is recorded every 3 minutes and observed data are fit with all five selected mathematical models shown as in equations 1-5 (Figure 4.1).

Hydration of navy beans: The selected five models predicted observed values with high correlation, $r > 0.975$ (Table 4.1). The total error was highest for exponential-1 and Harte-Venegas models (7.81 and 22.08 respectively), which is on average 10 to 30 times higher than other three models. The average modeled and measured volume readings for navy beans were significantly different in case of Weibull and exponential-1 models *(*$P < 0.05$), where no significant difference was found in case of Peleg, exponential-2 and Harte-Venegas models ($P >$ 0.81, 0.38, 0.08 respectively) for fixed bias error. Regression fixed error was highest in models exponential-1, exponential-2 and Harte-Venegas (76.78%, 8.42% and 89.12% respectively), with their slope for observed versus predicted volume readings being significantly different from 1. For Weibull and Peleg, the slope between measured and modeled values is not significantly different. For models Weibull, Peleg and exponential-2, most of error is concentrated in random error (> 75%), while exponential-1 and Harte-Venegas had less than 15%. All models had varying level of random error without normal distribution *(*$P < 0.01$). From error analysis, exponential-1and Harte-Venegas had highest regression fixed error, meaning that the models consistently under estimated the volume readings and these models can be considered not satisfying. Weibull and exponential-2 models had highest bias fixed error, meaning that the models consistently over estimated, but these models also had very high random error and lower total error for which they be considered satisfying models but with limitations. Peleg model had highest random error with very good correlation (r > 995) and low total error, in which case it can be considered most satisfying model to predict volume readings of navy beans while soaking at 55^{o} C for three hours.

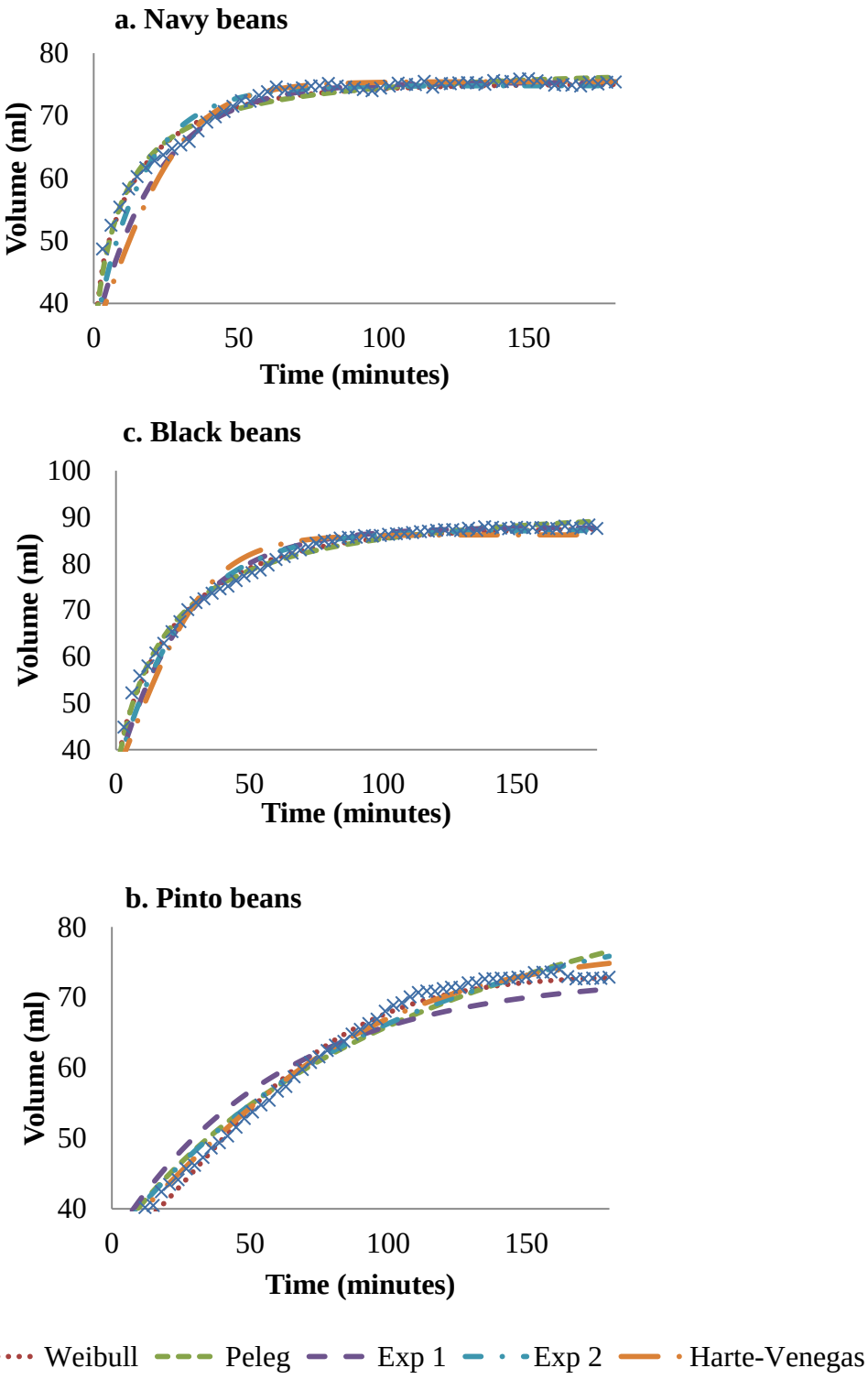

Figure 4.1. Observed and modeled volume data of navy (a), black (b) and pinto (c) beans during hydration at 55^{o} C of soak water temperature for 3 hours.

Table 4.1. Error analysis for five models used to predict the volume of navy

Model	Bias error		Reg error		Random error		Total error	r (%)
	P<	%	P<	%	P<	%		
Weibull	0.0138	16.3779	0.2624	8.2313	0.0001	75.3907	0.5416	99.30
Peleg	0.8100	0.1877	0.9815	1.1456	0.0001	98.6668	0.6150	99.01
Exponential-1	0.0016	9.1714	0.0001	76.7814	0.0001	14.0472	7.8092	98.70
Exponential-2	0.3795	10.4745	0.0127	8.4193	0.0001	81.1062	0.3342	98.26
Harte-Ven	0.0810	1.7732	0.0001	89.1186	0.0001	9.1083	22.0786	97.91

Hydration of black beans: Measured volume readings from seed hydration analyzing device fit with selected five models with high correlation, $r > 0.98$ (Table 4.2). The total error was highest for exponential-1, exponential-2 and Harte-Venegas models (5.00, 3.24, and 8.70 respectively), which is on average 10 to 15 times higher than other two models. The average modeled and measured volume readings for navy beans were significantly different in case of Weibull model ($P < 0.05$), where no significant difference was found in case of Peleg, exponential -1, exponential-2 and Harte-Venegas models ($P > 0.79$, 0.99, 0.27, and 0.19 respectively) for fixed bias error. Regression fixed error was highest in models exponential-1, exponential-2 and Harte-Venegas (81.11%, 65.89% and 60.12% respectively), with their slope for observed versus predicted volume readings being significantly different from 1. For Weibull and Peleg, the slope between measured and modeled values is not significantly different. For models Weibull, Peleg and exponential-2, most of error is concentrated in random error (> 75%), while exponential-1, exponential-1 and Harte-Venegas (18.89%, 32.49% and 37.41% respectively) which is less than 40%. All models had varying level of random error without normal distribution ($P < 0.01$). From error analysis, exponential-, exponential-2 and Harte-Venegas had highest fixed error which is concentrated regression fixed error, meaning that the

models consistently under estimated the volume readings and these models can be considered not satisfying. Weibull model had highest bias fixed error, meaning that the models consistently over estimated, but it also had very high random error and lower total error for which it can be considered satisfying models but with limitations. Peleg model had highest random error with very good correlation (r > 99.66%) and low total error, in which case it can be considered most satisfying model to predict volume readings of black beans while soaking at 55^{o} C for three hours.

Hydration of pinto beans: Pinto beans are soaked in seed hydration analyzing device and volume of beans are recorded to fit with selected five models with high correlation, $r > 0.98$ (Table 4.3). The total error was highest for model exponential-1 (17.63), which is 10 times higher than other models. The average modeled and measured volume readings for navy beans were significantly different in case of all models ($P < 0.05$) for fixed bias error. Regression fixed error was highest in all models ($P < 0.05$) except for Weibull, with their slope for observed versus predicted volume readings being significantly different from 1. All the models except for exponential-1 model had more significant amount random error, but they are not very high and without normal distribution ($P < 0.01$).

Table 4.2. Error analysis for five models used to predict the volume of black beans

Model	Bias Error		Reg Error		Random Error		Total Error	r (%)
	P<	%	P<	%	P<	%		
Weibull	0.0079	19.11	0.3876	5.56	0.0001	75.34	0.4346	99.76
Peleg	0.7857	0.24	0.6856	4.20	0.0001	95.57	0.4886	99.66
Exponential 1	0.9938	0.00	0.0001	81.11	0.0001	18.89	5.0004	99.41
Exponential 2	0.2703	1.62	0.0008	65.89	0.0001	32.49	3.2375	99.32
Harte-Ven	0.1910	2.47	0.0049	60.12	0.0001	37.41	8.6987	98.04

From error analysis, Peleg, exponential-1 and exponential-2 had more fixed error than random error which is concentrated regression fixed error, meaning that the models consistently under estimated the volume readings and these models can be considered not satisfying. Weibull model had highest bias fixed error, meaning that the models consistently over estimated, but it also had very high random error and lower total error for which it can be considered satisfying models but with limitations. Also Harte-Venegas had lower fixed error and overall error making it a satisfying model when compared to other models.

Hydration of oil seeds:

Two varieties of soy beans (black and yellow) are soaked at 24^{o} C of soak water temperature for 16 hours in seed hydration analyzing device. Volume of each bean variety is recorded every 10 minutes and observed data are fit with all five selected mathematical models shown as in equations 1-5 (Figure 4.2).

Hydration of black soybeans: The selected five models predicted observed values with high correlation, $r > 0.989$ (Table 4.4). The total error was highest for exponential-1, exponential-2 and Harte-Venegas models (10.03, 11.88 and 27.66 respectively), while other two models Weibull and Peleg had lower total error (3.36 and 3.58 respectively).

Table 4.3. Error analysis for five models used to predict the volume of pinto beans

Model	Bias error		Regression error		Random error		Total error	r (%)
	P<	%	P<	%	P<	%		
Weibull	0.0022	24.80	0.6941	2.90	0.0001	72.31	0.6015	99.71
Peleg	0.4140	1.28	0.0034	49.41	0.0013	49.31	2.5491	99.06
Exponential 1	0.3934	0.52	0.0001	93.18	0.0001	6.29	17.6297	98.87
Exponential 2	0.3205	1.73	0.0014	55.04	0.0004	43.23	2.1116	99.32
Harte-Ven	0.4148	1.55	0.0359	34.26	0.0001	64.19	0.6366	99.71

The average modeled and measured volume readings were significantly different in case of Weibull, Peleg, exponential-1, exponential-2, model ($P < 0.05$), where no significant difference was found in case of Harte-Venegas model ($P > 0.02$) for fixed bias error. Regression fixed error was highest in all models ($P < 0.05$) except for Weibull, with their slope for observed versus predicted volume readings being significantly different from 1. All the models except for exponential-2 model had more significant amount random error, and hypothesis for normal distribution is rejected ($P < 0.01$). From error analysis, Peleg, exponential-1 and exponential-2 and Harte-Venegas models have more fixed error than random error which is concentrated regression fixed error, meaning that the models consistently under estimated the volume readings and these models can be considered not satisfying. Weibull model had highest bias fixed error, meaning that the models consistently over estimated, but it also had very high random error and lower total error for which it can be considered satisfying model to predict volume of black soybeans when soaked at 24^{o} C of soak water temperature for 16 hours.

Table 4.4. Error analysis for five models used to predict the volume of black soybeans

Model	Bias error		Reg error		Random error		Total error	r (%)
	P<	%	P<	%	P<	%		
Weibull	0.0126	11.78	0.3180	3.27	0.0001	84.95	3.3563	99.07
Peleg	0.2763	1.67	0.0239	36.39	0.0001	61.94	3.5768	99.33
Exponential 1	0.0619	3.10	0.0001	62.12	0.0001	34.78	10.0258	99.02
Exponential 2	0.0762	2.36	0.0001	90.47	0.0001	7.17	11.8773	98.86
Harte-Ven	0.0218	3.91	0.0001	68.32	0.0001	27.77	27.6597	98.04

Hydration of yellow soybeans: Volume of yellow soybeans fit with selected five models had high correlation, $r > 0.95$, with Weibull and Peleg models having correlations greater than 0.99 (Table 4.5).

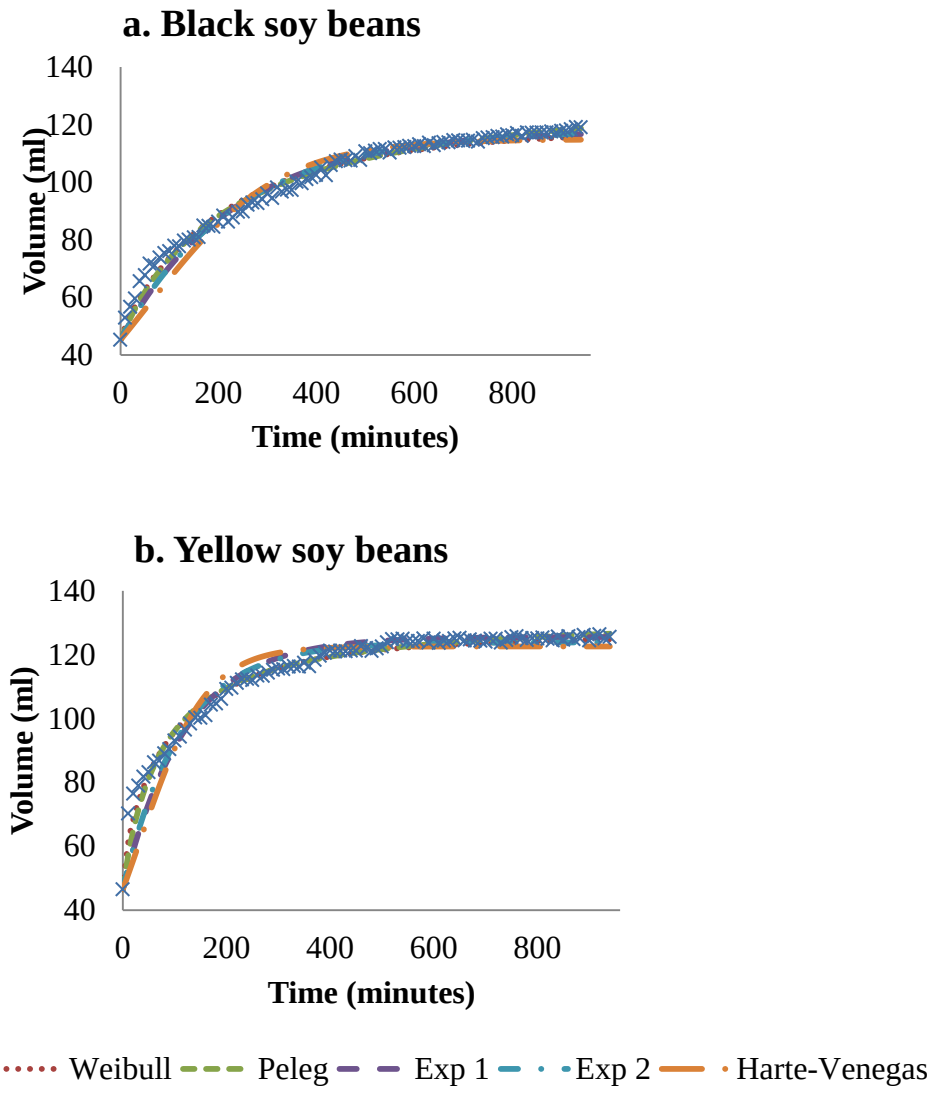

Figure 4.2. Observed and modeled volume data of black (a) and yellow (b) soybeans during hydration at 24^{o} C of soak water temperature for 16 hours.

The total error was highest for exponential-1, exponential-2 and Harte-Venegas models (36.52, 22.01and 37.46 respectively), while other two models Weibull and Peleg had lower total error (2.41 and 4.65 respectively). The average volume readings were significantly different in case of Weibull model ($P < 0.05$), while no significant difference was found in case of Peleg, exponential-1, exponential-2 and Harte-Venegas model ($P > 0.09$) for fixed bias error. .

Regression fixed error was highest in all models *(P* < 0.05) except for Weibull and Peleg, with their slope for observed versus predicted volume readings being significantly different from 1 *(P* < 0.05). All the models except for exponential-2 model had more significant amount random error and hypothesis for normal distribution is rejected *(P* < 0.01). From error analysis, Peleg, exponential-1 and exponential-2 and Harte-Venegas models have more fixed error than random error which is concentrated regression fixed error, meaning that the models consistently under estimated the volume readings and these models can be considered not satisfying. Weibull model had higher bias fixed error, but it also had very high random error, better correlation and lower total error for which it can be considered satisfying.

5.7.3. *Hydration of cereals:*

Barley are soaked for 4 hours at room temperature (24^{o} C) and volume of seeds are recorded every 5 minutes using seed hydration analyzing device. The observed data are fit with all five selected mathematical models shown as in equations 1-5 (Figure 4.3).

Hydration of barley: All the selected models had high correlation, *r* > 0.99, with Weibull and Peleg models having correlations greater than .0999 (Table 4.6).

Table 4.5. Error analysis for five models used to predict the volume of yellow soybeans

Model	Bias Error		Reg Error		Random Error		Total Error	r (%)
	P<	%	P<	%	P<	%		
Weibull	0.0072	13.65	0.4485	1.40	0.0001	84.95	2.4102	99.11
Peleg	0.4335	0.91	0.0552	32.08	0.0001	67.01	4.6456	98.77
Exponential 1	0.6548	0.10	0.0001	85.20	0.0001	14.70	36.5160	98.33
Exponential 2	0.1528	1.48	0.0001	90.82	0.0001	7.70	22.0055	97.62
Harte-Ven	0.0934	2.35	0.0004	63.38	0.0001	34.27	37.4630	95.96

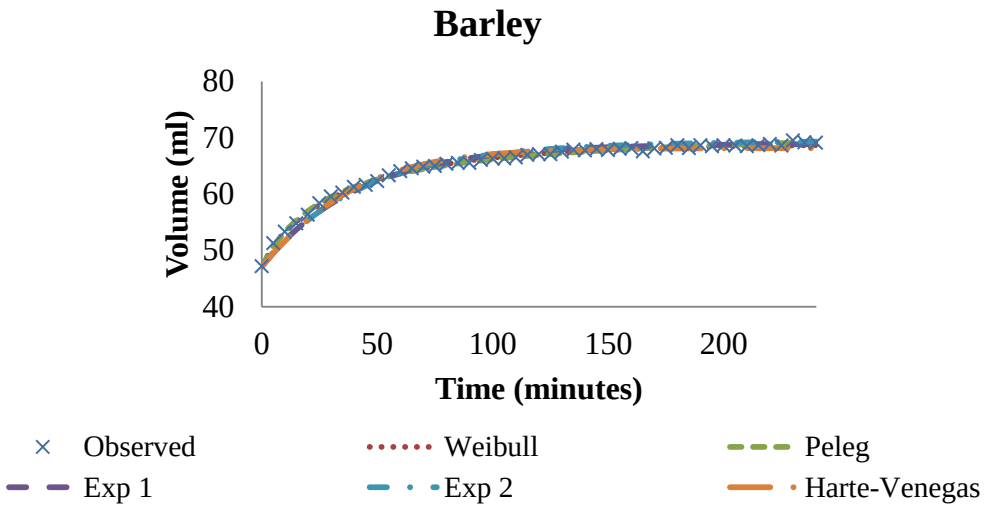

Figure 4.3. Observed and modeled volume data of barley during hydration at 24^{o} C of soak water temperature for 4 hours.

The total error was low for all model, but highest for Peleg, exponential-2 and Harte-Venegas models (0.45, 0.36 and 0.93 respectively), while other two models Weibull and exponential-1 had very lower total error (0.05 and 0.06 respectively). The average modeled and measured volume readings were significantly different in case of exponential-2 model ($P < 0.05$), while no significant difference was found in case of Weibull, Peleg, exponential-1 and Harte-Venegas model ($P > 0.33$) for fixed bias error. . Regression fixed error was highest in all models ($P < 0.05$) except for Weibull and exponential-1 with their slope for observed versus predicted volume readings being significantly different from 1 ($P < 0.05$). Peleg and exponential-2 models had very less significant amount random error, and hypothesis for normal distribution is rejected for all models ($P < 0.01$). From error analysis, Peleg, exponential-2 and Harte-Venegas models have more fixed error than random error which is concentrated regression fixed error, meaning that the models consistently under estimated the volume readings and these models can be considered not satisfying.

Table 4.6. Error analysis for five models used to predict the volume barley

Model	Bias error		Regression error		Random error		Total error	r (%)
	P <	*%*	*P* <	*%*	*P* <	%		
Weibull	0.3329	3.77	0.4063	8.13	0.0001	88.10	0.0448	99.84
Peleg	0.9960	0.00	0.7684	91.40	0.0001	8.60	0.4505	99.85
Exponential-1	0.7594	1.26	0.0001	3.16	0.0001	95.58	0.0619	99.60
Exponential-2	0.0414	13.01	0.0001	73.05	0.0001	13.94	0.3625	99.70
Harte-Ven	0.3391	1.09	0.0001	73.88	0.0001	25.03	0.9307	99.17

Weibull and exponential-1 models had more error concentrated in bias fixed error, but it also had very high random error, better correlation and lower total error for which they both can be considered satisfying models to predict volume of barley seeds when soaked at 24^{o} C of soak water temperature for 4 hours.

Conclusion

Theil error splitting method to analyze different empirical models is successfully demonstrated with navy, black, pinto, black soy, yellow soy and barley seeds. From the analysis, no one model predicted the measured volume readings for all tested seeds satisfactorily. For Weibull model worked well for all the tested seeds, although in all cases, except it had higher bias fixed error for of dry beans. Peleg model had best prediction for navy and black beans, while it was very poor with all other seeds tested. Exponential-1worked well for only barley, while exponential-2 worked well only for navy beans. Harte-Venegas model showed better prediction for pinto beans. Models Weibull and Peleg have two parameters, while rest of the models has only one parameter, which can be useful for application. Thus, from the current study, it is recommended that models that are being considered to predict hydration kinetics (volume, moisture, weight) of

seeds during soaking should be thoroughly analyzed with demonstrated error splitting methods for its validity and robustness, in addition to its ability to correlate with the measured data.

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CHAPTER V

Studies on Steeping Process of Malt – using Seed Hydration Analyzing Device

Abstract

Barley is one of the important cereal grains in food industry and malting is an essential step in preparation of beer. Steeping is a critical step in malt preparation. Maltsters undertake small scale trails to determine the effect of steeping on barley. The trails measure the intrinsic characteristics of barley seeds and current practices in industry allow very little scope to change established recipes for malt making. Continuous monitoring of seeds in the device can provide with exact time of germination and rate of hydration which can in turn be correlated with process and seed parameters. The objective of this study was to use a novel device to measure volume, weight and density of barley seeds during steeping. Steeping studies were carried out on three barley varieties (Copeland, Metcalf and Thoroughbred) and effect of temperature, temperature cycles and air rest periods. Data from seed hydration analyzing device indicated drop in weight of barley seed during, and further data process enabled accurate prediction of the beginning of weight drop, which is in turn used to surmise onset of germination. From the study, Thoroughbred and Metcalf varieties required least germination time (45.25 hours) during steeping, while Copeland required 51.50 hours. Among studied temperatures, 18^{o} C resulted in least germination time, while it was least for the temperature cycle of 20^{o} C for initial 5 hours followed by 18^{o} C for 79 hours. Air rest period of 60 minutes among studied immersion intervals had the least germination time.

Introduction

Barley is very important cereal in food industry with potential applications in alcoholic and non-alcoholic beverages and baked foods. They are rich source of proteins, minerals and carbohydrates (Ford & Hewitt, 1979). Malting is an essential step in preparation of beverages using barley. During malting, seeds are carefully germinated to enable production of enzymes, which break down complex starches into simple sugars (e.g.: glucose and sucrose). Also, proteins are broken down by protease enzymes, which are released during germination and are later utilized by yeast during fermentation (C. W. Bamforth, 2003). Malting process is tailored for different end products by controlling level of germination and subsequent preparation methods. For example, proteins are not favored in beer production, whereas in preparation of dietary supplements and nutritional powders, proteins need to be conserved to add value (Zhou, 2010). A simple malting process is illustrated schematically in Figure 1 (C. W. Bamforth, 2006).

Steeping is the most critical stage in malt preparation. Moisture required to allow barley seeds to germinate is provided in controlled environment with water temperatures ranging from 16^{o} C to 20^{o} C with periods of 'air rests' with no water (Cozzolino, Roumeliotis, & Eglinton, 2012). 'Air rests are necessary to provide sufficient oxygen to support respiration in the seeds. Barley is steeped for 48-60 hours during which seeds attain 45 – 50% moisture content. Absorption of water during steeping facilitates release of gibberellins (Gas) from embryo. Gas help trigger release of amylase enzymes which help convert starch present in endosperm to sugars, which are essential for germination (C. W. Bamforth, 2006). Figure 2 shows the physiological changes in barley during steeping process. Steeping aids such as potassium bromate (200-300ppm), is used to hinder growth of rootlets, along with other anti-microbial agents such as hypochlorite or dilute hydroxides. In United States, potassium bromate is allowed for treatment with concentrations

less than 75 ppm (21 CFR § 172.730). Gibberellic acid (GA) is also used to supplement native gibberellins of barley to aid faster germination.

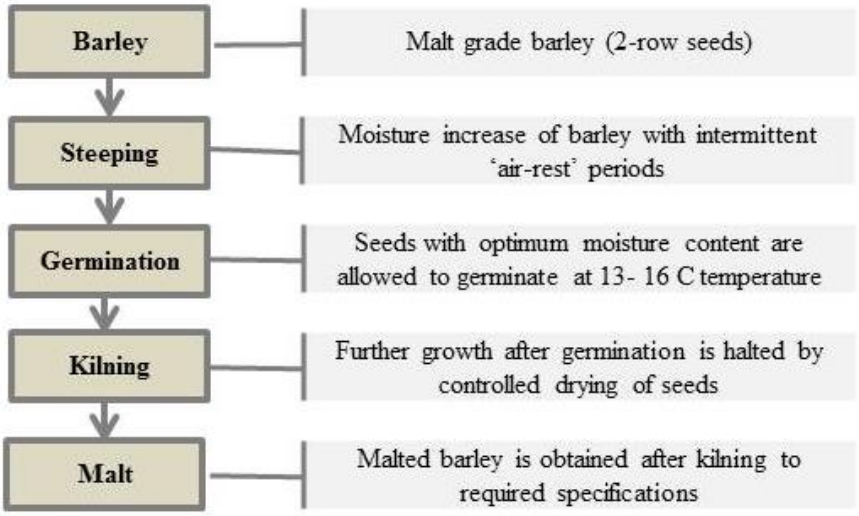

Figure 5.1. Schematic illustration of barley malting process

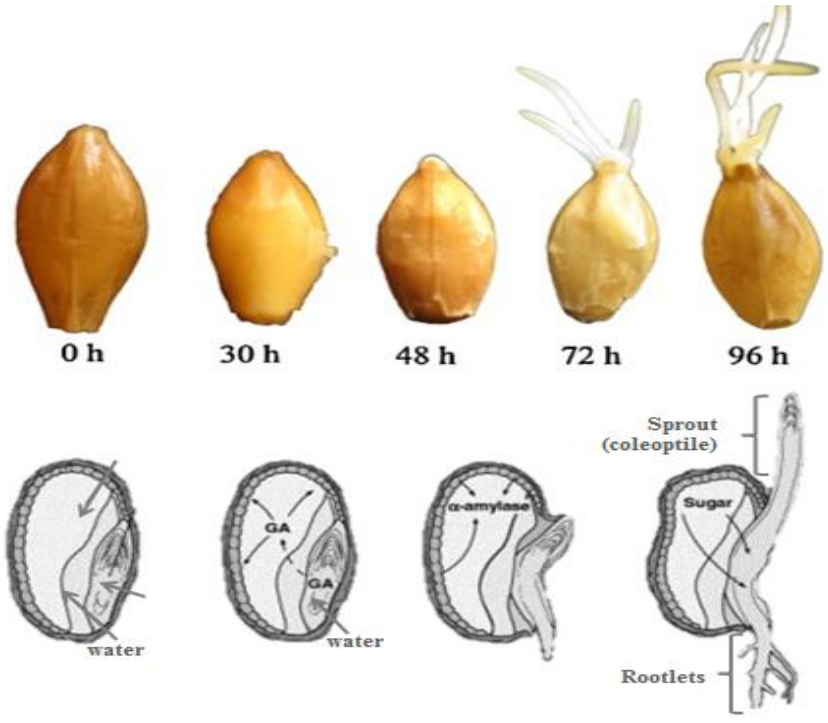

Figure 5.2. Barley seed physiological changes during steeping process

The quality of malt heavily depends on seed quality. The following factors play prominent role in obtaining high quality malt (Eagles, Bedggood, Panozzo, & Martin, 1995; Han et al., 1997; Henry & Cowe, 1990). Steeping process is designed based on barley variety and required final malt product.

a. *Barley variety:* Barley varieties with required enzyme systems are necessary for efficient production of malt.
b. *Germination:* Germination is essential factor in malt quality barley. Barley seeds must be able to pass a 3-day 4 ml germination test with a minimum of 95% germination rate.
c. *Protein content:* High protein content in barley results in slower water absorption during steeping, and very low protein content reduces enzyme modification required for germination. Standard protein content is determined to be in range of 11-13%.
d. *Moisture content:* Barley seeds are stored with moisture content around 14%. Storage condition impact moisture content, with lower levels resulting is seed damage and higher levels tending to increase microbial growth and enzyme activity, resulting in low malt quality seeds.
e. *Other parameters:* Seed plumpness is considered as a good indicator of high starch content in barley and thus desired to produce more beer. Seed uniformity is also considered to be a good quality resulting in uniform germination.

Maltsters undertake small scale trails to determine the effect of steeping on barley along with determination of germination energy test. The trails measure the intrinsic characteristics of barley seeds and current practices in industry allow very little scope to change established recipes for malt making. In process testing such as moisture analysis and farinator tests are conducted to understand extent of hydration (Chandra et al., 2001). Moisture analysis is

generally carried out using standard moisture analyzers to determine if the seeds achieved optimum moisture content. Farinator tests are used to monitor endosperm structure. The farinator device cuts through barley seeds and are examined for amount of mealy or steely seeds. Ability to study steep process conditions along with seed parameters can provide malt producers with valuable information on optimizing steeping. Extrinsic parameters such as water quality, water temperature, and process additives can be altered and tested for effectiveness. Such studies have been limited due to lack of proper analyzing techniques and instrumentation.

Materials and Methods

A hydration analyzing device developed to measure volume and weight of seeds while soaking is proposed to mimic steeping process in malt preparation (C. W. Bamforth, 2006; Briggs, 1998). Continuous monitoring of seeds in the device can provide with exact time of germination and rate of hydration which can in turn be correlated with process and seed parameters, such as volume, weight and density. The recorded data are presented in appendix I. Once steeping process is well understood using the device, the parameters are altered to establish concrete relationship with water uptake and germination rates and optimize them to achieve high quality malt. Using the seed hydration analyzing device, the following specific objectives are suggested for studies on the steeping process: a) determine germination time for different barley varieties using the device and compare results with regular steeping process, b) determine the effect of steeping parameters such as water temperature, 'air-rest' period on germination of barley

Studies on germination time for barley varieties: Three barley varieties, two (Metcalf and Copeland) from Canadian Malting Barley Technical Centre in Winnipeg, Manitoba, Canada and one (Thoroughbred) from Riverbend Malt House, Asheville, North Carolina are used to test varietal differences using the seed hydration analyzing device. 25 grams of barley seeds are

steeped in seed hydration device with three replicates for 84 hours, with seeds being covered in water every 15 minutes. Volume and weight measurements of the seeds are recorded every 15 minutes. The average weight and volume measurements recorded are used to analyze and determine the germination time of barley seeds during steeping.

Studies on effect of temperature: Different steeping temperatures are studied to see their impact on germination time on Thoroughbred barley variety. The barley seeds (25 grams) are steeped in seed hydration analyzing device with three replicated for 84 hours at temperatures of 16^{o} C, 18^{o} C and 20^{o} C, with water immersions every 15 minutes. Volume and weight measurements of the seeds are recorded by the seed hydration analyzing device every 15 minutes. The average weight and volume measurements recorded are used to analyze and determine the germination time of barley seeds at different temperatures during steeping.

Studies on effect of temperature cycle: The effect of temperature cycle son steeping process of barley (25 grams of Thoroughbred variety with three replicates) was studied using the seed hydration analyzing device. For cycle one: barley is initially steeped for 5 hours at 20^{o} C followed by 18^{o} C for 79 hours. For cycle two and three, the initial temperatures of 24^{o} C and 1^{o} C are used to understand impact of high and low initial temperatures of steep water. During the temperature cycle studies the seeds are covered in water every 15 minutes with volume and weight measurements recorded every 15 minutes. The average weight and volume measurements recorded are used to analyze and determine the germination time of barley with different temperature cycle during steeping.

Studies on effect of air rest periods: The effect of water immersion intervals on barley steeping process is studied using 25 grams of Thoroughbred variety and three replicates in seed hydration

analyzing device. Barley seeds are steeping in 18^{o} C temperature with immersion intervals (air rest periods) of 10, 15, 20, 30, and 60 minutes and their volume and weight readings are recorded every 10, 15, 20, 30, and 60 minutes respectively. The recorded readings are later analyzed to determine germination time of each rest period trail.

Results and Discussion

Determination of germination time: Steeping of barley is carried out in seed hydration analyzing device, which measures and records volume and weight of seeds. From literature, physiological changes in barley indicate loss weight, with leaching of solids into water, and conversion of sugars to provide energy for enzymatic break down of proteins with release of carbon-dioxide (C. Bamforth & Barclay, 1993; C. W. Bamforth, 2006; Mayolle, Lullien-Pellerin, Corbineau, Boivin, & Guillard, 2011). The process of weight loss is triggered with germination, and development of sprout or coleoptile (Figure 5.2). Volume of barley seeds increases during this time, with growth of roots and sprouts. This reported phenomenon is recorded during steeping process of barley with continuous recording of volume and weight of seeds using seed hydration analyzing device. Figure 5.3 shows the volume, weight and density (weight/volume) of barley seeds (Metcalf variety) for every 15 minutes during steeping at 18^{o} C for 84 hours. The boxed region of the graph shows the beginning of decline in weight and density, indicating beginning of germination. From the weight and density data of barley, it is unclear of certain point and to distinctly identify where the weight decline begins, exponential of weight readings were plotted against the steeping time for barley (Figure 5.4). Data processing magnified change in weight drop to allow simple determination of the germination time for the steeping process using the data from seed hydration analyzing device. It's important to note the significance of identifying the onset of germination of barley seeds during steeping, as it is used to stop the steeping process

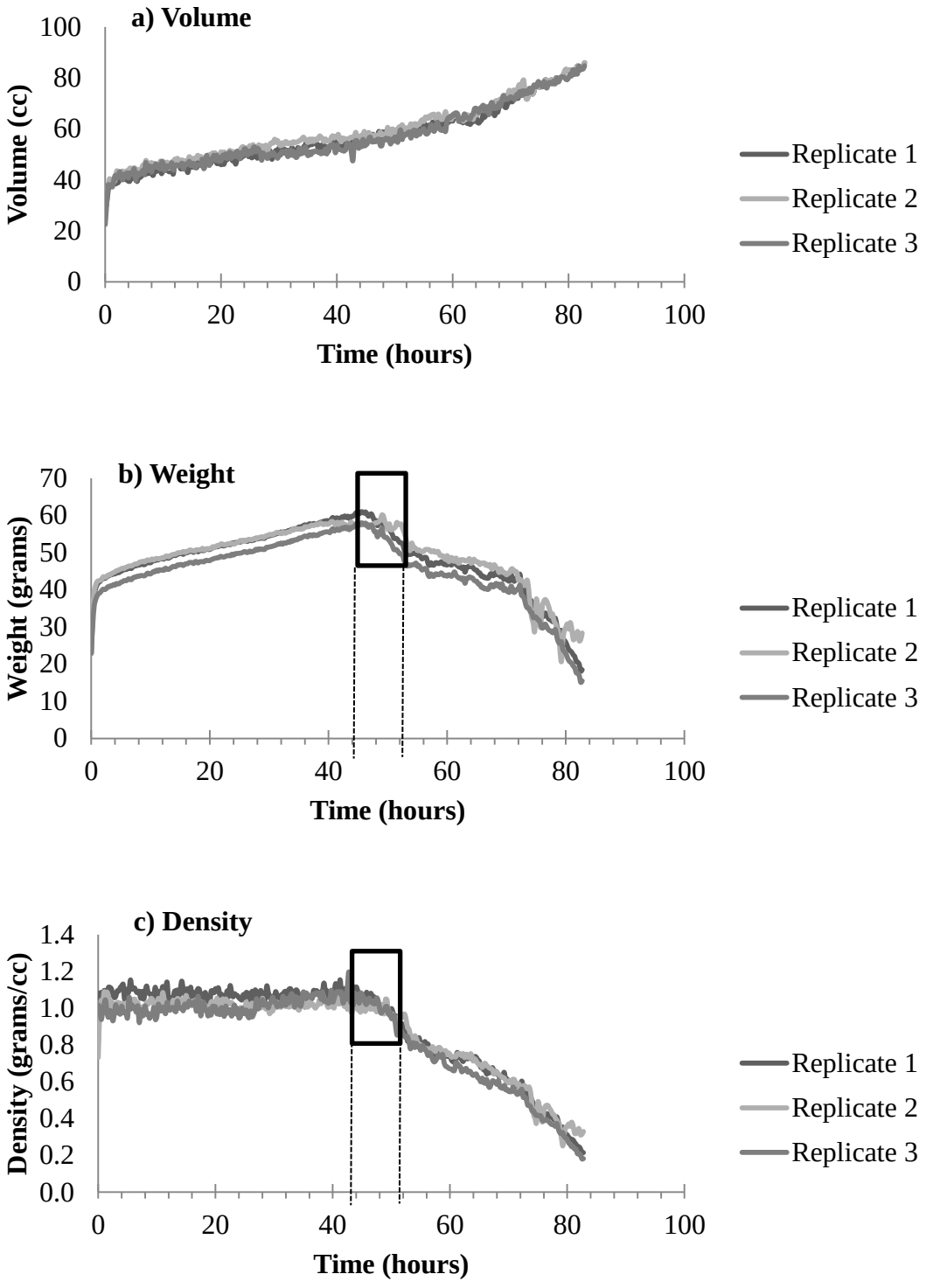

Figure 5.3. Graphs showing volume (a), weight (b) and density (c) of barley over time. The boxed region in data shows the beginning of decline of weight and density.

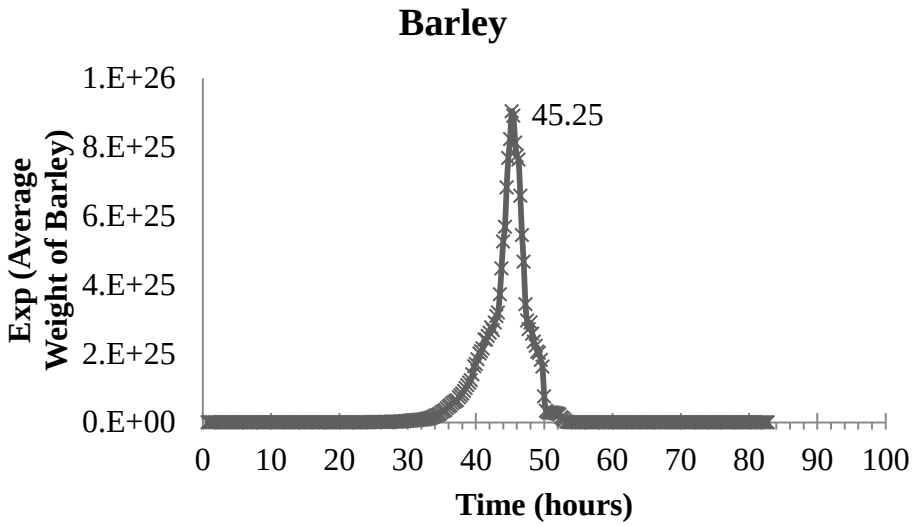

Figure 5.4. Exponential values of barley weight data over time, clearly showing distinct change in hydration profile to indicate as germination signatures, with designated highest point of this signature.

To withhold any further physiochemical changes in barley along with offsetting complete development of roots and sprout growth (Briggs, 1998).

Studies on germination time for barley varieties: The predicted germination time for three different barley varieties, Copeland, Metcalf, and Thoroughbred through exponential of weight data signatures obtained from seed hydration analyzing device as shown in figure 5.5. Varieties Metcalf and Thoroughbred had a predicted germination time of 45.25 hours. Copeland had higher germination time with 51.50 hours, indicating that it requires longer steeping time in preparation of malt when compared to other two barley varieties. The differences between Copeland and Metcalf, which are both Canadian malting barley varieties, suggest the varietal differences among same regions can impact steeping time during malt production.

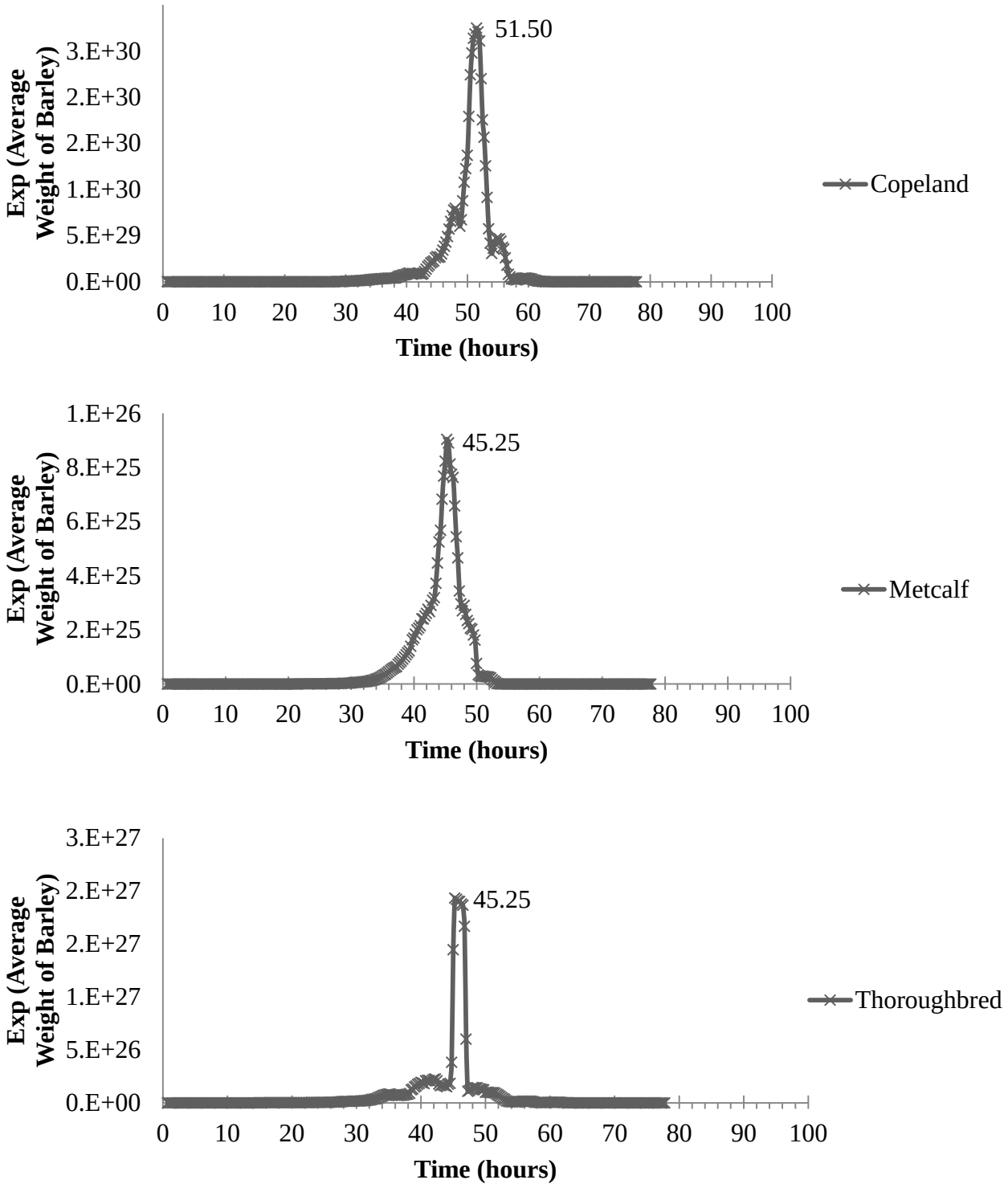

Figure 5.5. Signatures of three different barley varieties (Copeland, Metcalf and Thoroughbred) during steeping, indicating time required for completion of germination.

Studies on effect of temperature: Thoroughbred barley is used to study the effect of steep water temperatures of 16^{o} C, 18^{o} C and 20^{o} C on germination time. The exponential weight signatures of all three temperature trails are shown in figure 5.6. The data from seed hydration analyzing device suggests that steeping of barley is highly dependent on steep water temperature as indicated by different predicted germination times of 66.75 hours, 45.25 hours and 53.75 hours at 16^{o} C, 18^{o} C and 20^{o} C respectively. From the current study, steeping carried out at 18^{o} C of steep water temperature had faster germination time for barley.

Studies on effect of temperature cycle: The impact of higher or lower initial steep water temperature on germination times of Thoroughbred barley variety is studied using seed hydration analyzing device. Figure 5.7 shows the exponential weight signatures of three different temperature cycles. Cycle one and two with higher initial temperatures of 20^{o} C and 24^{o} C for first 5 hours followed by 18^{o} C for 79 hours of steeping had a predicted germination time of 52.75 hours and 83.75 hours respectively. The initial lower temperature of 1^{o} C for 5 hours followed by 18^{o} C for 70 hours of steeping had a predicted germination time of 62.75 hours. The predicted germination times of all three temperature cycles were higher than regular steep water temperature of 18^{o} C, which is for Thoroughbred variety 45.25 hours, indicating that the variable initial steep water temperature increases the germination time of barley seeds during steeping.

Studies on effect of air rest periods: Thoroughbred barley is steeped in seed hydration analyzing device for 84 hours at 18^{o} C of steep water temperature to study the effect of water immersion intervals on germination time. The exponential weight readings versus time of the rest period (immersion interval trails) are show in figure 5.8, with predicted germination time data point labeled.

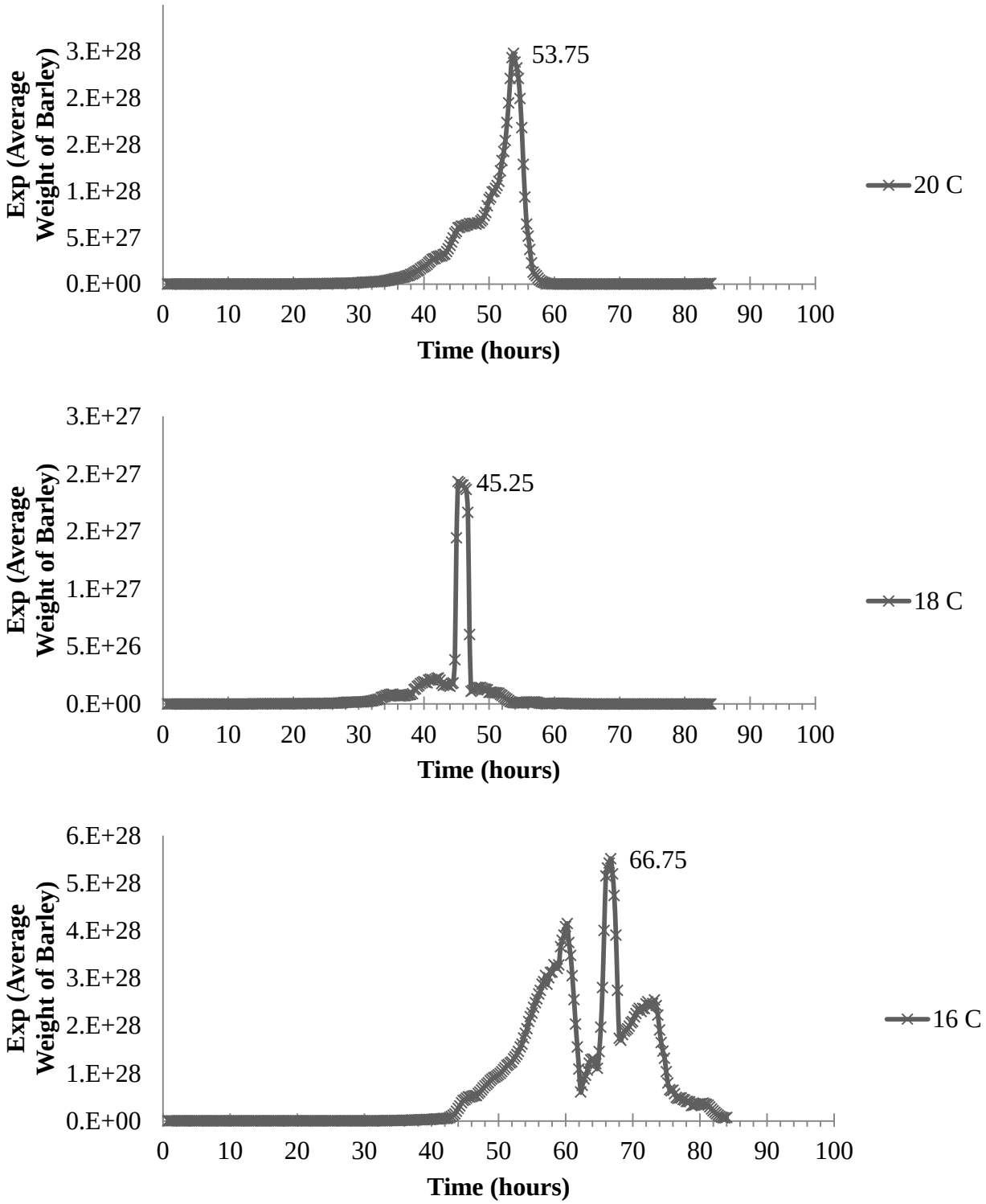

Figure 5.6. Signatures Thoroughbred barley variety at different temperatures (16^{o} C, 18^{o} C and 20^{o} C) of steeping, indicating time required for completion of germination.

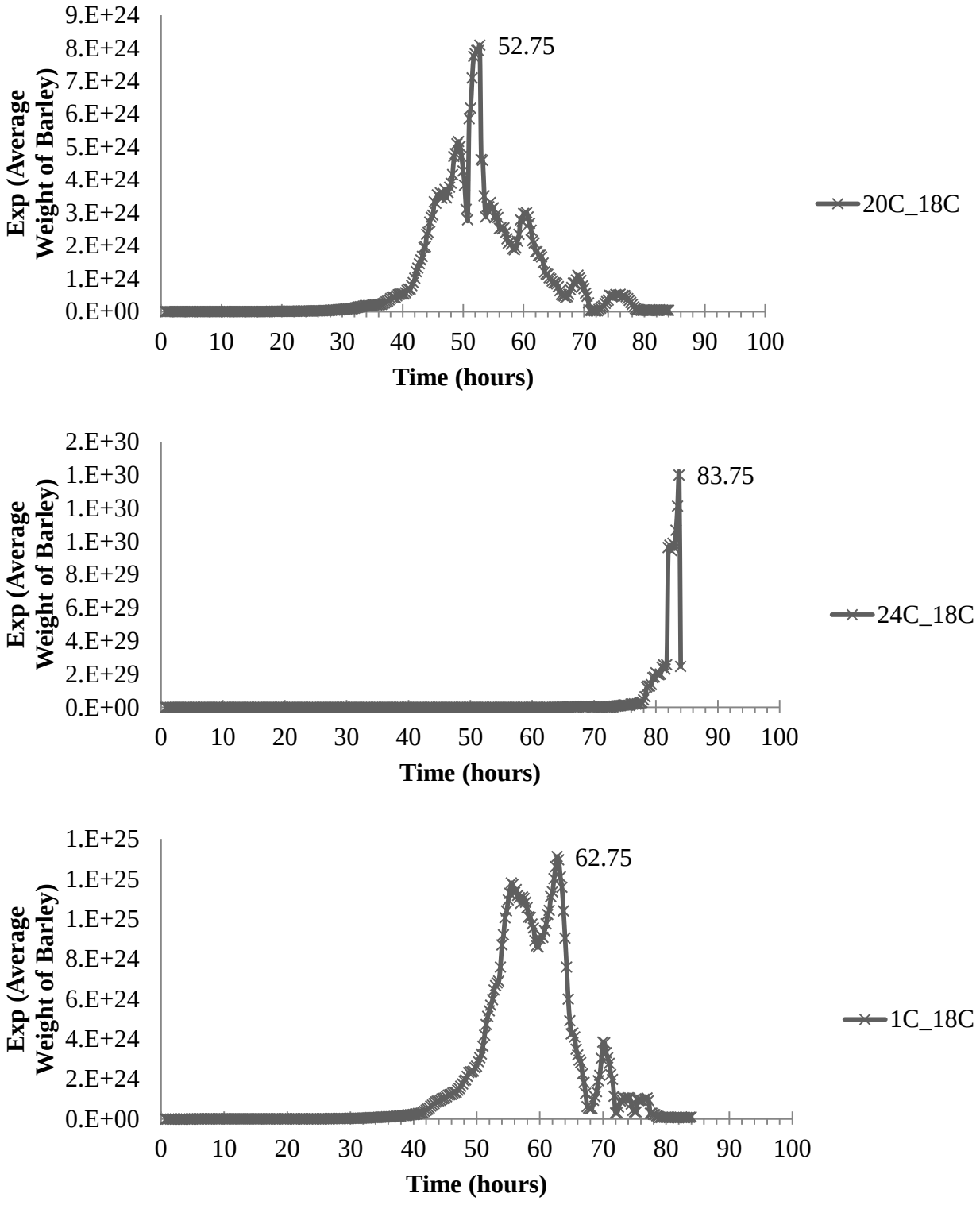

Figure 5.7. Signatures Thoroughbred barley variety at three different temperature cycles of steeping, indicating time required for completion of germination.

The germination time is lower for barley with 60 minute (45 hours), 15 minute (45.25 hours) and 30 minute (45.50 hours) rest periods, while 10 minutes (57 hours) and 20 minutes (52.50 hours) were higher. The studies indicate that the interval between water immersions of barley seeds during steeping has effect on their germination time during steeping. While shorter than 10 minutes of air rest period might deprive oxygen barley seeds, which is required for respiration, sufficient water is necessary to break down the cell walls to hydrolyze starch molecules. Thus, 15 minute rest periods for Thoroughbred barley variety could optimum immersion interval for shorter germination time.

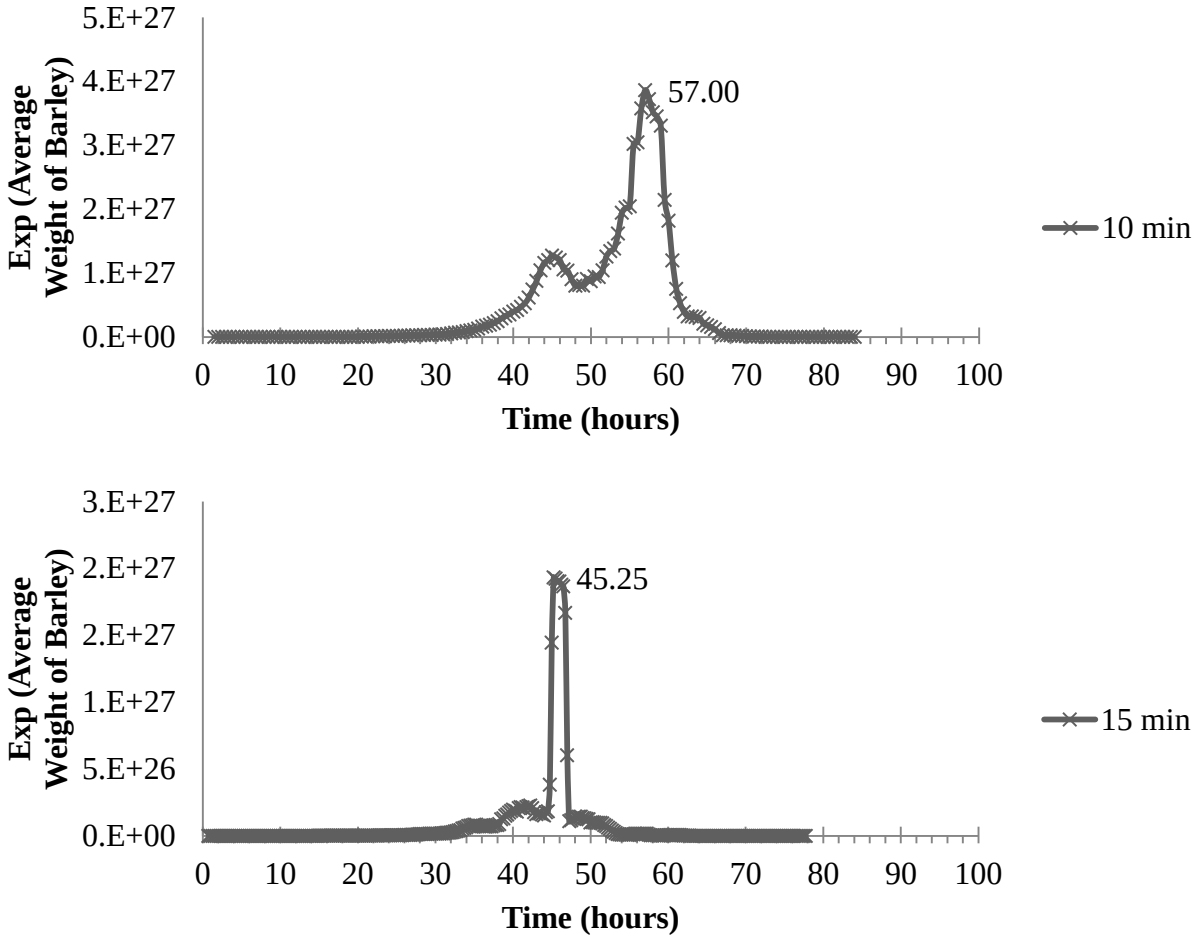

Figure 5.8: Signatures Thoroughbred barley variety at different air rest periods of steeping, indicating time required for completion of germination

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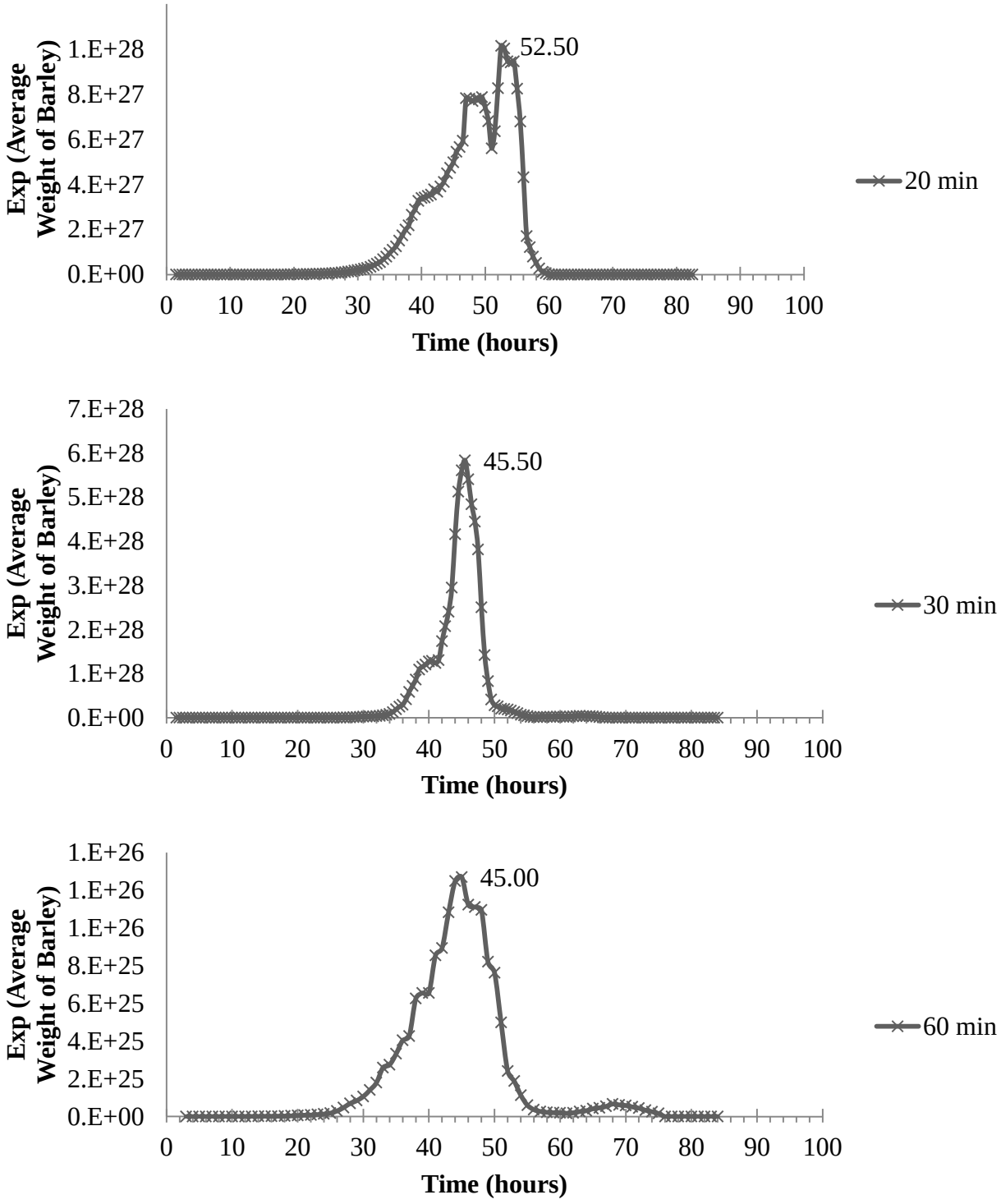

Figure 5.8: Signatures Thoroughbred barley variety at different air rest periods of steeping, indicating time required for completion of germination

Conclusion

Seed hydration analyzing device is successfully employed to understand germination time of barley seeds during steeping process of malt preparation, by accurate prediction of weight drop that occurs during higher respiration activity with onset of sprout growth. Among three barley varieties studied: the germination times of Copeland, Metcalf and Thoroughbred are 51.50 hours, 44.25 hours and 44.25 hours. Among studied steep water temperatures of 16^{o} C, 18^{o} C and 20^{o} C, 18^{o} C had the least germination time of 45.25 hours. Different steep water temperature cycles, with higher temperatures of 20^{o} C and 24^{o} C and a lower temperature of 1^{o} C followed by 18^{o} C were studied and found no improvement in germination time when compared to single temperature of 18^{o} C. Barley seeds are studied at 18^{o} C temperature with immersion intervals (air rest periods) of 10, 15, 20, 30, and 60 minutes, and germination time of 60 minutes was lowest of 45 hours, along with 15 minutes (45.50 hours) and 30 minutes (45.50 hours).

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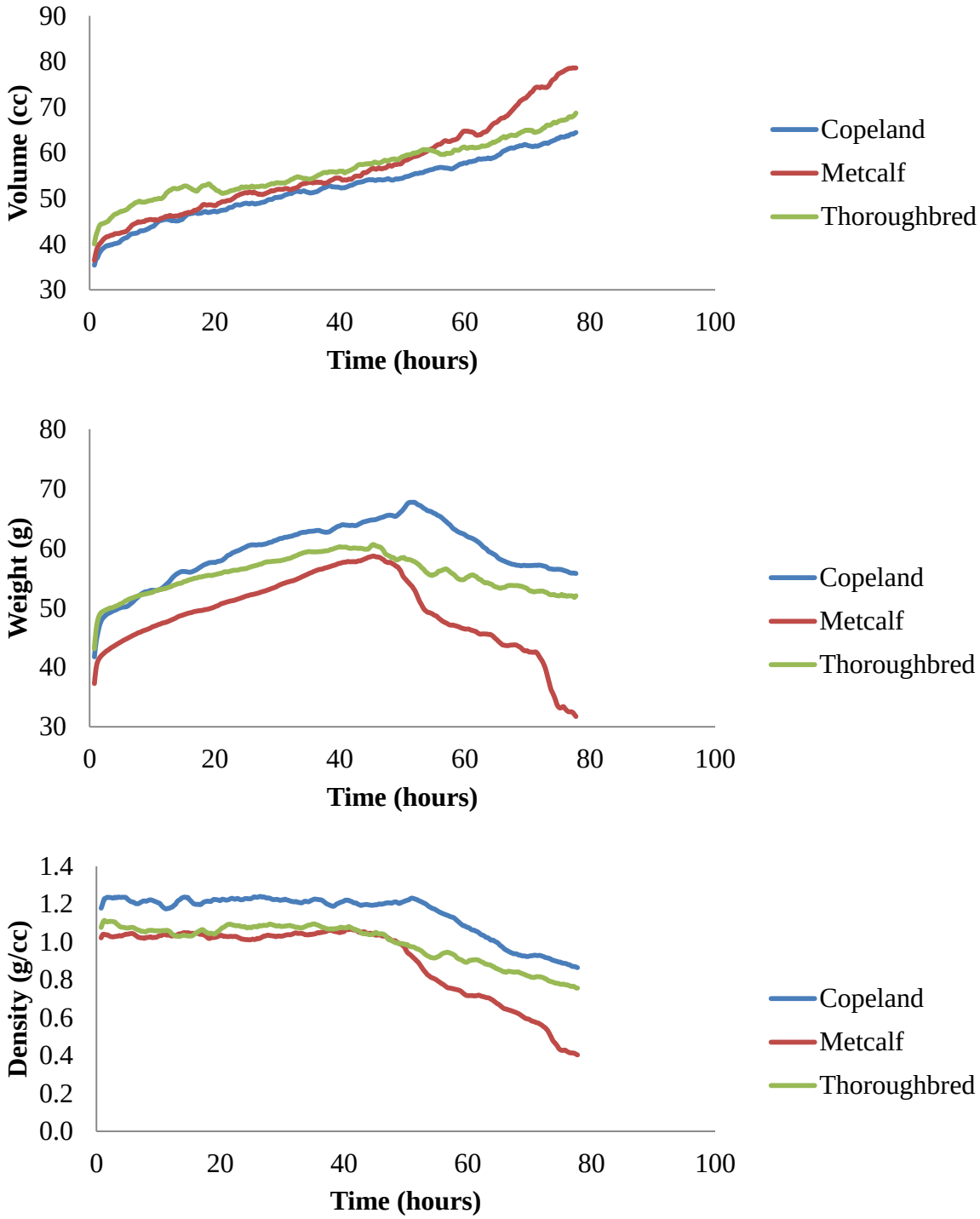
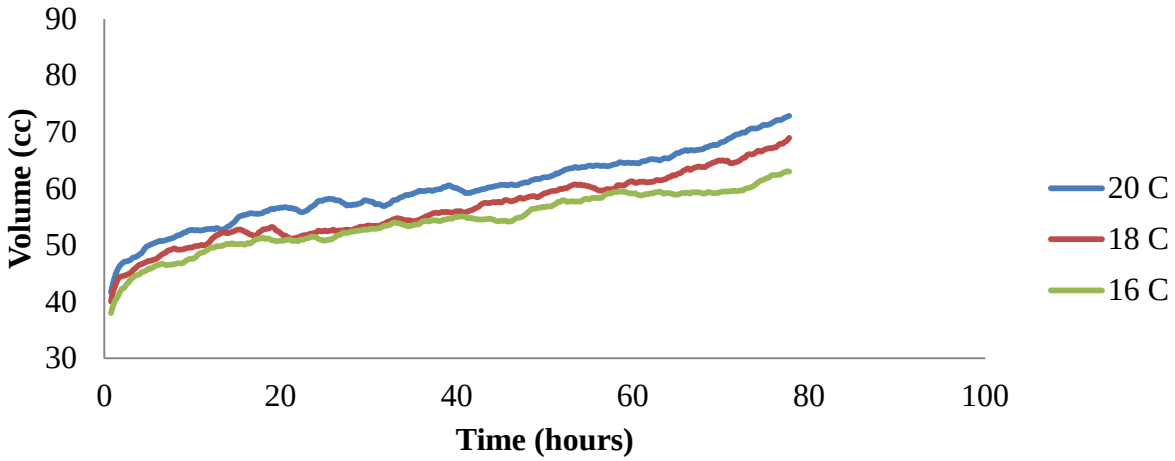
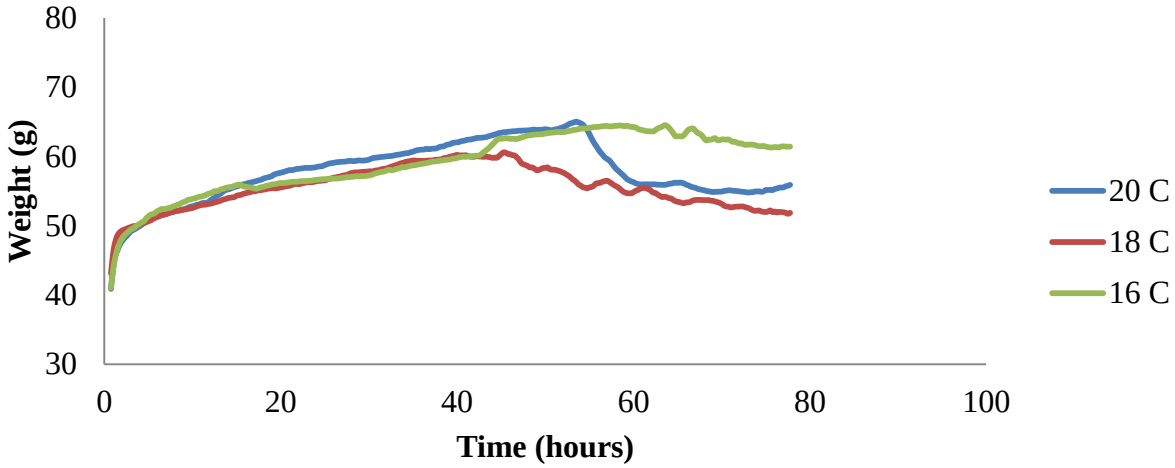
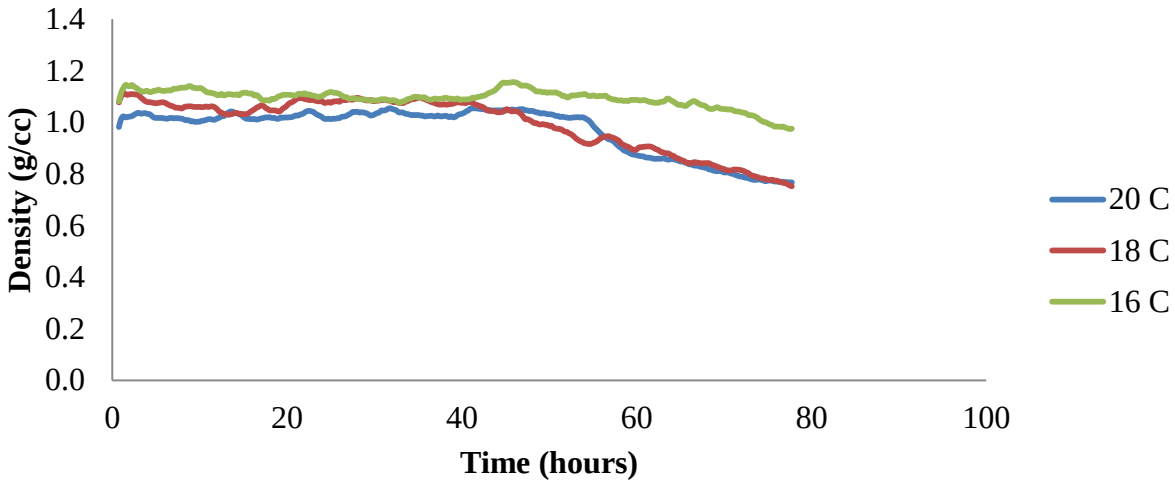
Appendix II



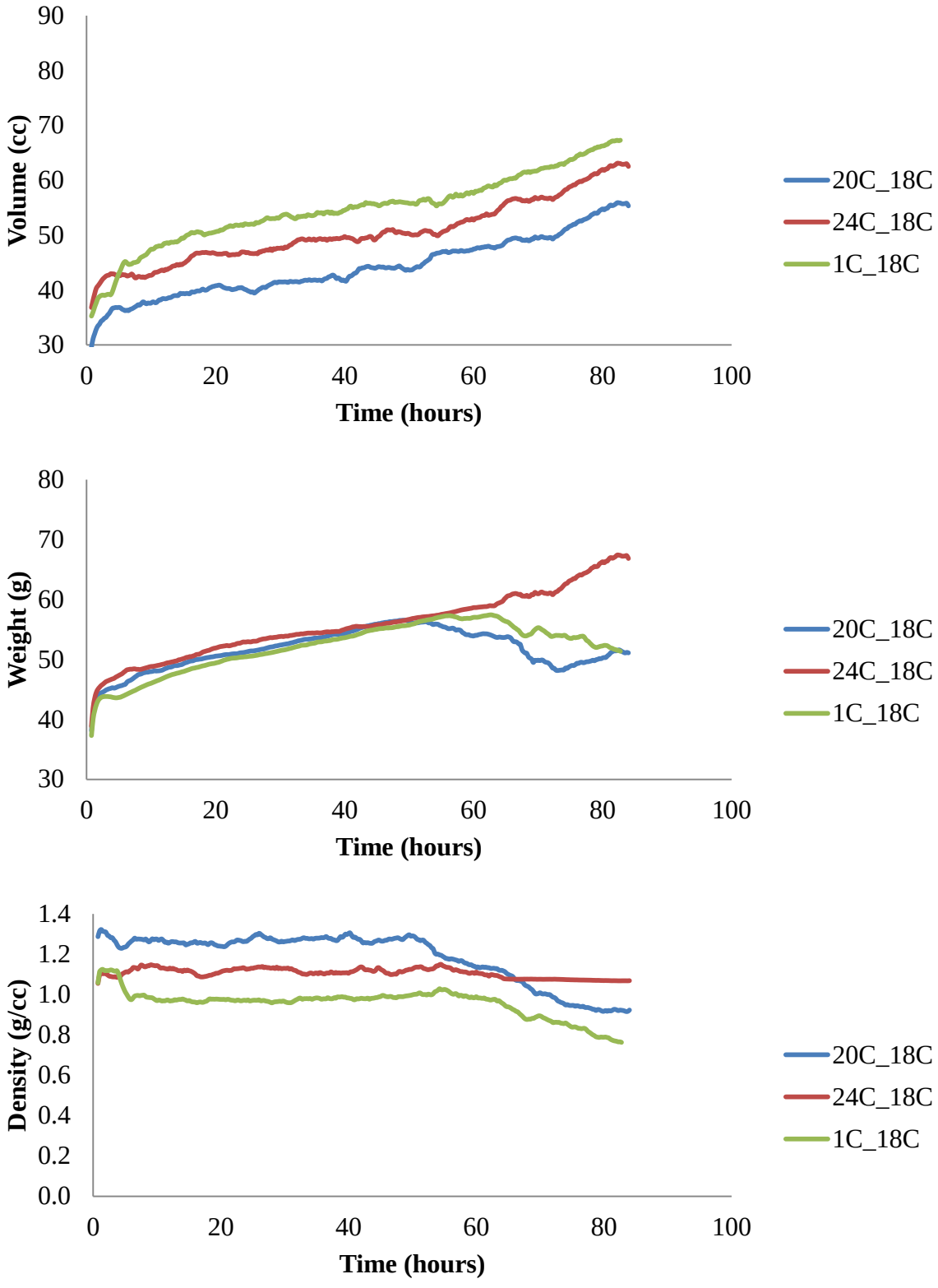
Volume, weight and density of three barley varieties Copeland, Metcalf and Thoroughbred.



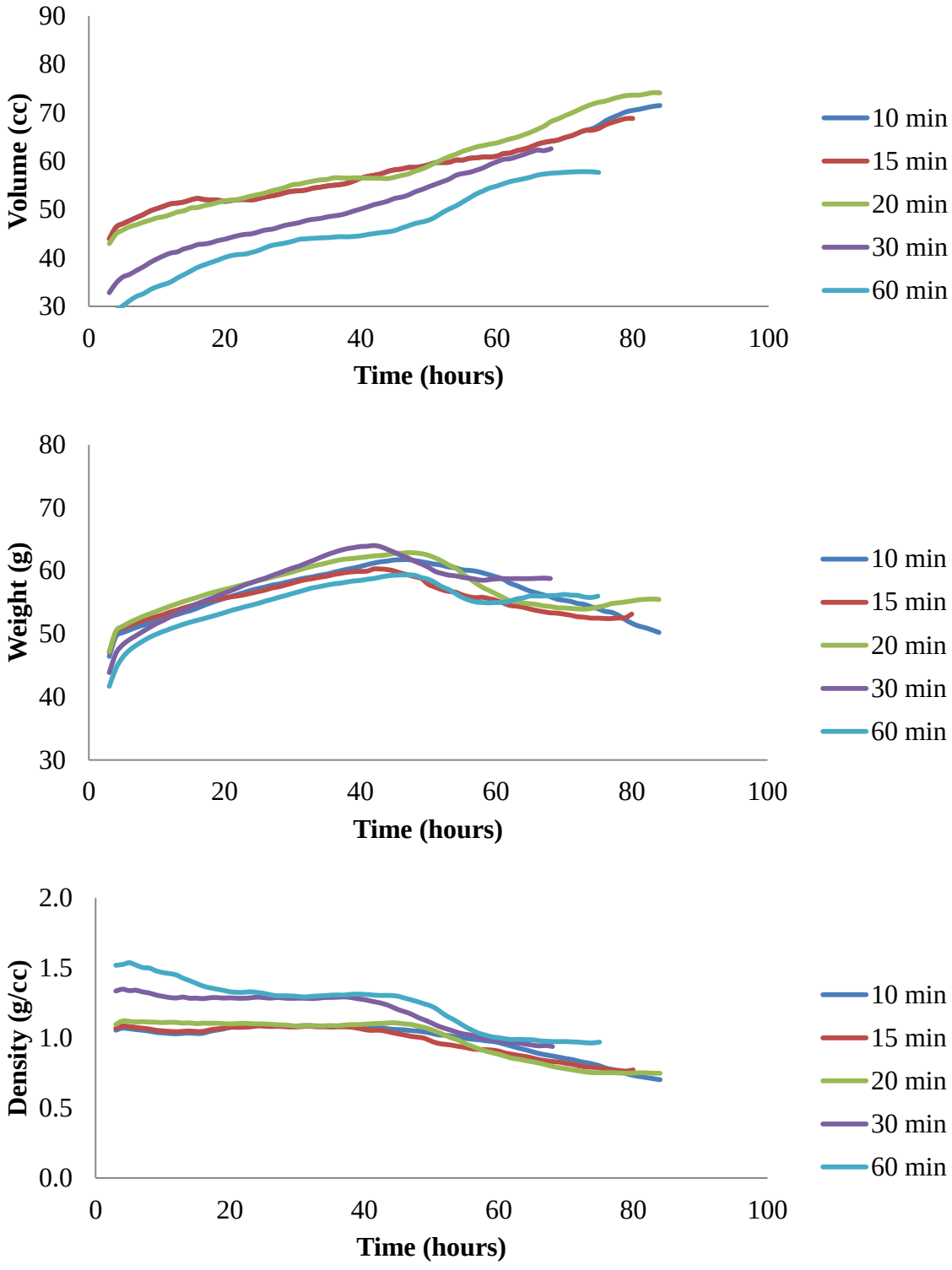




Volume, weight and density of Thoroughbred barley variety at different temperatures (16^{o} C, 18^{o} C and 20^{o} C) of steeping



Volume, weight and density of Thoroughbred barley variety at three different temperature cycles of steeping



Volume, weight and density of Thoroughbred barley variety at different air rest periods of steeping, indicating time required for completion of germination.

CHAPTER VI

Conclusion

Conclusion

An analytical tool is designed and developed to analyze hydration and dehydration cereals and legumes. The device is able to simultaneously soak seeds and measure volume, weight and density. The device also records and presents data in real time in a computer that is paired. Accuracy of the device is calculated by measuring a constant volume and weight of marbles for 2 hours at two minute interval. For volume measurements, the average accuracy of the system over 2 hour period with three replicates is 97.87%., and for weight it is 99.08%. The variability (covariance) of repeated measurements of the device is calculated to be 1.6% for volume and 0.29% for weight. Measurement variation among replicates varied from each replicates taken from three soak chambers by a maximum of 2.2% for volume and 0.2% for weight.

Navy bean, black bean and pinto bean cultivars grown in the United States have shown little or no difference in hydration profiles as determined by Weibull parameters and time required to reach 95% of equilibrium volume. International navy bean cultivars from Ethiopia, Argentina and China showed differences in hydration profiles when compared to a control navy bean cultivar. Effects of salt treatments on physiological characteristics of navy beans that are both stored and regular are studied with addition of NaCl, $CaCl_2$ to soft water. Initial experiments with addition of Na^+ and Ca^{2+} ions concentrations separately showed maximum volume at optimum ppm of NaCl, and maximum weight for optimum ppm of $CaCl_2$ for regular navy beans. Combined optimum treatments of NaCl and $CaCl_2$ have improved weight and volume of stored beans, indicating possible application of these salts in improving bean yield for low initial moisture content beans.

Theil error splitting method to analyze different empirical models is successfully demonstrated using data collected from seed hydration analyzing device with navy beans, black beans, pinto

beans, black soy beans, yellow soy beans and barley seeds. From the analysis, no one model predicted the measured volume readings for all tested seeds satisfactorily. For Weibull model worked well for all the tested seeds, although in all cases, except it had higher bias fixed error for of dry beans. Peleg model had best prediction for navy and black beans, while it was very poor with all other seeds tested. Exponential-1worked well for only barley, while exponential-2 worked well only for navy beans. Harte-Venegas model showed better prediction for pinto beans. Models Weibull and Peleg have two parameters, while rest of the models has only one parameter, which can be useful for application. Thus, from the current study, it is recommended that models that are being considered to predict hydration kinetics (volume, moisture, weight) of seeds during soaking should be thoroughly analyzed with demonstrated error splitting methods for its validity and robustness, in addition to its ability to correlate with the measured data.

Seed hydration analyzing device is successfully employed to understand germination time of barley seeds during steeping process of malt preparation, by accurate prediction of weight drop that occurs during higher respiration activity with onset of sprout growth. Among three barley varieties studied: the germination times of Copeland, Metcalf and Thoroughbred are 51.50 hours, 44.25 hours and 44.25 hours. Among studied steep water temperatures of 16^{o} C, 18^{o} C and 20^{o} C, 18^{o} C had the least germination time of 45.25 hours. Different steep water temperature cycles, with higher temperatures of 20^{o} C and 24^{o} C and a lower temperature of 1^{o} C followed by 18^{o} C were studied and found no improvement in germination time when compared to single temperature of 18^{o} C. Barley seeds are studied at 18^{o} C temperature with immersion intervals (air rest periods) of 10, 15, 20, 30, and 60 minutes, and germination time of 60 minutes was lowest of 45 hours, along with 15 minutes (45.50 hours) and 30 minutes (45.50 hours).

VITA

Vinay Kumar Mannam was born in a small town called Kanigiri in southern state of Andhra Pradesh, India. He grew up in Ongole and Hyderabad, India, where he completed high school. He joined Acharya N G Ranga Agricultural University (ANGRAU) to pursue a degree in Agricultural Engineering from College of Agricultural Engineering, Bapatla, India. While at ANGRAU he developed interest in higher education, and with encouragement of his mentors (TVS Satyanaraya, Ch VV Satayanarayana, BVS Prasad), he applied for Masters in Biosystems Engineering in the University of Tennessee, Knoxville (UTK). In the Fall of 2007, he came to UTK with a scholarship from J N TATA endowment award for higher studies and support from National Institutes of Health under Dr. Douglas Hayes. He graduated with his M. S. in Biosystems Engineering in December, 2009. During his Master's program, he was fortunate to take a course provided by Dr. Federico Harte in Food Engineering which led to his eventual decision to pursue doctorate degree in Food Science and Technology at the University of Tennessee, Knoxville with food engineering concentration. Since, fall of 2009 he worked under guidance of Dr. Harte on various projects concerning cereal and legume hydration, the fruit of which is this dissertation. In the future, he plans to pursue a career in food industry to progressively evolve innovative and advanced technology ideas and accomplish them for development of healthy and sustainable foods. In process, he would also like to care for lot of pets, travel the world, care and love his family, start new businesses and restaurants, and bring peace to earth.