RELATIONSHIPS BETWEEN PHYSICOCHEMICAL PROPERTIES AND HARD-TO-COOK PHENOMENON OF DRY COMMON BEANS

By

ROSETTE NAKALEMA

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Food Science - Master of Science

2015
ABSTRACT

RELATIONSHIPS BETWEEN PHYSICOCHEMICAL PROPERTIES AND HARD-TO-COOK PHENOMENON OF DRY BEANS

By

ROSETTE NAKALEMA

Common beans (*Phaseolus vulgaris*) are one of the most important legumes cultivated worldwide today. Despite the fact that all beans have a long cooking time, some beans cook faster than others. The objective of this study was to determine the physicochemical properties of dry common beans influencing their cooking time. Three genotypes of yellow and three of red mottled beans from both Puerto Rico and Tanzania locations were used in the study. In order to ascertain the relationships among physiochemical properties and cooking times, the properties such as seed coat weight and thickness, calcium and magnesium contents, protein content, starch content, starch gelatinization and protein denaturation temperatures, and pectin content were determined.

Ascending order of cooking time for yellow bean varieties was ADP-521, ADP-111 and ADP-513, and for red mottled bean varieties was ADP-443, ADP-436 and ADP-434. There was no significant difference in cooking time of soaked and dehulled studied bean varieties. Country of origin had no effect on the trend of cooking time. Among the studied physicochemical properties, seed coat thickness, starch content, total pectin content, and hot water soluble pectin content each was associated with cooking time of the studied dry bea
ACKNOWLEDGEMENTS

I am very much grateful to the Almighty God for his grace, love and care throughout the two years at Michigan State University. My sincere gratitude goes out to MasterCard Foundation Scholars program for offering me a great opportunity to study here at MSU.

My earnest appreciation goes to my adviser Professor Perry Ng and my research committee members, Dr. Karen Cichy and Professor Gale Strasburg, for all their guidance, encouragement and supervision they offered in accomplishing this work. I am appreciative of their confidence in me, patience, correction and aid during the course of this project. Great thanks go to Dr. YongFeng Ai, Dr. Jin Yining, Yasmin Salat and Jason Wiesinger for sharing with me their technical expertise and guidance. Heartfelt gratitude goes to my family and friends who have given me pecuniary, devout and moral support even when I was a thousand miles away.
TABLE OF CONTENTS

LIST OF TABLES ..................................................................................................................... vii

LIST OF FIGURES ................................................................................................................ viii

KEY TO ABBREVIATIONS .................................................................................................... x

CHAPTER 1 ............................................................................................................................... 1

INTRODUCTION AND PROBLEM STATEMENT ................................................................... 1

CHAPTER 2 ............................................................................................................................... 4

LITERATURE REVIEW ........................................................................................................... 4

2.1. Physicochemical properties influencing cooking time of beans ........................................ 4

2.1.1 Introduction .................................................................................................................. 4

2.1.2. Bean seed coat ........................................................................................................... 4

2.1.3. Bean cotyledon .......................................................................................................... 8

2.1.4. Proteins ..................................................................................................................... 8

2.1.5. Starch ......................................................................................................................... 10

2.2. Proposed mechanisms of the HTC Phenomenon ............................................................. 13

2.3. Factors influencing the HTC phenomenon ..................................................................... 15

2.3.1.1 Time ....................................................................................................................... 15

2.3.1.2. Relative Humidity and Temperature .................................................................. 15

2.3.2.1 Polyphenolic compounds ................................................................................. 16

2.3.2.2. Phytic acid ........................................................................................................... 18

2.3.2.3. Dietary fiber ......................................................................................................... 19

2.3.2.4. Pectin .................................................................................................................... 20

2.3.2.5. Divalent cations ................................................................................................. 21

2.3.2.6. Starch content and starch gelatinization temperature .................................... 25

2.4. Methods used to reduce cooking time of dry common beans .......................................... 26

2.4.1. Soaking ..................................................................................................................... 26

2.4.2. Micronization ........................................................................................................... 27

2.4.3. Dehulling .................................................................................................................. 27

2.5. Knowledge gaps ............................................................................................................ 28
LIST OF TABLES

Table 1. Overall Mineral Content of Phaseolus vulgaris (Common beans). Source: Salunkhe and Kadam (1989) .................................................................24

Table 2. Cooking times of raw tempered yellow (Y) and red mottled (R) beans from Puerto Rico (PR) and Tanzania (TZ); beans were raw (not soaked or dehulled), soaked, or dehulled before cooking

Table 3. Seed coat weight (%) and thickness (µm) of fast, moderate and slow cooking yellow and red mottled beans from Puerto Rico and Tanzania

Table 4. Correlation coefficients between cooking time of raw common beans and their physicochemical properties: protein content, gelatinization temperature, denaturation temperature, seed coat weight, seed coat thickness, magnesium content, and calcium contents, starch content, hot water soluble pectin, hot water insoluble pectin, and total pectin for dry common beans from Tanzania and Puerto Rico locations

Table 5. Calcium and magnesium contents of whole seeds of fast, moderate, and slow cooking yellow and red mottled common dry beans from Puerto Rico and Tanzania

Table 6. Protein concentration of fast, moderate, and slow cooking yellow and red mottled common dry beans varieties from Puerto Rico and Tanzania

Table 7. Starch contents of raw fast, moderate, and slow cooking yellow and red mottled dry common beans from Puerto Rico and Tanzania

Table 8. Hot water soluble pectin (HWSP), hot water insoluble pectin (HWIP), and total pectin (Total) of six fast (F), moderate (M), and slow (S) cooking common bean varieties of yellow (Y) and red mottled (R) beans grown in Puerto Rico and Tanzania locations

Table 9. Starch gelatinization and protein denaturation temperatures of dry common bean varieties of yellow and red mottled beans from Puerto Rico and Tanzania

Table 10. Correlation coefficients among physicochemical properties of dry common beans: cooking time (CK) protein content (PC), protein solubility (PS), Gelatinization temperature (GT), Denaturation temperature (DT), seed coat weight (SCW), seed coat thickness (SCT), magnesium content (MC) and calcium content (CA), starch content (SC), HWSP, HWSIP and total pectin (PC) for dry common beans
LIST OF FIGURES

Figure 1. A cross section of a mature broad bean seed with one cotyledon removed. Source: Salunkhe and Kadam (1991)................................................................. 5

Figure 2. A microstructure of mung bean seed coat and cotyledon: PC= Palisade cells; MC= Mesophyll cells; SP= Spongy cells. Source: Salunkhe and Kadam (1991)................................. 7

Figure 3. Scanning electron microscope (SEM) photomicrographs for chick pea cotyledon; (A) dry and (B) soaked; S-starch globule, ECS-Extra cellular space. Source: Tiwari and others (2011)........................................................................ 9

Figure 4. Chemical structure of amylose and amylopectin Source: Tester and others (2004). .... 12

Figure 5. Scanning Electron Microscope images of the middle lamella between three cells of bean cotyledon stored under different conditions for 6.5 months. (A) Control (5°C/40% RH) and (B) HTC (35°C/75% RH). Source: Mohan and others (2011).................................................................. 14

Figure 6. The polyphenol units commonly found in common beans. Source: Petry and others, (2015)........................................................................................................ 17

Figure 7. Dry common bean genotypes used in the study. ................................................. 31

Figure 8. Cross sections of raw beans mounted on aluminum stubs in a Turbo Pumped Coater for platinum coating........................................................................... 34

Figure 9. SEM image of a cross section of a raw ADP-513-S common bean seed from Puerto Rico with four seed coat thickness measurements indicated (in orange). ......................... 35

Figure 10. Cross-sections of seed coats for fast, moderate and slow cooking common beans by Scanning Electron Microscope (SEM). Y-521-F = fast cooking, Y-111-M = moderate cooking and Y-513-S = slow cooking for yellow beans. R-443-F = fast cooking, R-436-M = moderate cooking and R-434-S = slow cooking for red mottled beans ........................................... 53

Figure 11. The peak gelatinization (first peak) and peak denaturation (second peak) temperatures of dry common beans from Tanzania. ................................................................. 74

Figure 12. The peak gelatinization (first peak) and peak denaturation (second peak) temperatures of dry common beans from Puerto Rico. ................................................................. 75

Figure 13. Cooking time of raw tempered yellow (Y) and red mottled beans (R) from Puerto Rico (PR) and Tanzania (TZ) that were raw (not soaked or dehulled), soaked and dehulled before
cooking. Data represent means and standard deviations. Values of each bar topped by the same letter are not significantly different ($P \leq 0.05$) from each other.

Figure 14. Hot water soluble pectin (HWSP), hot water insoluble pectin (HWIP), and total pectin (Total) of six fast (F), moderate (M), and slow (S) cooking common bean varieties of yellow (Y) and red mottled (R) beans grown in Puerto Rico and Tanzania locations. Data represent means and standard deviations of n=2. Values of each bar topped by the same letter are significantly different ($P \leq 0.05$) from each other.
KEY TO ABBREVIATIONS

ADP- Andean Diversity Panel

HTC - Hard-to-cook

ETC - Easy-to-cook

HWSP - Hot water soluble pectin

HWIP - Hot water insoluble pectin

TP – Total Pectin

SEM - Scanning Electron Microscope

Tg - Gelatinization temperature

Td – Denaturation temperature
CHAPTER 1

INTRODUCTION AND PROBLEM STATEMENT

Common beans (*Phaseolus vulgaris* L.) are one of the most important legumes cultivated worldwide today (El-Tabey Shehata, 1992) and a major source of food to large populations of people in the world. They originated from South and Central America. There are different market classes of beans which are differentiated according to seed size, color and shape. Examples include yellow beans, red mottled beans, pinto beans, kidney beans, black beans, etc. They are a good source of dietary protein, fiber, starch, vitamins and minerals (Kaur and others, 2013).

Unlike fresh common beans, dry beans require a long cooking time, which has been a problem for centuries. Cooking dry beans is time consuming and, most importantly, energy consuming. Beans being a staple food commonly eaten as a protein source, they are the biggest component of most school children’s diet in Uganda. In most African countries, particularly Uganda, firewood is the main source of energy for cooking since the biggest percentage of population cannot afford gas and electricity. This implies that a lot of trees are cut down per day for this cause, which has detrimentally degraded the environment. For developed countries that mainly use gas or electricity, cooking beans is costly since more gas or electricity is needed to cook dry beans than other foods like rice or beef. This justifies the importance of studying relationships among cooking time and physiochemical properties of dry beans in hopes of eventually producing varieties with shorter cooking times.

Past studies have proven that long cooking time of dry beans is a result of beans hardening during storage. This is known as the hard-to-cook (HTC) phenomenon. Long storage time, high
temperatures and high relative humidity are factors that were reported to enhance the hardening phenomenon of beans (Aguilera and Stanley, 1985). There are two possible mechanisms by which storage hardens the beans: (1) pectin-phytate crosslinking in the cell walls and (2) lignification (Aguilera and Stanley, 1985). The latter is a result of deposition of lignin-like material into the cell walls from the seed coat, thus hardening them (Stanley and others, 1989; Stanley, 1992). Pectin-phytate crosslinking is promoted by high temperature and relative humidity storage conditions. Under these conditions, phytic acid undergoes hydrolysis, releasing the bound divalent cations, i.e., calcium and magnesium. These cations then move to the middle lamella where they participate in crosslinking of the enzymatically demethoxylated pectin substances and phenolic compounds, thus hardening the cell walls (Del Valle and Stanley, 1995). This hardening of the bean cell walls increases the bean’s cooking time. In an effort to save energy and time, several methods have been adopted to reduce the cooking time (i.e., easy-to-cook, ETC, methods), such as soaking (in water, sodium and potassium salt solutions), micronization, pressure cooking, and dehulling.

The HTC effect develops in all dry beans regardless of the variety. However, some varieties have inherently longer cooking times than others. Maryange and others (2010) reported a significant difference in cooking time among 30 bean lines that were tested, with times ranging from 29-83 minutes using the Matson method (Maryange and others, 2010). These differences were maintained even when dry beans were preconditioned (soaking of dry beans in distilled water for 4 hours) before cooking. Cooking time of beans is said to be a genetic trait (Singh, 1999), but it is also associated with factors such as seed size, seed coat color and thickness, the cotyledon and seed coat chemical compositions, and the size of the micropyle and hilum (Mkanda and others,
2007). However, it is still not fully understood why some varieties cook faster than others. The aim of this study was to understand if the physicochemical properties influencing the HTC mechanism are associated with the varietal differences in cooking time of different market classes of dry common beans. Information generated from this study could be used by plant breeders to perform targeted genetic engineering in order to develop bean varieties with shortened cooking times.
CHAPTER 2
LITERATURE REVIEW

2.1. Physicochemical properties influencing cooking time of beans

2.1.1 Introduction

A mature dry common bean seed (hereafter referred to as bean) is mainly comprised of two cotyledons and a seed coat. The cotyledons comprise 80-90% and the seed coat 8-20% of the total seed weight (Sathe and Deshpande, 2003). There are different market classes of beans, which are differentiated according to seed size, color and shape. The cooking time of beans is greatly influenced by both physicochemical properties and environmental factors. The physicochemical properties include seed size, seed coat color and thickness, the cotyledon and seed coat chemical compositions, and size of the micropyle and hilum (Mkanda and others, 2007). The environmental factors are temperature and relative humidity of the storage environment.

2.1.2. Bean seed coat

A seed coat is an outer layer of a bean seed (Figure 1) that protects the inner part of the seed from the external environment (Reyes-Moreno and Paredes-Lopes, 1993). It is rich in minerals such as calcium and iron (Sathe and Dashpande, 2003). In addition, the cell walls of the seed coat are reported to have high amounts of fiber including cellulose (59.4 to 60.7%), hemicelluloses (17.4 to 25.8%), pectin substances (11.1 to 15.9%) and lignin (1.4 to 1.9%).
Figure 1. A cross section of a mature broad bean seed with one cotyledon removed. Source: Salunkhe and Kadam (1991)
The seed coat has also been reported to contain phenolic compounds, especially for the colored common beans (*Phaseolus vulgaris*), e.g., red, black and bronze beans (Bressani and Elias, 1980). Depending on the variety of the bean, the microstructure of the seed coat may have amorphous palisade cells (Figure 2) as observed in the black eyed peas, or organized palisade cells, as observed in adzuki beans (Dedeh and Stanley, 1979). The organized palisade cells could be related to thick seed coats as a result of old age/maturity (Sathe and Deshpande, 2003). On the other hand, the amorphous palisade cells could be associated with thin seed coats with loosely packed palisade cells (Sefa-Dedeh and Stanley, 1979). Each seed coat has a micropyle (Figure 1), a small pore through which water enters the seed for germination. This pore, too, plays a role in hydration of the seed. Agbo and others (1987) reported that beans with open micropyles and pores in their seed coats showed rapid water uptake as compared to those with closed micropyles and no pores in the seed coat. Also, thick seed coats are associated with low water uptake since the packed palisade cells act as a barrier to water penetration. In addition, thick seed coats were reported to be associated with high lipid content of seed coats (Sathe and Deshpande, 2003) which would further hinder hydration of the bean cotyledon. This would imply that beans with thick seed coats could have longer cooking times than those with thin seed coats. In contrast, Pirhayati and others (2011) reported that ETC beans had thicker seed coats (38.00 μm) than HTC beans (32.00 μm), and therefore cooking time was not necessarily attributed to seed coat thickness. In addition, there were no significant differences between the microstructures of the seed coats of the ETC and HTC beans in their study. On the contrary, Bhatti (1995) reported structural differences between the microstructures of seed coats of HTC and ETC lentils. More research is needed to clarify these contradictions.
Figure 2. A microstructure of mung bean seed coat and cotyledon: PC= Palisade cells; MC= Mesophyll cells; SP= Spongy cells. Source: Salunkhe and Kadam (1991)
2.1.3. Bean cotyledon

A common bean seed is composed of two cotyledons covered with a seed coat. The cotyledons contain parenchyma cells (Figure 3) which are the storage units for most of the seed’s nutrients, e.g., protein bodies and starch granules (Sefah-Dedeh and Stanley, 1979b). Each cell wall contains 15 to 25% protein, 50 to 75% carbohydrates and 0.4 to 0.6% lignin (Sathe and Deshpande, 2003). Reyes and Paredes-Lopez (1993) reported that cotyledon cell walls also contain non-starch polysaccharides including cellulose (25.9 to 30.9%) and, pectin substances (28.5 to 41.2%) in the middle lamella.

2.1.4. Proteins

The common bean (Phaseolus vulgaris L.) contains 18-25% proteins, most of which are salt-soluble globulins (45 to 70%) and water-soluble albumins (10 to 30%) (Chung and others, 2008). Proteins play a major role in water absorption in the parenchyma cells of the cotyledon. However, the cells do not have direct contact with water because of the seed coat which is a barrier to water movement (Sefah-Dedeh and Stanley, 1979b). Proteins form a matrix around the starch granules, which hinders hydration of the granules. The different packing densities of the parenchyma cells also affect hydration (Jones and Boutler, 1983). Bernal-Lugo and others (1997) discovered that an easy-to-cook (ETC) bean (variety Michigan 800) had a higher protein content (252.4 mg/g) than a HTC bean (variety Ojo de Cabra, 219.0 mg/g).
Figure 3. Scanning electron microscope (SEM) photomicrographs for chick pea cotyledon; (A) dry and (B) soaked; S-starch globule, ECS-Extra cellular space. Source: Tiwari and others (2011).
It is not known however, if other genotypes of the same market classes would behave in the same way in terms of cooking time. If protein content does not have an influence on cooking time, perhaps its functional properties such as denaturation temperature could play a role. Reyes-Moreno and others (1994) reported that ETC beans (variety Michigan 800) had a lower protein denaturation temperature (88.6°C) than that of HTC beans (variety Ojo de Cabra, 95.3°C), and indicated that this affected the cooking time of beans. On the contrary, Bernal-Lugo and others (1997) reported that both ETC and HTC beans had similar protein denaturation temperatures (103°C and 104°C, respectively) and therefore protein denaturation temperature did not appear to contribute to cooking time.

The other functional property that may influence cooking time of beans is protein solubility. Coelho and others (2011) reported that in their studied beans, a decrease in soluble proteins was associated with increases in storage time and cooking time. They concluded that the high storage temperature induced protein denaturation, which caused coagulation of protein thus reducing its solubility. It is not known if common beans stored under similar conditions (e.g., temperature and time), but with different cooking times, will exhibit similar protein solubility properties.

2.1.5. Starch

Legumes contain about 13-49% starch content on dry matter basis. Starch is the most abundant carbohydrate in the common bean, constituting about 22-45% by weight (Chibbar and others, 2010). It is a polymer of a few thousands of D-glucose units and it consists mainly of two polysaccharides, amylose and amyllopectin (Figure 4). Amylose is a linear molecule composed of
D-glucose units linked by $\alpha$ 1-4 linkages. Amylopectin is a branched molecule of D-glucose units with about 5% $\alpha$ 1-6 branch linkages. It has a molecular weight of $10^9$ g/mole (Jane, 2012).
Figure 4. Chemical structure of amylose and amyllopectin Source: Tester and others (2004).
2.2. Proposed mechanisms of the HTC Phenomenon

The HTC phenomena of common beans are attributed to various mechanisms, with “lignification” (cell wall thickening) and pectin-phytate crosslinking” being the most possible mechanisms (Hincks and Stanley, 1987). These mechanisms result in increased difficulty in achieving cell separation during cooking (Stanley and Aguilera, 1985). Soft texture of the seed coat simplifies separation of cell walls during cooking of beans, which signifies the role of the middle lamella and cell wall in this phenomenon (El-Tabey Shehata, 1992). This was confirmed when Garcia and others (1998) reported with scanning electron micrographs of common bean cell walls stored at 5°C/40% RH and 35°C/75% RH which showed thickening of the middle lamella (Figure 5).

Pectin-phytate crosslinking is promoted by high temperature and relative humidity storage conditions. Under these conditions, pectin methylesterase (PME) hydrolyses pectin molecules forming pectic acid and methanol. Also, phytase hydrolyses phytic acid releasing the bound divalent cations, i.e., calcium and magnesium. These cations then migrate to the middle lamella where they react with pectic acid and phenolic compounds forming insoluble substances thus hardening the cell walls (El-Tabey Shehata, 1992).
Figure 5. Scanning Electron Microscope images of the middle lamella between three cells of bean cotyledon stored under different conditions for 6.5 months. (A) Control (5°C/40% RH) and (B) HTC (35°C/75% RH). Source: Mohan and others (2011).
2.3. Factors influencing the HTC phenomenon

2.3.1.1 Time

The cooking time of dry beans depends not only on the genotype of the common bean (Singh, 1999), but also on storage conditions, including storage time, temperature and relative humidity (Nasar-Abbas and others, 2008). Duration of storage of beans is one of the main factors influencing cooking time of beans. The longer the storage time, the harder the texture of the dry beans. However, rate of bean texture hardening depends on the storage conditions such as relative humidity and temperature. Moscosco and others (1984) reported an increase in hardness with increasing storage time of red kidney beans stored for 9 months. Furthermore, Curtis (1991) studied a relationship between bean texture hardness and soaking time before cooking, where fresh and aged beans were soaked for 0, 20 and 44 hours and their texture hardnesses determined. Aged beans were observed to have the greatest hardness at each of the soaking times.

2.3.1.2. Relative Humidity and Temperature

Jones and Boulter (1983) reported that high relative humidity (RH) of the storage environment is an initiation stage of the hardening effect of beans. It allows metabolic breakdown of the cell wall allowing bivalent ions from hydrolyzed phytate to access pectin making the latter insoluble. Hentges and others (1991) studied the effects of storage temperature and humidity on physical and chemical components of beans. They reported that seeds stored at 29°C and 65% RH required a longer cooking time than seeds stored at 5°C and 30% RH, or 29°C and 30% RH, or 5°C and 65% RH. There is a linear relationship between HTC effect and high storage temperature. Nasar-Abbas and others (2008) reported a linear increase in the HTC effect on Faba beans with high storage
temperatures of > 37°C. In addition, Reyes-Moreno and others (2001) reported a long cooking time of chick peas after storing them at temperatures > 25°C and relative humidities > 65%. Furthermore, storage of beans under high temperature and relative humidity conditions was reported by Kon and Sanshuck (1981) to have increased cooking time by about 5-fold. Similarly, prolonged storage of dry beans at high temperatures (30-35°C) and high relative humidities (60-80%) has been reported to elevate the HTC phenomenon (Taiwo, 1998). It is not clear which one of the three factors (time, temperature, or relative humidity) is the major contributor to the HTC effect.

2.3.2.1 Polyphenolic compounds

Polyphenolic compounds are a heterogeneous class of compounds derived from secondary plant metabolism. They protect the plant against pathogens and UV radiation; they are also responsible for pigmentation in beans. In addition, they play an important role in pollination by insects (Petry and others, 2015). Some of the polyphenols present in common beans include phenolic acids, anthocyanidins, and flavonoids (Figure 6) (Petry and others, 2015). Flavanols are commonly referred to as proanthocyanidins or condensed tannins; they are the major polyphenols found in colored beans and are located in the seed coat (Reddy and others, 1985). Diaz and others (2010) determined polyphenol concentration in bean seed coats and they reported an average concentration of about 20% of seed coat weight, with an overall average of about 20% in 250 bean varieties.
Figure 6. The polyphenol units commonly found in common beans. Source: Petry and others, (2015).
Phenolic compounds are also responsible for the browning of the seed coat of legumes during storage (Nozzolillo and Bezada, 1984). Browning is ascribed to the polymerization of soluble tannins forming insoluble brown high molecular weight tannins. The presence of condensed tannins in the seed coat of lentils was supported by the deep color (brown) of not-soaked seeds after four days of storage as compared to the yellow color of the presoaked seeds after the same storage time. Phenolics in these lentil samples were abundant in the not-soaked seeds and most originated from the seed coat (Nozzolillo and Bezada, 1984).

2.3.2.2. Phytic acid

Phytic acid is a natural reservoir of phosphorous in the bean seed and it has been implicated in influencing the HTC effect in legumes. Stanley and Aguilera (1985) reported that phytic acid chelates divalent ions (Ca\(^{2+}\) and Mg\(^{2+}\)), hindering them from precipitating pectic acid in the cell wall. More evidence was reported by Chang and others (1997) who studied the phytase enzyme (myo-inositol hexakisphosphate phosphohydrolase, EC. 3.1.3.8). The activity of this enzyme increases with storage time at higher temperatures and relative humidities. Phytase hydrolyses phytate, releasing the divalent cations that move to the cell walls where they precipitate pectic acid and phenolic compounds resulting in insoluble substances forming in the middle lamella, thus hardening the bean. Upon blanching the beans before storage, the hardening effect was reduced, implying that the enzyme had been inactivated (Vindiola and others, 1986). Bhatta and Slinkard (1989) studied the effect of phytic acid and cooking quality of 36 samples of lentils; they reported that the cooking time was predominantly influenced by phytic acid. Seeds with a long cooking time had a low phytic acid content which implied that most of it had been hydrolyzed. Increasing
phytic acid levels in legumes would be a remedy to HTC effect; on the contrary, phytic acid binds divalent cations making them unavailable for human nutrition.

Moscosco and others (1984) reported a decrease in the phytic acid content of beans during storage for 9 months under elevated temperatures and high relative humidity conditions which was associated with a subsequent increase in cooking time. After the ninth month, beans were left with less phytic acid and fewer of the monovalent ions (Na\(^+\) and K\(^+\)) that are typically responsible for solubilizing the pectin substances via ion exchange and chelation during cooking. It was concluded that the degraded cell wall allowed redistribution of divalent ions to the middle lamella during storage where they formed insoluble substances with pectin. This was in agreement with the pectin-phytate theory. Furthermore, Chitra (1994) studied the effect of storage on phytic acid and cooking time. He reported a notable loss of phytic acid associated with elevated cooking time for chick peas and soy beans stored at 25°C and 37°C, respectively. For short cooking time, a high phytic acid content is needed, however, it binds divalent ions making them unavailable for human nutrition. It was suggested that genotypes with high phytic acid could be identified and bred to improve their cooking times (Chitra, 1994).

2.3.2.3. Dietary fiber

Dietary fiber is defined as the component of plant cells that is resistant to hydrolysis by human digestive enzymes. Dietary fiber includes cellulose, hemicellulose, pectin, lignin, and resistant starch. Dietary fiber can be classified according to its solubility, i.e., water-soluble and water-insoluble (Salunkhe and Kadam, 1989). The dietary fiber content of legumes can be affected by variety, environmental conditions and agronomical practices (Wang and others, 2008). The total dietary fiber (TDF) of pulses ranges from 8-27.5%, with soluble dietary fiber (SDF) in the range
of 3.3-13.8% (Gullion and Champ, 2002). Gullion and Champ further reported that DF is located in both the cotyledons and the seed coat of the common bean. The cell walls of the cotyledons contain pectin substances (approximately 55% w/w) and non-lignified cellulose (about 9%). On the other hand, the seed coat contains a large amount of cellulose (35-57% w/w) and small amounts of hemicellulose and pectin (Van-Laar and others, 1999). Kutos and others (2003) determined contents of SDF, IDF (insoluble dietary fiber), TDF and resistant starch (RS) of raw and processed dry beans. The results indicated that thermal processing reduced IDF content and the overall TDF of the beans. Thermal processing also increased RS of beans. Shiga and others (2009) investigated effects of cooking on fibers of HTC beans, and reported that the HTC effect did not change the amounts of soluble and insoluble fiber contents in cooked beans, but rather the physico-chemical characteristics of the bean carbohydrates like solubility of the fiber. Furthermore, Shiga and others (2011) studied the effect of storage on the solubility of bean hull fiber. It was observed that aging resulted in a decrease in water-soluble fiber and an increase in water-insoluble fiber. This was caused by the insolubilization of galacturonans and xyloglucans. Hulls make up 7% of all water-insoluble fiber of the whole bean while cotyledons contribute 10% of the water-insoluble fiber.

2.3.2.4. Pectin

Pectin is a complex polysaccharide in plant cell walls and consists of three components i.e., rhamnogalacturonan I, homogalacturonan with homogalacturonan accounting for about 60% of the total pectin in the cell walls (Mohnen, 2008). Pectin molecules can also be tightly bound to other polysaccharides like hemicellulose, wall proteins and phenolic compounds (Caffall and Mohnen, 2009). It consists of about 100-200 D-galacturonic acids linked via (1-4) α-D-galacturonic acid linkage. Shiga and others (2006) reported that the pectin in common beans consists of rhamnogalacturonans, galactans, xylogalacturonans and ramified arabinans. During
storage of beans under higher temperature and relative humidity, phytase hydrolyses phytate releasing calcium and magnesium cations. These cations migrate to the cell walls where they bind demethylated pectin molecules, forming insoluble substances thickening the cell wall (Reyes-Moreno and Paredes-Lopez, 1993). There are changes in pectin during storage of beans at high temperature and humidity, which result in increased bean cooking time.

Jones and Boulter (1983) observed a reduction in water-solubility of pectin during storage at 4°C and RH of 65%. In addition, Salvador (2007) observed reduced pectin water-solubility of cowpeas stored at an elevated temperatures of 42°C and RH of 67% and this was associated with increased cooking time from 89 to more than 270 minutes. Bernal-Lugo and others (1997) reported that the fast cooking common beans (variety Michigan 800) and the slow cooking beans (variety Ojo de Cabra), both grown in Mexico, had similar total pectin contents (25.0 and 25.7 mg/g, respectively). On the other hand, the fast cooking beans (variety Michigan 800) had more hot-water-soluble pectin (13.6±1.5 mg/g) than the slow cooking beans (variety Ojo de Cabra; 6.0±1.0 mg/g).

2.3.2.5. Divalent cations

Common beans contain 70-210 μg/g calcium and 160-230 μg/g magnesium (Table 2.1). The cations of these minerals have been reported to be involved in the HTC effect of common beans. Soaking cow peas in Ca^{2+} salt solutions prior to cooking enhanced the hardening effect (Shewfelt and McWatters, 1992). Aguilera and Rivera (1992) also reported that there was a positive correlation between calcium content and the HTC effect, however, it was small. Furthermore, Kyriakidis and others (1997) boiled beans in both plain water and divalent cation solutions (CaCO_3 and MgCO_3) and they reported that beans boiled in the divalent solutions were much harder than
those boiled in plain water. However, they did not report which of the two solutions, i.e., \( \text{CaCO}_3 \) or \( \text{MgCO}_3 \) solution, caused more hardness.
Table 1. Overall Mineral Content of *Phaseolus vulgaris* (Common beans). Source: Salunkhe and Kadam (1989)

<table>
<thead>
<tr>
<th>Common beans</th>
<th>Ca (µg/g)</th>
<th>Cu (µg/g)</th>
<th>Fe (µg/g)</th>
<th>Mg (µg/g)</th>
<th>Mn (µg/g)</th>
<th>P (µg/g)</th>
<th>K (µg/g)</th>
<th>Na (µg/g)</th>
<th>Zn (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw</td>
<td>70-210</td>
<td>0.0-1.40</td>
<td>3.34-8.0</td>
<td>160-230</td>
<td>1.0-2.0</td>
<td>380-570</td>
<td>1320-1780</td>
<td>4.0-21.0</td>
<td>1.9-6.5</td>
</tr>
<tr>
<td>Cooked</td>
<td>70-260</td>
<td>0.50-1.10</td>
<td>2.88-7.93</td>
<td>130-220</td>
<td>1.0-2.1</td>
<td>360-510</td>
<td>1100-1710</td>
<td>1.5-6.90</td>
<td>1.9-4.0</td>
</tr>
</tbody>
</table>
2.3.2.6. Starch content and starch gelatinization temperature

There are two physicochemical properties of starch that are proposed to influence cooking time, i.e., starch content and starch gelatinization temperature. Bernal-Lugo and others (1997) reported that the fast-cooking common beans of variety Michigan 800 had a lower starch content (240.0 mg/g) than the slow-cooking bean variety Ojo de Cabra (260.0 mg/g), indicating that starch content influenced cooking time. Furthermore, the fast-cooking bean variety was reported to have a lower amylose content (38.0%) than the slow-cooking bean variety (44%). This implied that amylose content could influence cooking time.

Starch gelatinization is the disruption of molecular orders within the starch granule when heated in water and it results in irreversible changes in properties such as granular swelling, native crystalline melting, loss of birefringence, and starch solubilization (Jane, 2012). Gelatinization is usually followed by a process known as pasting. When starch is heated in water, there is disruption of hydrogen bonds between starch molecules, which weakens the granules. This allows the starch granules to imbibe water and swell, which contributes to an increased viscosity of the starch-water solution (Jane, 2012). The initial temperature at which gelatinization begins is known as the onset gelatinization temperature. Studies have shown that high onset gelatinization temperatures are exhibited by starches with long-branch chains of amylopectin as these can form much more stable crystalline structures than the short chains (Jane and others, 1999). Lower onset gelatinization temperature has been associated with short amylopectin chains (Ovando-Martinez and others, 2011). The peak gelatinization temperatures of varieties Black 8025 and Pinto Durango beans grown in two different locations and with different watering regimes were reported to range from
70.14°C to 75.42°C; starch of beans grown in a rainy location had a lower peak gelatinization temperature than that of those grown in the irrigation locality (Ovando-Martínez and others, 2011).

When cooking beans, starch granules present in the beans are heated in water and therefore undergo gelatinization. It has been proposed that if beans have a low peak gelatinization temperature, they could have a shorter cooking time. Reyes-Moreno and others (1994) studied physicochemical properties influencing cooking time of fresh, storage-hardened, and chemical-hardened variety Mayocoba (fast-cooking) and variety Flor de Mayo (slow-cooking) common beans. He reported that the fast-cooking beans had a lower peak gelatinization temperature (76.6°C) than the slow-cooking beans (78.7°C), thus influencing cooking time of the beans. On the contrary, Bernal-Lugo and others (1997) reported that both fast and slow-cooking beans had similar peak gelatinization temperatures (86°C and 87°C, respectively), therefore peak gelatinization temperature may not relate much to differences in cooking time. In both cases, it is not mentioned if the two varieties studied belonged to the same market class; it would be important to know how bean genotypes of the other market classes grown in the same location would behave.

2.4. Methods used to reduce cooking time of dry common beans

2.4.1. Soaking

Soaking beans before cooking is a common practice used in many parts of the world with a goal of reducing subsequent cooking time. The amount of water absorbed depends on the permeability of the seed coat (Valle and others, 1992). Soaking dry beans in monovalent (Na+) cation solutions has also been used to reduce the cooking time of beans. De LeoÂ and others (1992) reported that soaking HTC beans in a salt solution of 2.5% K₂CO₃ or of 0.5% NaHCO₃, w/v, significantly
reduced their cooking time. Furthermore, Salvador (2007) reported that cowpeas soaked in a sodium chloride solution were observed to have improved pectin water-solubility, which was associated with reduced cooking time. Soaking activates rhamnogalacturonase, galactanase and polygalacturonase cell wall enzymes of beans. These enzymes break down the pectin polysaccharides, rhamnogalacturonan I and polygalacturonan giving cell walls new polysaccharide arrangements that result in higher thermostability hence shorter cooking time (Martinez-Manrique, 2011).

2.4.2. Micronization

Micronization of legumes is a process in which moisture-conditioned seeds are treated with infrared heat (1800 to 3400 nm) to soften their texture by breaking the pectin molecules into smaller and more soluble molecules before cooking. This increases water uptake during cooking thus reducing cooking time of beans (Mwangwela and others, 2007). According to Fasina and others (2001), the infrared rays cause vibration of water molecules at 60000 to 150000 MHz resulting in internal heating of the bean. This is a high efficiency method as compared to other technologies (Bellido and others, 2003). Salvador (2007) preconditioned cow peas using sodium ion solution prior to micronization, and the combination was seen to reduce cooking time from more than 270 to 59 minutes.

2.4.3. Dehulling

Dehulling has been reported to reduce cooking time of pulses. Kon and others (1973) reported a 70% reduction in cooking time of pinto beans resulting from dehulling. This was attributed to the removal of the impermeable seed coat that hinders water uptake of the bean during cooking. However, dehulling has been reported to result in a significant reduction in minerals, especially
calcium, copper, magnesium and manganese (Wang and others, 2009), which are important for human health.

2.5. Knowledge gaps

Pirhayati and others (2011) reported that the seed coat thickness of the fast-cooking beans (38.00 μm) was greater than that of the HTC beans (32.00 μm); therefore, cooking time might not be solely attributed to seed coat thickness. There were also no significant differences in the microstructures of the seed coats of the fast and slow-cooking beans. On the contrary, according to the other published literature, varieties with thick seed coats are expected to have longer cooking times. In addition, Bhattay’s (1995b) observations on fast and slow-cooking lentils showed structural differences in seed coats of slow-cooking lentils. To resolve this contradiction, this current thesis research was aimed at characterizing the dry common bean seed coat thickness and weight, and their relationships with cooking time.

The influence of calcium and magnesium salt solutions on the cooking time of beans has been extensively studied. Pirhayati and others (2011) reported an increase in hardness of beans after soaking them in tap water (Ca^{2+} and Mg^{2+}). This was attributed to the assumption that the calcium and magnesium ions in the tap water migrated to the middle lamella and participated in the crosslinking of pectate substances and phenolic compounds hardening the cell walls. However, the influence of the inherent calcium and magnesium of the entire bean is not yet known. Protein solubility of dry common beans was reported to decrease with increase in storage time, and result in an increase in the cooking time of dry beans. Coelho and others (2012) concluded that the high storage temperature induced protein denaturation which caused coagulation of protein thus reducing its solubility in water. It is not known, however, if protein from other dry common bean
varieties will behave in a similar manner. Slow-cooking beans were reported to have less hot water soluble pectin (HWSP) and higher starch content than the fast cooking beans (Bernal-Lugo and others, 1997). However, determinations were done on only two varieties grown in Mexico. It is not known if other varieties from other locations would follow the same trend.

2.6. Hypothesis
Dehulling reduces cooking time of raw dry common beans more than does soaking the whole raw bean. Cooking time of dry common beans is associated with seed coat thickness and weight, starch content, starch gelatinization and protein denaturation temperatures, protein content, hot water soluble pectin content, and inherent calcium and magnesium contents in the entire bean.

2.7. Objectives
Overall objective
To determine the physicochemical properties of components in dry common beans associated with cooking time of dry common beans.

Specific objectives
1. To determine the seed coat weight and thickness and their correlations with cooking time of dry common beans.
2. To determine the thermal properties of various components of dry common beans and to quantify their correlations with cooking times of the studied varieties.
3. To quantify the storage protein, starch, and pectin contents, and to determine the contents of inherent calcium and magnesium of dry common beans and to quantify their correlations with cooking time.
CHAPTER 3

METHODS AND PROCEDURES

3.1. Materials: Dry common bean samples

Dry common beans (*Phaseolus vulgaris* L.) belonging to the Andean Diversity Panel (ADP) were used in this study. Bean varieties in the ADP are large seed beans that are commonly consumed in Southern and Eastern Africa. Two market classes were used in this study, i.e., yellow beans and red mottled beans. These market classes were chosen because they showed large variations in cooking time (Cichy and others, 2015). Each market class had three genotypes, one slow-cooking (S), one moderate-cooking (M), and one fast-cooking (F) variety. Yellow beans included Cebo cela (ADP-521-F) that originated from a market Place in Angola, Uyole 98 (ADP-111-M) that originated from Tanzania and was developed by the Tanzania National breeding Program in 1999 and Canario (ADP-513-S) that originated from Angola (hereafter referred to as Y-521-F, Y-111-M, and Y-513-S, respectively). Genotypes of red mottled beans all originated from Puerto Rico. JB178 (ADP-443-F) was released for its superior agronomic traits and disease resistance, Vazon 7 was released in 2005 (ADP-436-M) and PR0737-1 (ADP-434-S) is a virus resistant variety developed jointly by University of Puerto Rico, Haiti National Program and USDA-ARS in 2013 (Cichy and others, 2015). Hereafter referred to as R-443-F, R-436-M, and R-434-S, respectively (Figure 7). All six genotypes were grown in two locations, i.e., Puerto Rico and Tanzania (Arusha) in 2014. These beans were sorted and cleaned to remove dirt and foreign matter.
Figure 7. Dry common bean genotypes used in the study.
3.2. Sample preparation

The bean samples were tempered by storing whole beans in a moisture equalizer at 14% moisture content at room temperature for 3 weeks (hereafter referred to as raw beans) prior to any analyses. For analyses requiring ground sample, raw beans were freeze-dried (Refrigeration for Science, Inc. Island Park, New York) for 48 hours, then ground by a Wiley laboratory mill (Arthur H. Thomas Co., Scientific Apparatus, Philadelphia, PA., USA) to pass through a 0.5 mm screen before analysis. The ground samples were stored in airtight plastic tubes at room temperature.

3.3. Determination of cooking time

Cooking time was determined using a Mattson cooker (Customized Machining and Hydraulics Co., Winnipeg, Canada) (Wang and Daun, 2005). For each bean variety, 25 raw beans were cooked as is, another 25 raw beans were soaked for 12h before cooking, and another 25 raw beans were dehulled manually using a razor blade before cooking. The Mattson cooker has 25 metallic pins under which beans are to be placed, one under each pin. The device is connected to a monitor with the Mattson software. Distilled water (1800 ml) was boiled in the pan of the Mattson cooker. The Mattson device, loaded with 25 raw, soaked, or dehulled beans of one variety, was placed in the boiling water, the start button pressed, and the starting time recorded. During cooking, the beans softened and the pins dropped as they pierced the beans. The time at which each pin dropped was recorded. When 80% of the pins had dropped (i.e., 20 beans), the time was recorded as the end cooking time of that particular treated bean variety. Cooking time was calculated by subtracting time at the beginning of cooking from end cooking time. Cooking time determinations were done in two replicates for each bean variety and treatment as described above.
3.4. Determination of the percentage of seed coat weight

The seed coat weight of the beans was determined gravimetrically. Total weight of five raw whole beans from each variety was determined. The beans were then dehulled manually using a sharp razor blade and the total weight of the five dehulled beans was determined. The total seed coat weight of the five beans was the calculated difference between the total weight of five raw beans and the total weight of the same five beans dehulled. The percentage of seed coat weight was calculated as total weight of the five seed coats divided by total weight of the five raw beans multiplied by 100. This was done in two replicates and results for each variety averaged.

3.5. Measurement of the seed coat thickness

The thickness of the common bean seed coat was measured using the Scanning Electron Microscope (SEM). Raw whole beans of each variety were first freeze dried for 48h (Refrigeration for Science, Inc. Island Park, New York) to 3% moisture content. Cross sections of six raw beans of each variety were made using sharp razor blades. The cross sections (2 per bean) were then mounted on aluminum stubs using high vacuum carbon tabs (SPI Supplies, West Chester, PA). They were then coated with platinum for sixty seconds while rotating to a thickness of 8nm in a Quorum Technologies/ Electron Microscopy Sciences Q15OT Turbo Pumped Coater (Quorum Technologies, Laughton, East Sussex, and England BN8 6BN) (Figure 8). They were then examined using a JEOL JSM-7500F (cold field emission electron emitter) scanning electron microscope (JOEL Ltd, Tokyo, Japan). Images of bean cross sections (Figure 9) were taken and the seed coat thicknesses measured using the micrograms. This was done in duplicate (i.e., for a total of 12 beans examined per variety), with two micrograms produced per bean (one for each cross section). The averages for each variety were recorded.
Figure 8. Cross sections of raw beans mounted on aluminum stubs in a Turbo Pumped Coater for platinum coating.
Figure 9. SEM image of a cross section of a raw ADP-513-S common bean seed from Puerto Rico with four seed coat thickness measurements indicated (in orange).
3.6. Determination of calcium and magnesium contents

Calcium and magnesium contents were determined using Inductively Coupled Argon Plasma Emission Spectroscopy (ICAPES) following AOAC Method 985.01. Calcium or magnesium standards or ground raw whole beans (2 g each, prepared according to the description in 3.1) were each weighed into microwave digestive tubes and 2.0 mL of concentrated nitric acid was added into each tube. Samples were chemically digested according to the Open Vessel Microwave SW846-3051A procedure (AOAC 991-10D) at 175°C for 15 minutes. After the digestion, samples were diluted to a volume of 25 ml with deionized water and analyzed on a Thermo iCAP 6000 Series ICAPES. This analysis was done in duplicate and the averages for each variety were recorded.

3.7. Determination of total protein content

Ground freeze dried bean samples were analyzed by A & L Great Lakes Laboratories, Inc, Fort Wayne, USA. Total protein was calculated by determining total nitrogen of the bean samples according to the Dumas Method (AOAC 990.03). For each variety, ground raw bean sample (about 120 mg) was weighed and a sample press was then used to compress the sample to remove any atmospheric air. The sample weight was recorded and checked using the “Sample View Window” in the Dumas software to verify that the weight was recorded accurately. During the run, 100 mg of aspartic acid was used in the blank position to calibrate throughout the run. The samples were loaded into the rapid nitrogen cube auto-sampler carousel for analysis. The percentage nitrogen
content was recorded and converted to protein content by multiplying by 6.25. All determinations were conducted in duplicate and the averages for each variety were recorded.

3.8. Determination of total starch content of the common bean varieties

Beans of each variety were prepared for analysis by placing raw whole beans in a freeze drier (Refrigeration for Science, Inc., Island Park, New York) for 48 h, then grinding each sample using a Wiley laboratory mill to pass through a 0.5 mm screen. Total starch content was determined according to Megazyme Total Starch Kit procedure (Megazyme International, Wicklow, Ireland). For each variety, ground bean sample (about 100 mg) was weighed accurately into a glass tube. It was then wetted with 0.2 ml of 80% v/v aqueous ethanol to aid dispersion and the mixture was mixed vigorously on a vortex mixer (Fisher Vortex, Genie 2™) for 30 seconds. Immediately, 2 ml of dimethyl sulfoxide (DMSO) were added and the mixture was shaken vigorously using the above vortex mixer. The tube was then placed in a vigorously boiling water bath and was removed after five minutes. Immediately, 3 ml of thermal stable α-amylase (contents of bottle 1 diluted 1:30 in Reagent 1; 100 mM sodium acetate buffer, pH 5.0) was added to the tube. The tube was then incubated in a boiling water bath for 6 minutes, and stirred vigorously using the above vortex mixer after 2, 4 and 6 minutes. The tube was then placed in a water bath (Julabo SW22, USA) at 50°C and 0.1 ml of the contents of bottle 2 of the kit (amyloglucosidase, 300 U of starch) was added. The tube was vortexed and incubated at 50°C for 30 minutes. The entire contents of the test tube was transferred to a 100 ml volumetric flask using a funnel to assist transfer, and a wash bottle with distilled water to rinse out the tube’s contents thoroughly. The volume was adjusted to the volume mark with distilled water and mixed thoroughly by hand swirling. An aliquot of this solution (10 ml) was centrifuged using the above centrifuge at 3000 x g for 10 minutes and the
clear undiluted filtrate was used for the assay. Duplicate aliquots of 0.1 ml of each sample were transferred to glass test tubes. Glucose oxidase/peroxidase (GOPOD) reagent (3.0 ml) was added to each tube. In addition, D-glucose controls consisting of 0.1 ml of D-glucose standard solution (1 mg/ml) and 3.0 ml of GOPOD reagent were prepared and stirred. Furthermore, a blank solution was prepared by adding 0.1 ml distilled water and 3.0 ml of GOPOD reagent to a glass test tube and vortexed. All the tubes were then incubated at 50°C for 20 minutes. Absorbance for each sample and D-glucose control was read at 510 nm against the reagent blank using a spectrophotometer (Spectronic Genesys™ 5, USA). Starch % was calculated using the formula:

\[ \text{starch} \% = \Delta A \times \frac{F}{W} \times FV \times 0.9 \]

where \( \Delta A \) = absorbance against the reagent blank, \( F = 100 \mu g \) of D-glucose/absorbance of 100 \( \mu g \) of D-glucose, \( FV = 100 ml \), \( W = \) weight of sample flour in mg.

This experiment was done in duplicate.

### 3.9. Thermal properties of common dry beans

Both starch gelatinization and protein denaturation temperatures were measured using a differential scanning calorimeter (DSC) (TA Instruments, DSC Q100, DE, USA) and the method reported by Yin and others (2008). Raw whole freeze dried beans were ground to pass through a 0.5 mm screen. For each study variety, the sample (5 mg) was weighed into a standard DSC aluminum pan and distilled water (15 \( \mu l \)) was added to the sample. A rubber ring was placed on the edge of an aluminum standard DSC lid, the lid positioned on top of the pan, and the pan then sealed tightly with a standard sealer. The samples were then left in the pan to stand at room temperature for 2h before analysis. A reference was prepared by sealing an empty pan to be a control. The reference pan and a sample pan were placed in the DSC machine with the reference
pan taking the back position. The DSC was closed and analysis was run between 30°C and 140°C at a temperature increase rate of 10°C per minute. This was done in duplicate.

3.10. Extraction and determination of contents of hot water soluble pectin and hot water insoluble pectin and total pectin of dry common beans.

Pectin content was determined spectrophotometrically using the metahydroxydiphenyl method of Blumenkrantz and Asboe-Hansen (1973) and using galacturonic acid as a standard (Berna-Lugo and others, 1996). Raw bean samples were dehulled manually with a blade and freeze dried for 48 hours. The raw-dehulled, freeze-dried beans were then ground to pass through a 0.5 mm screen, and stored in airtight plastic tubes at room temperature until analysis. Before extraction of pectin, soluble sugars were removed from the ground samples by extracting with 75% ethanol. Ground sample (3 g) was weighed into a beaker and 30 ml of 75% ethanol was added. The mixture was stirred at 25°C for 2h. It was then centrifuged by the above centrifuge for 15 minutes at 4000 x g at 20°C to remove soluble sugars. The residue, alcohol insoluble solids (AIS), was washed with 30 ml of absolute ethanol twice. It was then oven dried for 24h at 25°C. Hot water soluble pectin (HWSP) and hot water insoluble pectin (HWIP) were extracted in the following manners. The HWSP was extracted by weighing 1g of AIS into a beaker, adding 10 ml of distilled water, and then stirring for 2hrs at 85°C using the VWR 575 Digital Hotplate Stirrer made in the USA. The mixture was then centrifuged for 15 minutes at 4000 x g and 20°C. The extract was frozen overnight and then freeze dried for 48h using the above freeze drier. The HWIP was extracted using 2% sodium hexametaphosphate. One g of AIS was weighed into a glass beaker and 10 ml of 2% hexametaphosphate were added to the sample. The mixture was stirred at 90°C for 6h. The mixture was centrifuged with the above centrifuge for 15 minutes at 4000 x g and 20°C. The extract
in the supernatant was frozen overnight and then freeze dried for 48 hours using the above freeze
drier. The samples were kept in airtight plastic tubes at room temperature until further analysis.
Pectin content was expressed as galacturonic acid. This is because galacturonic acid is mainly used
as a representative of acid mucopolysaccharides (including pectin) in biological substances, since
it is the main component of repeating units of most acid mucopolysaccharides. The colorimetric
assay using m-hydroxydiphenyl solution for analysis of galacturonic acids was used. While all
carbohydrates react in concentrated acid to form pink-colored compounds, galacturonic acids react
with m-hydroxydiphenyl solution in a strong basic environment to form pink-colored complexes.
Reagents used included the following: (1) galacturonic acid stock solution, prepared by dissolving
100 mg dry galacturonic acid powder in 100 ml distilled water to give a solution concentration of
1 mg/ml (this was kept refrigerated); (2) M/80 sodium tetraborate in sulphuric acid (0.0125M); (3)
0.5% sodium hydroxide; (4) m-hydroxydiphenyl solution (0.15%), covered in a container with
aluminium foil to protect it from the light, and kept refrigerated.
Solutions for the standard curve were made as follows: 2 ml stock galacturonic acid solution were
pipetted into a 100 ml volumetric flask and diluted to final volume with distilled water. The
concentration of galacturonic acid in this sample was 20 µg/ml. This was subsequently repeated
using 4 ml, 6 ml, 8 ml, 10 ml, 12 ml, 14 ml and 16 ml of stock galacturonic acid solutions to make
40, 60, 80,100, 120, 140 and 160 µg/ml concentrations of standard solutions.
To determine the pectin contents of each bean variety, clean glass test tubes were placed in an ice
bath to cool before being used. The sulfuric acid/sodium tetraborate solution was kept in an ice
bath throughout the experiment. An aliquot (1.0 ml) of the standard solution or sample was pipetted
into a test tube and allowed to cool for 1 minute. The tetraborate solution (6.0 ml) was then added
to the test tube. This was mixed thoroughly using a vortex mixer (Fisher Vortex, Genie 2™, USA)
for 1 minute. The glass test tubes were then heated at 80°C in a water bath (Julabo SW22, USA) for precisely 6 minutes. The tubes were then returned to the ice bath and allowed to cool to 4°C. M-hydroxydiphenyl solution (0.1 ml) was pipetted into each tube and mixed thoroughly using the above vortex mixer. A blank was prepared by mixing 10 ml deionized water plus 6.0 ml tetraborate solution and 0.1 ml of 0.5% sodium hydroxide solution. The glass tubes were then allowed to stand for 15-20 minutes at room temperature to allow any bubbles formed to dissipate. Absorbance was measured at 520 nm using a spectrophotometer (Spectronic Genesys™5, USA) by reading sample against the blank. A calibration curve of absorbance (y-axis) against concentration of galacturonic acid (x-axis) was plotted and was used to determine the pectin contents of the bean varieties studied. The experiment was done in duplicate and the averages were recorded.

3.1.1. Statistical analysis of data

All data obtained from above experiments were analyzed using Statistical Package for Social Sciences (SPSS version 2009, International Business Machines, New York, USA). Multiple Linear regression and Analysis of Variance (ANOVA) were performed and means were separated by Tukeys HSD test (p < 0.05).
CHAPTER 4

RESULTS AND DISCUSSION

4.1. Effect of processing on cooking time of common beans

Cooking times of tempered raw, tempered soaked and tempered dehulled common beans (hereafter referred to as raw, soaked and dehulled, respectively) from Puerto Rico and Tanzania are presented in Table 2. Generally, cooking time of the studied bean varieties was specific to variety for both yellow and red mottled beans. For the raw yellow (Y) beans from Puerto Rico, it was confirmed that the Y-521-F had the shortest cooking time (80.93±0.87 minutes) followed by Y-111-M (83.33±1.27 minutes) and Y-513-S had the longest cooking time (106.37±1.69 minutes); F, M, and S, refer to fast, moderate and slow cooking time, respectively. The cooking time of variety Y-521-F was 2.4 minutes less than that of Y-111-M and 25 minutes less than that of variety Y-513-S. This implies that less energy and money is required to cook variety Y-521-F than variety Y-513-S. These results show that genotypes within a market class have significantly different cooking times.

All the yellow genotypes maintained the same cooking time trend when grown in Tanzania, i.e., Y-521-F, Y-111-M, and Y-513-S in ascending order. The cooking time of variety Y-521-F was about 5 minutes less than that of Y-111-M and 9 minutes less than that of variety Y-513-S. However, the cooking time of raw yellow beans grown in Tanzania were significantly greater than their counterparts from Puerto Rico (P<0.05). The cooking times of genotypes Y-521-F, Y-111-M, and Y-513-S grown in Tanzania were 31, 33, and 14 minutes longer than those of their counterparts grown in Puerto Rico.
Table 2. Cooking times in minutes of raw tempered yellow (Y) and red mottled (R) beans grown in Puerto Rico and Tanzania; beans were raw (not soaked or dehulled), soaked for 12 hours, or dehulled before cooking\(^1\)

<table>
<thead>
<tr>
<th>Bean Sample(^2)</th>
<th>Puerto Rico</th>
<th>Tanzania</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Raw</td>
<td>Soaked</td>
</tr>
<tr>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-521-F</td>
<td>80.93±0.87(^a)</td>
<td>31.37±2.71(^d)</td>
</tr>
<tr>
<td>Y-111-M</td>
<td>83.33±1.27(^b)</td>
<td>42.24±2.89(^de)</td>
</tr>
<tr>
<td>Y-513-S</td>
<td>106.37±1.69(^c)</td>
<td>42.55±2.94(^de)</td>
</tr>
<tr>
<td>Red Mottled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-443-F</td>
<td>95.14±1.51(^f)</td>
<td>42.28±4.95(^m)</td>
</tr>
<tr>
<td>R-436-M</td>
<td>103.09±2.28(^g)</td>
<td>46.24±9.32(^l)</td>
</tr>
<tr>
<td>R-434-S</td>
<td>154.88±14.97(^y)</td>
<td>105.56±0.63(^n)</td>
</tr>
</tbody>
</table>

\(^1\)Data represent means and standard deviations of n=36. Values within the same column or row followed by the same superscript letter are not significantly different (P ≤ 0.05) from each other.

This could be attributed to environmental differences like temperature and relative humidity during their growth. The variety Y-513-S had the least difference in cooking time between Tanzania and Puerto Rico.

As expected, soaking significantly reduced the cooking time of beans ($P<0.05$) for all the yellow genotypes from Puerto Rico or Tanzania, however, the magnitude of this decrease varied among genotypes. There were 61.2%, 49.3%, and 60% reductions in cooking time of Y-521-F, Y-111-M, and Y-513-S bean genotypes, respectively, from Puerto Rico when soaked before cooking. When grown in Tanzania, there was a 73.7%, 65%, and 60% reduction in cooking time of Y-521-F, Y-111-M, and Y-513-S bean genotypes, respectively, when soaked before cooking.

Similar to soaking, dehulling significantly reduced the cooking time for all the yellow genotypes from Puerto Rico or Tanzania ($P<0.05$). The magnitude of this decrease also varied among genotypes. There were 71%, 68%, and 70% reductions in cooking time of Y-521-F, Y-111-M, and Y-513-S bean genotypes, respectively, from Puerto Rico when dehulled before cooking. When grown in Tanzania, there were 73%, 74%, and 71% reductions in cooking time of Y-521-F, Y-111-M, and Y-513-S bean genotypes, respectively, when dehulled before cooking. Generally, Y-521-F exhibited a greater reduction in cooking time when soaked than Y-513-S. This could imply that perhaps it had a better soaking ability than Y-513-S. There were no significant differences between the cooking times of soaked yellow beans and their dehulled counterparts from Puerto Rico or Tanzania.

For the raw red mottled (R) beans from Puerto Rico, it was confirmed that the R-443-F had the shortest cooking time followed by R-436-M and R-434-S had the longest cooking time; F, M, and S, refer to fast, moderate and slow cooking time, respectively. The cooking time of variety R-443-F was 8 minutes less than that of R-436-M and 60 minutes less than that of variety R-434-S. This
implies that less energy and money is required to cook variety R-443-F than varieties R-436-M and R-434-S. As yellow beans, red mottled beans maintained the same cooking time trend when grown in Tanzania, i.e., R-443-F, R-436-M, and R-434-S in ascending order. The cooking time of variety R-443-F was about 8 minutes less than that of R-436-M, and 31 minutes less than that of variety R-434-S. However, the cooking time of raw red mottled beans grown in Tanzania were significantly greater than their counterparts from Puerto Rico (P<0.05). The cooking times of genotypes R-443-F, R-436-M, and R-434-S grown in Tanzania were 23, 23 and 6 minutes longer than those of their counterparts grown in Puerto Rico. This could be attributed to environmental differences like temperature and relative humidity during their growth. Like yellow beans, the slow-cooking genotype, R-434-S, had the least difference in cooking time when grown in Tanzania and Puerto Rico. This could imply that environmental differences did not have much effect on its cooking time.

Like yellow beans, soaking significantly reduced the cooking time of red mottled beans (P<0.05) for all the genotypes from Puerto Rico or Tanzania, however, the magnitude of this decrease varied among genotypes. There were 56%, 55%, and 32% reductions in cooking time of R-443-F, R-436-M, and R-434-S bean genotypes, respectively, from Puerto Rico when soaked before cooking. When grown in Tanzania, there were 47%, 65%, and 44% reductions in cooking time of R-443-F, R-436-M, and R-434-S bean genotypes, respectively, when soaked before cooking. As yellow beans, the fast-cooking red mottled genotype, R-443-F, grown in Puerto Rico had the greatest reduction in cooking time when soaked implying that it could have the best soaking ability among the three genotypes. However, for Tanzania red mottled beans, the moderate-cooking genotype, R-436-M, had the greatest reduction in cooking time when soaked before cooking.
Similar to soaking, dehulling significantly reduced the cooking time of beans (P<0.05) for all the red mottled genotypes from Puerto Rico or Tanzania. The magnitude of this decrease also varied among genotypes. There were 65%, 64%, and 74% reductions in cooking time of R-443-F, R-436-M, and R-434-S bean genotypes, respectively from Puerto Rico when dehulled before cooking. When grown in Tanzania, there were 67%, 68%, and 70% reductions in cooking time of R-443-F, R-436-M, and R-434-S bean genotypes, respectively, when dehulled before cooking. Among the three red mottled genotypes grown in Puerto Rico or Tanzania, the slow-cooking genotype, R-434-S, had the greatest reduction in cooking time when dehulled before cooking. There were no significant differences between the cooking times of dehulled red mottled genotypes and their soaked counterparts for both locations. This implies that dehulling reduced cooking time of beans as soaking.

The results in the present study show that genotypes within a market class have significantly different cooking times. It is commonly known that yellow beans cook faster than red mottled beans however this is not true for all genotypes. The raw genotype R-443-F had a shorter cooking time (95.14±1.51) than the yellow genotype Y-513-S (106.37±1.69 minutes). This information is very vital when making a choice among many bean varieties for consumption since fast-cooking beans will require less energy and money to cook them.

The findings in the present study are in agreement with past studies that have shown that soaking reduced cooking time of legumes, (Taiwo and others, 1998; Martinez-Manrique and others, 2011). The results are also consistent with the common experience of reduced cooking time in households. Soaking activates the pectic enzymes in the cell walls of beans which hydrolyze the main pectin polymer rhamnogalacturonan I reducing its degree of polymerization and increasing
polygalactururonan and galactan solubility. This results into a reduced thermosolubility of pectic polysaccharides thus shorter cooking time (Martinez-Manrique and others, 20011).

The results of the present study are also in line with Kon and others (1973) who reported that dehulling reduces cooking time of pulses. This is attributed to the removal of the impermeable seed coat that hinders water uptake of the bean during cooking. It has also been reported that dehulling results in a significant reduction of minerals especially calcium, copper, magnesium and manganese (Wang and others, 2009) and dietary fiber. Removal of these minerals compromises the nutritional quality of the beans, thus soaking is often preferred. From the above results of the current study, we can conclude that the studied bean genotypes within a market class have different cooking times, and that soaking and dehulling each reduced cooking time, relative to raw bean cooking, in a similar way. Since dehulling is very cumbersome and compromises the nutritional quality of the beans, soaking is the easiest way to reduce cooking time. Although the cooking times of the studied bean genotypes from different locations may be different, the trends within each location were consistent, i.e., the variety that cooked fast when grown in Puerto Rico, still cooked fast when grown in Tanzania.
4.2. Percentage seed coat weight

The percentages seed coat weight of the studied raw beans are presented in Table 3. Generally, beans from Puerto Rico had larger percentage seed coat weights than their counterparts from Tanzania. The percentage seed coat weights of yellow beans were not significantly different among fast, moderate and slow cooking genotypes for both locations (P>0.05). Except for the fast-cooking red mottled beans variety R-433-F being significantly smaller than the slow-cooking variety R-434-S from Puerto Rico, the percentage seed coat weight values of all the three genotypes from both locations were not significantly different (P>0.05). This implies that percentage seed coat weight did not have an association with cooking time of bean genotypes within a market class and between market classes in the studied varieties. Also, locations did not display significant effect on the percentage seed coat weight values.
Table 3. Seed coat weight (%) and thickness (µm) of fast, moderate and slow cooking yellow and red mottled beans from Puerto Rico and Tanzania.

<table>
<thead>
<tr>
<th>Bean Variety</th>
<th>Seed Coat Weight (%) 3</th>
<th>Seed Coat Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puerto Rico</td>
<td>Tanzania</td>
</tr>
<tr>
<td><strong>Yellow</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-521-F</td>
<td>8.65±1.02</td>
<td>7.47±0.57</td>
</tr>
<tr>
<td>Y-111-M</td>
<td>9.18±0.6</td>
<td>7.66±0.51</td>
</tr>
<tr>
<td>Y-513-S</td>
<td>9.27±0.82</td>
<td>7.89±0.11</td>
</tr>
<tr>
<td><strong>Red Mottled</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-443-F</td>
<td>8.29±0.89</td>
<td>7.92±0.31</td>
</tr>
<tr>
<td>R-436-M</td>
<td>9.55±1.04</td>
<td>8.71±0.43</td>
</tr>
<tr>
<td>R-434-S</td>
<td>10.23±0.59</td>
<td>9.71±0.34</td>
</tr>
</tbody>
</table>

1 Data represent means and standard deviations of n=24. For seed coat weight and n=72 for seed coat thickness. Values within the same column or row followed by the same superscript letter are not significantly different (P ≤ 0.05) from each other.

2 F: fast-cooking genotype; M: moderate-cooking genotype; S: slow-cooking genotype.

3 Seed coat weight (%) = Total weight of seed coats of 5 beans divided by total weight of 5 whole beans x 100.
4.3. Seed coat thickness

The seed coat thicknesses of the studied raw beans are presented in Table 3, and Figure 10 shows images of cross sections of raw beans with seed coats. Generally, red mottled beans had thicker seed coats than yellow beans. Also, beans from Tanzania had thicker seed coats than their counterparts from Puerto Rico. The seed coat thickness of yellow beans from Puerto Rico was significantly different among genotypes (P<0.05) and increase in thickness was associated with an increase in cooking time. The seed coat thickness of the slow-cooking genotype Y-513-S from Puerto Rico was 1.4-fold thicker than genotype Y-521-F. On the contrary, there were no significant differences in the seed coat thicknesses of fast, moderate and slow cooking yellow bean genotypes (P>0.05) from Tanzania. For red mottled beans, the fast cooking genotype R-443-F had a significantly smaller seed coat thickness than the moderate and slow cooking genotypes for both Puerto Rico and Tanzania locations. Generally, seed coat thicknesses of all the three red mottled bean genotypes from Puerto Rico and Tanzania were not significantly different (P>0.05). This would imply that location had no effect on seed coat thickness.
Figure 10. Cross-sections of seed coats for fast, moderate and slow cooking common beans by Scanning Electron Microscope (SEM).

Regardless of the market class, increase in seed coat thickness was associated with an increase in bean cooking time. There was a 74.3% positive correlation (P<0.05) between seed coat thickness and cooking time of raw beans (Table 4), which implies that cooking time of beans was largely associated with the seed coat thickness. Furthermore, seed coat thickness made a unique association with cooking time (beta coefficient = .824) (P<0.05); however, this value was lower than that of hot water soluble pectin (beta coefficient = 3.548) implying that it made less of a contribution when the variance explained by all other variables in the model is controlled for.

These results are in agreement with (Sathe and Deshpande, 2003) who reported that thick seed coats hinder hydration of the bean cotyledon. Hydration is a requirement for any bean to cook. The results from the current study generally show that red mottled beans have thicker seed coats than yellow beans. This could explain why yellow beans cook faster than red mottled beans. Selecting for a thinner seed coat would be a good method of reducing cooking time of beans, however, it is a protective mechanism against pests, and so perhaps selecting for thin but tough seed coats would address the problem.
Table 4. Correlations and beta coefficients between cooking time (CT) of raw common beans and their physicochemical properties: seed coat thickness (SCT), protein content (PC), starch content (SC), hot water soluble pectin (HWSP), hot water insoluble pectin (HWIP), and total pectin (TP) for six varieties of dry common beans grown in Tanzania and Puerto Rico

<table>
<thead>
<tr>
<th>Physicochemical properties</th>
<th>Correlation coefficient (r)</th>
<th>Unstandardized coefficients (B)</th>
<th>Standardized coefficients (Beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Constant)</td>
<td>10.995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seed coat thickness</td>
<td>0.743*</td>
<td>1.270*</td>
<td>0.824*</td>
</tr>
<tr>
<td>Protein content</td>
<td>-0.260</td>
<td>-1.756</td>
<td>-0.251</td>
</tr>
<tr>
<td>Starch content</td>
<td>0.417*</td>
<td>-0.344</td>
<td>-0.150</td>
</tr>
<tr>
<td>Hot water soluble pectin</td>
<td>-0.484*</td>
<td>5.590*</td>
<td>3.548*</td>
</tr>
<tr>
<td>Hot water insoluble pectin</td>
<td>-0.346*</td>
<td>6.096</td>
<td>2.462</td>
</tr>
<tr>
<td>Total pectin</td>
<td>-0.668*</td>
<td>-8.421*</td>
<td>-3.987*</td>
</tr>
</tbody>
</table>

1 Data represent correlation and beta coefficients for n = 24. Values followed by * are significant (P ≤ 0.05).

The R square for this model is 70.2% (P<0.05). 

\[ CT = 10.995 + 1.27 \times (SCT) - 0.344 \times (SC) + 5.590 \times (HWSP) + 6.096 \times (HWIP) - 8.421 \times (TP) \]
4.4. Calcium and magnesium contents

The calcium and magnesium contents of the studied raw common beans are presented in Table 5. The calcium content ranged between 0.089-0.188 g/100 g of beans, and magnesium content ranged between 0.172-0.22 g/100 g. There were no significant differences in the calcium and magnesium contents of fast, moderate and slow cooking yellow or red mottled beans from either the Puerto Rico or the Tanzania location (P>0.05). Kyriakidis and others (1997) reported that beans cooked in divalent cation solutions (CaCO$_3$ and MgCO$_3$) had a longer cooking time than those boiled in plain water. This could imply that the total inherent calcium and magnesium contents of dry beans is not associated with cooking time of beans, but rather only those found in cooking solutions. Since these minerals were proved to play a role in the HTC phenomenon (Kyriakidis and others, 1997), perhaps it is about how much of these minerals are available for a chemical reaction. Fast and slow cooking beans might inherently have similar amounts of these minerals, but perhaps the slow cooking beans release more divalent cations during phytic acid hydrolysis which in turn participate in crosslinking of pectin molecules, as compared to the fast cooking beans that might be releasing fewer of these divalent ions. Growing location did not have an effect on calcium and magnesium contents of the beans according to the findings in the current study. These findings are in agreement with Muyonga and others (2008) who reported that calcium and magnesium contents of common beans grown in different locations (Kabale, Lira and Naari) were not significantly different (P>0.05).
Table 5. Calcium and magnesium contents of whole seeds of fast, moderate, and slow cooking yellow and red mottled common dry beans from Puerto Rico and Tanzania locations

<table>
<thead>
<tr>
<th>Bean Sample</th>
<th>Calcium (% by weight)</th>
<th>Magnesium (% by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puerto Rico</td>
<td>Tanzania</td>
</tr>
<tr>
<td>Yellow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-521-F</td>
<td>0.188±0.026&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.150±0.020&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Y-111-M</td>
<td>0.117±0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.114±0.001&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Y-513-S</td>
<td>0.155±0.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.124±0.009&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Red Mottled</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-443-F</td>
<td>0.154±0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.089±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>R-436-M</td>
<td>0.135±0.007&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.156±0.008&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>R-434-S</td>
<td>0.137±0.018&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.145±0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Data represent means and standard deviations of n=24. Values within the same column or row followed by the same superscript letter are not significantly different (P ≤ 0.05) from each other.

2 F: fast-cooking genotype; M: moderate-cooking genotype; S: slow-cooking genotype.
Therefore inherent calcium and magnesium contents in beans are not associated with cooking time of the six common beans studied here. Perhaps the distribution of these minerals in the bean cells might be different for the fast and slow cooking beans. Further research should be conducted to investigate this.
4.5. Protein content

The protein contents of the studied raw common beans are presented in Table 6. They ranged between 20.17 and 25.97 g/100 g of raw beans. The protein contents of the fast, moderate, and slow cooking yellow beans were not significantly different from each other (P>0.05) for both Puerto Rico and Tanzania locations. However, genotypes Y-111-M and Y-513-S from Puerto Rico had 4 g and 2.3 g, respectively, greater protein content than their counterparts from Tanzania. This could be attributed to the fertility of the soil where they were grown. Except for the genotype R-443-F, the protein contents of all the red mottled genotypes were not significantly different from each other (P>0.05) for both Puerto Rico and Tanzania locations. The fast-cooking genotype R-443-F from Puerto Rico had about 3 g/100 g of protein greater than the slow-cooking genotypes. There was also no correlation between protein content and cooking time. This however does not necessarily imply that protein has no association with cooking time of beans. Perhaps the structure of the proteins in the fast and slow cooking beans could be different. Further research should be done to study the structure of protein in these beans.
Table 6. Protein content of fast, moderate, and slow cooking yellow and red mottled common dry bean varieties from Puerto Rico and Tanzania locations

<table>
<thead>
<tr>
<th>Common Bean Varieties</th>
<th>Protein Content (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puerto Rico</td>
<td>Tanzania</td>
<td></td>
</tr>
<tr>
<td><strong>Yellow</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-521-F</td>
<td>22.34±0.04 (^{fb})</td>
<td>21.67±0.05 (^{b})</td>
<td></td>
</tr>
<tr>
<td>Y-111-M</td>
<td>24.15±0.23 (^{fe})</td>
<td>20.17±0.14 (^{b})</td>
<td></td>
</tr>
<tr>
<td>Y-513-S</td>
<td>23.57±0.16 (^{f})</td>
<td>21.28±0.36 (^{b})</td>
<td></td>
</tr>
<tr>
<td><strong>Red mottled</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-443-F</td>
<td>25.97±0.39 (^{e})</td>
<td>23.82±0.17 (^{f})</td>
<td></td>
</tr>
<tr>
<td>R-436-M</td>
<td>23.03±0.04 (^{f})</td>
<td>23.42±0.12 (^{f})</td>
<td></td>
</tr>
<tr>
<td>R-434-S</td>
<td>23.29±0.03 (^{f})</td>
<td>22.59±0.03 (^{fb})</td>
<td></td>
</tr>
</tbody>
</table>

1 Data represent means and standard deviations of n=24. Values within the same column and row followed by the same superscript letter are not significantly different (P ≤ 0.05) from each other.

2 F: fast-cooking genotype; M: moderate-cooking genotype; S: slow-cooking genotype.
4.6. Starch content

The starch contents of the studied raw common bean genotypes ranged between 22.8 and 39.75 g/100 g (Table 7). There was a significant difference among the starch contents of fast, moderate and slowing-cooking yellow bean genotypes (P<0.05) from Puerto Rico and Tanzania. As expected, starch content increased with increase in cooking time for all yellow genotypes in both locations except genotype Y-521-F from Puerto Rico that had 7 g and 5 g higher starch content than genotypes Y-111-M and Y-513-S, respectively. The slow-cooking genotype Y-531-S from Tanzania had 4.3 g greater starch than the fast-cooking genotype Y-521-F. The genotypes Y-111-M and Y-513-S from Tanzania had 27% and 26%, respectively, greater starch content than their counterparts from Puerto Rico.

Like yellow beans, starch content of fast, moderate and slow-cooking red mottled beans was significantly different from each other for both locations (P<0.05) with fast-cooking beans having the lowest starch content and the slow-cooking beans having the highest content. Like yellow beans, the starch content of red mottled beans from Tanzania was significantly higher than their counterparts from Puerto Rico however the differences varied according to genotypes. The genotypes R-443-F, R-436-M, and R-434-S from Tanzania had 8 g, 10 g, and 9 g, respectively, greater starch content than their counterparts from Puerto Rico.

There was a 41.7% positive correlation (P<0.05) between starch content and cooking time of raw beans (Table 4), which implies that cooking time of beans was associated with their starch content. These results are in agreement with Reyes-Moreno and others (1994) who reported that the fast cooking common bean variety Michigan 800 had a lower starch content (24.0 g/100 g) than that
of the slow cooking bean variety Ojo de Cabra (26.0 g/100 g), thus starch content could be associated with cooking time. Regardless of the positive correlation with cooking time, the present study also showed that starch content did not make a significant contribution to cooking time (beta coefficient = -.150) (P>0.05) in this model. This could have been due to the small number of samples used in the present study. Further research should be conducted using a larger number of samples, and perhaps the different components of starch and the starch: protein ratio could be studied in relation to cooking time.
Table 7. Starch contents of raw fast, moderate, and slow cooking yellow and red mottled dry common beans from Puerto Rico and Tanzania locations

<table>
<thead>
<tr>
<th>Common Bean Varieties</th>
<th>Starch Content (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puerto Rico</td>
<td>Tanzania</td>
<td></td>
</tr>
<tr>
<td><strong>Yellow</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-521-F</td>
<td>34.30±1.23&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>35.48±1.89&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Y-111-M</td>
<td>26.84±0.99&lt;sup&gt;e&lt;/sup&gt;</td>
<td>36.59±1.29&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Y-513-S</td>
<td>29.31±0.06&lt;sup&gt;f&lt;/sup&gt;</td>
<td>39.75±4.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Red mottled</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-443-F</td>
<td>25.31±0.95&lt;sup&gt;e&lt;/sup&gt;</td>
<td>33.44±3.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>R-436-M</td>
<td>22.84±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32.94±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>R-434-S</td>
<td>27.10±0.44&lt;sup&gt;e&lt;/sup&gt;</td>
<td>36.01±2.57&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>Data represent means and standard deviations of n=24. Values within the same column or row followed by the same superscript letter are not significantly different (P ≤ 0.05) from each other.

<sup>2</sup>F: fast-cooking genotype; M: moderate-cooking genotype; S: slow-cooking genotype.
4.7. Hot water soluble pectin (HWSP) content

Total pectin content and its two fractions for the studied raw common beans are presented in Table 8. All bean genotypes for both market classes from both locations had less HWSP than HWIP. The HWSP contents of fast, moderate and slow cooking yellow beans from Puerto Rico were significantly different (P<0.05), with moderate cooking beans having the highest HWSP. The HWSP content of Y-521-F was about 2.4-fold greater than the slow cooking beans with the lowest HWSP value. HWSP content of fast, moderate, and slow-cooking yellow from Tanzania location were not significantly different from each other (P>0.05).

HWSP contents of red mottled beans from Puerto Rico were not significantly different from each other (P>0.05). HWSP content of fast, moderate, and slow-cooking red mottled beans from Puerto Rico and Tanzania location were not significantly different from each other except for the fast-cooking red mottled bean genotype, R-443-F, that was significantly greater than all of them and had about 2.5-fold greater HWSP content (14.78±0.62 mg/g) than the slow-cooking red mottled bean genotype (5.94±1.26 mg/g).

There was a 48.4% (P<0.05) negative correlation between HWSP and cooking time of raw beans (Table 4), which implies that cooking time of beans decreased with increase in HWSP. Furthermore, HWSP made the greatest contribution to cooking time (beta coefficient = 3.548) (P<0.05) when the variance explained by all other variables in the model is controlled for.

These results were in agreement with Bernal-Lugo and others (1996) and Njoroge and others (2014) who reported that fast cooking bean (variety Rose coco) had more hot water soluble pectin
(8.44 mg/g) than the slow cooking beans (variety Pinto, 5.51 mg/g). Further research with a large sample size should be conducted to verify these findings.
Table 8. Hot water soluble pectin (HWSP), hot water insoluble pectin (HWIP), and total pectin (Total) of fast (F), moderate (M), and slow (S) cooking common bean varieties of yellow (Y) and red mottled (R) beans grown in Puerto Rico and Tanzania locations\(^1\)

<table>
<thead>
<tr>
<th>Bean Sample(^2)</th>
<th>Puerto Rico</th>
<th>Tanzania</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HWSP (mg/g)</td>
<td>HWIP (mg/g)</td>
</tr>
<tr>
<td><strong>Yellow</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-521-F</td>
<td>11.69±0.23(^{b})</td>
<td>14.50±2.19(^{c})</td>
</tr>
<tr>
<td>Y-111-M</td>
<td>16.54±1.20(^{c})</td>
<td>16.86±0.11(^{e})</td>
</tr>
<tr>
<td>Y-513-S</td>
<td>6.91±0.80(^{a})</td>
<td>13.77±1.28(^{c})</td>
</tr>
<tr>
<td><strong>Red Mottled</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-443-F</td>
<td>8.62±0.51(^{a})</td>
<td>14.43±2.43(^{e})</td>
</tr>
<tr>
<td>R-436-M</td>
<td>6.45±0.87(^{a})</td>
<td>17.60±1.22(^{e})</td>
</tr>
<tr>
<td>R-434-S</td>
<td>7.79±0.74(^{a})</td>
<td>12.13±0.67(^{de})</td>
</tr>
</tbody>
</table>

\(^1\) Data represent means and standard deviations of n=24. Values within the same column or row followed by the same superscript letter are not significantly different (P ≤ 0.05) from each other.

\(^2\) F: fast-cooking genotype; M: moderate-cooking genotype; S: slow-cooking genotype.
4.8. Hot water insoluble pectin (HWIP) content

The HWIP of the studied raw beans ranged between 9.98±1.78 mg/g and 17.60±1.22 mg/g (Table 8). Except for R-443-F, each of the bean varieties of both yellow and red mottled beans from Puerto Rico and Tanzania had a greater HWIP content than HWSP content. The HWIP contents of fast, moderate and slow-cooking beans of both market classes within one location were generally not significantly different from each other (P>0.05). This could mean that HWIP is formed at a more uniform rate during bean growth, and these beans were all about the same “age” therefore they had similar amounts of HWIP. If some had been stored longer, then perhaps they would have higher HWIP contents.

There was a slight negative correlation 34.6% (P>0.05) between HWIP and cooking time (Table 4). This implies that cooking time increased with decrease in HWIP. These results are not in line with the findings of Bernal-Lugo and others (1996) who reported that the slow cooking beans had a higher HWIP pectin content than fast cooking beans. Some pectin molecules are tightly bound to other polysaccharides like hemicellulose, wall proteins and phenolic compounds (Caffall and Mohnen, 2009). Perhaps most of the HWIP in the hard to cook beans in the present study were tightly bound to other polysaccharides like hemicellulose, wall proteins and phenolic compounds making it hard to be extracted. Further research with a larger sample size should be conducted to verify these findings.
4.9. Total Pectin

The total pectin of the studied raw beans ranged between 15.92 mg/g and 33.39 mg/g (Table 8). The total pectin contents of yellow beans from Puerto Rico were significantly different with Y-111-M having the highest pectin and Y-513 having the lowest pectin content. On the contrary, TP of yellow beans from Tanzania was not significantly different. Also, TP of yellow beans from Puerto Rico were slightly higher than their counterparts from Tanzania.

The red mottled fast, moderate and slow cooking beans from Puerto Rico were not significantly different (P>0.05) from each other. On the contrary, the red mottled fast, moderate and slow cooking beans from Tanzania were significantly different (P<0.05) from each other. The genotype R-443-F had 5 mg and 10 mg higher TP content than genotypes R-436-M and R-434-S, respectively. For both market classes in both locations, fast cooking beans were associated with a high total pectin content. There was a 66.8% (P<0.05) negative correlation between cooking time and total pectin (Table 4) and it made the largest contribution to cooking time (beta coefficient = 3.987) (P<0.05). Since the largest composition of TP is HWIP, it could mean that most of the pectin polymers in the slow cooking beans are tightly bound to the cell wall polymers like hemicellulose, phenolic compounds and wall proteins making it hard to extract enough of it. These results also imply that no matter the amount of pectin present, its soluble fraction is what is associated with the cooking time. Total pectin in this study is the sum of HWSP and HWIP so the strong negative correlation of TP with cooking time could be largely influenced by HWSP which has been reported in this study to also have a strong negative correlation with cooking time.
4.10. Starch gelatinization temperature

Starch gelatinization temperatures of the studied raw beans are presented in Table 9. Except for genotype R-443-F from Puerto Rico, there were no significant differences in Tg (P>0.05) among fast, moderate and slow cooking yellow and red mottled beans from Puerto Rico and Tanzania. The Tg of genotype R-443-F from Puerto Rico was at least 10°C lower than genotypes R-436-M and R-434-S. Except for R-443-F, each of the bean genotypes of both yellow and red mottled beans from Puerto Rico had 5-6°C higher gelatinization temperature (Tg) than its counterpart from Tanzania. This suggests that location could have an influence on the gelatinization temperatures of these dry bean varieties.

These results are in agreement with Bernal-Lugo and others (1996) who reported that both fast and slow cooking beans had similar gelatinization temperatures (86°C and 87°C, respectively) and therefore that Tg did not have much contribution to cooking time. Their Tg values were 4 to 6°C higher than Tg values in the present study probably because of the environmental difference in which the beans were grown. Since the beans had similar gelatinization temperatures, it can be concluded that Tg was not associated with their cooking time.
Table 9. Starch gelatinization and protein denaturation temperatures of dry common bean varieties of yellow and red mottled beans from Puerto Rico and Tanzania locations.

<table>
<thead>
<tr>
<th>Bean Variety</th>
<th>Tg(^3) (°C)</th>
<th>Td(^4) (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Puerto Rico</td>
<td>Tanzania</td>
</tr>
<tr>
<td><strong>Yellow</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Y-521-F</td>
<td>82.23±0.05(\text{e})</td>
<td>76.97 ±0.50(\text{a})</td>
</tr>
<tr>
<td>Y-111-M</td>
<td>80.46 ±0.60(\text{e})</td>
<td>75.78 ±1.29(\text{a})</td>
</tr>
<tr>
<td>Y-513-S</td>
<td>81.85±0.41(\text{e})</td>
<td>76.58±0.12(\text{a})</td>
</tr>
<tr>
<td><strong>Red Mottled</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R-443-F</td>
<td>70.29±0.07(\text{d})</td>
<td>73.80±0.37(\text{a})</td>
</tr>
<tr>
<td>R-436-M</td>
<td>81.34±0.12(\text{e})</td>
<td>75.79±0.42(\text{a})</td>
</tr>
<tr>
<td>R-434-S</td>
<td>81.38±0.47(\text{e})</td>
<td>75.75±0.14(\text{a})</td>
</tr>
</tbody>
</table>

\(1\) Data represent means and standard deviations of n=24. Values within the same column or row followed by the same superscript letter are not significantly different (P ≤ 0.05) from each other.

\(2\) F: fast-cooking genotype; M: moderate-cooking genotype; S: slow-cooking genotype.

\(3\) Tg = starch gelatinization temperature, \(4\) Td = protein denaturation temperature
4.11. Protein denaturation temperature

Protein denaturation (Td) temperatures of the studied raw beans are presented in Table 9. There were no significant differences in Td among fast, moderate and slow cooking yellow beans within the Puerto Rico location and within the Tanzania location (P>0.05). For red mottled beans, with the exception of R-443-F, there were also no significant differences in Td among fast, moderate and slow cooking yellow beans within the Puerto Rico location and within the Tanzania location (P>0.05). R-443-F had at least 3°C higher Td than other varieties in Puerto Rico. For both yellow and red mottled beans, each genotype grown in Puerto Rico had a slightly significant higher Td (2-3°C) than its counterpart grown in Tanzania.

Moreno and others (1994) reported that fast cooking beans (variety Michigan 800) had a lower protein denaturation temperature (88.6±0.8°C) than that of slow cooking bean (variety Ojo de Cabra, 95.3±0.7°C), and indicated that this affected the cooking time of beans. On the contrary, Bernal-Lugo and others (1996) reported that both fast and slow cooking beans had similar protein denaturation temperatures (103°C and 104°C, respectively) and that therefore denaturation temperature does not contribute to cooking time. The results of this thesis, in Table 9, are in agreement with Bernal-Lugo and others (1996) although the Td values for their bean varieties were at least 11°C higher than those of the beans in the present study. The results imply that Td did not have an association with cooking time of beans.

Based on results in the present study (Table 9), a difference in growing location had a slight effect on protein denaturation temperature of most of the counterpart varieties. Due to a limited number of bean varieties, further tests are needed from more different locations to verify the findings.
CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

The findings of this study showed that yellow beans cooked slightly faster than red mottled beans and location had no effect on the cooking time trend. There was no significant difference in cooking times of soaked and dehulled beans, therefore soaking is as good as dehulling. Seed coat thickness and starch content were positively correlated with cooking time. Hot water soluble pectin and total pectin content were negatively correlated to cooking time. Starch gelatinization temperature, protein denaturation temperature, protein content, and calcium and magnesium contents each was not associated with cooking time of beans.

Households should always soak dry common beans before cooking to shorten cooking time. Also, selecting for bean varieties with a thinner seed coat would be a good method of reducing cooking time of beans; however, the seed coat is a protective mechanism against pests, and so maybe selecting for thin but tough seed coats would result into varieties with a shorter cooking time. Further investigation on the structure of the studied components of fast and slow cooking common beans will provide more knowledge towards understanding the physicochemical properties influencing cooking time of dry common beans. Also larger number of bean genotypes and market classes should be used.
APPENDIX
Figure 11. The peak gelatinization (first peak) and peak denaturation (second peak) temperatures of dry common beans from Tanzania.
Figure 12. The peak gelatinization (first peak) and peak denaturation (second peak) temperatures of dry common beans from Puerto Rico.
Table 10. Correlation coefficients among physicochemical properties of dry common beans: cooking time (CT) protein content (PC), seed coat thickness (SCT), starch content (SC), hot water soluble pectin (HWSP), hot water insoluble pectin (HWIP) and total pectin (TP) for dry common beans

<table>
<thead>
<tr>
<th></th>
<th>CT</th>
<th>PC</th>
<th>SCT</th>
<th>SC</th>
<th>HWSP</th>
<th>HWSP</th>
<th>TP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Correlation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>-.226</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCT</td>
<td>.743</td>
<td>-.144</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SC</td>
<td>.417</td>
<td>-.605</td>
<td>.288</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWSP</td>
<td>-.484</td>
<td>.423</td>
<td>-.372</td>
<td>-.573</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HWIP</td>
<td>-.346</td>
<td>.172</td>
<td>-.567</td>
<td>.065</td>
<td>-.184</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>TP</td>
<td>-.668</td>
<td>.470</td>
<td>-.663</td>
<td>-.514</td>
<td>.816</td>
<td>.410</td>
<td>1.000</td>
</tr>
<tr>
<td>Sig. (1-tailed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td></td>
<td>.144</td>
<td>.000</td>
<td>.021</td>
<td>.008</td>
<td>.049</td>
<td>.000</td>
</tr>
<tr>
<td>PC</td>
<td>.144</td>
<td></td>
<td>.252</td>
<td>.001</td>
<td>.020</td>
<td>.210</td>
<td>.010</td>
</tr>
<tr>
<td>SCT</td>
<td>.000</td>
<td>.252</td>
<td></td>
<td>.086</td>
<td>.037</td>
<td>.002</td>
<td>.000</td>
</tr>
<tr>
<td>SC</td>
<td>.021</td>
<td>.001</td>
<td>.086</td>
<td></td>
<td>.002</td>
<td>.381</td>
<td>.005</td>
</tr>
<tr>
<td>HWSP</td>
<td>.008</td>
<td>.020</td>
<td>.037</td>
<td>.002</td>
<td></td>
<td>.195</td>
<td>.000</td>
</tr>
<tr>
<td>HWIP</td>
<td>.049</td>
<td>.210</td>
<td>.002</td>
<td>.381</td>
<td>.195</td>
<td></td>
<td>.023</td>
</tr>
<tr>
<td>TP</td>
<td>.000</td>
<td>.010</td>
<td>.000</td>
<td>.005</td>
<td>.000</td>
<td>.023</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>
Figure 13. Cooking time of raw tempered yellow (Y) and red mottled beans (R) from Puerto Rico (PR) and Tanzania (TZ) that were raw (not soaked or dehulled), soaked and dehulled before cooking. Data represent means and standard deviations. Values of each bar topped by the same letter are not significantly different (P ≤ 0.05) from each other.
Figure 14. Hot water soluble pectin (HWSP), hot water insoluble pectin (HWIP), and total pectin (Total) of six fast (F), moderate (M), and slow (S) cooking common bean varieties of yellow (Y) and red mottled (R) beans grown in Puerto Rico and Tanzania locations. Data represent means and standard deviations of n=2. Values of each bar topped by the same letter are significantly different (P ≤ 0.05) from each other.
REFERENCES
REFERENCES


