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EFFECT OF COOKING AND PROCESSING VARIABLES ON QUALITY CHARACTERISTICS OF DIVERSE NAVY BEANS (*Phaseolus vulgaris L.*)

presented by

Dominique Savio Nkunda

has been accepted towards fulfillment
of the requirements for the

Master's degree in Food Science

Mark A. Uebersax
Major Professor's Signature

August 22,2003
Date

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EFFECTS OF COOKING AND PROCESSING VARIABLES ON QUALITY CHARACTERISTICS OF DIVERSE NAVY BEANS (*Phaseolus vulgaris L.*)

By

Dominique Savio Nkunda

A THESIS

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

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

2003

ABSTRACT

EFFECT OF COOKING AND PROCESSING VARIABLES ON

QUALITY CHARACTERISTICS OF DIVERSE NAVY BEANS

By

Dominique Savio Nkunda

Three series of experiments were carried out on Navy beans (*Phaseolus vulgaris L*) to assess cooking and canning quality.

In the first, cooking times were measured in aqueous solutions of calcium and phosphate ions. Calcium and phosphate ions in the cooking water respectively increased and decreased significantly ($p<0.05$) the cooking time to a plateau. The soaking methods affected differently the texture of canned beans.

In the second, beans were soaked in various conditions, thermally processed in cans. The calcium ion in the soaking water increased significantly ($p<0.05$) the firmness of canned beans whereas the phosphate ion decreased it significantly. However the phosphate ion in brine had no significant effect ($p>0.05$) on the texture of processed beans.

In the third the PME activity was tested on beans soaked in various conditions. Beans displayed various levels of pectin methylesterase (PME) activity. Soaked beans showed a higher PME activity in the temperature range of 46.1-54.5°C. However a negative correlation was found between the PME activity and the firmness of processed beans, which corroborated the findings of many researchers that the PME activity was of limited impact in the development of the HTC phenomenon.

ACKNOWLEGDEMENT

I would like to express my profound and sincere gratitude to my advisor, Dr Mark Uebersax. I appreciated his encouragement, his patience, his enthusiasm and his availability for help despite his responsibility as a chairperson. I feel fortunate to have benefited a lot from his teaching and his experience.

My thanks go to Drs Joseph Levine and Gale Strasburg who, not only accepted to be members of my guidance comity, but also contributed by their teaching.

I should be ungrateful if I did not acknowledge the teachers who participated in broadening my horizons through my Master's program: Drs John Linz, James Pestka, Thomas Wilson, John Partridge, Zeynep Ustonol, Perry Ng and Alden Boreen. Their help was highly appreciated.

Many thanks go to the USAID-KIGALI, which funded my studies and my two-years stay in United States through the Partnership for Enhancing Agriculture in Rwanda through Linkages (P.E.A.R.L. project). The availability of Dr Daniel Clay, Mr David Weight, Mrs Julie McDaniels and Mr Kyle Myers to make the stay easier was fairly appreciated.

Finally, my thanks go to my wife Julienne who accepted, without complaint, such a long separation. Could she find here the expression of my gratitude. This thesis is dedicated to my daughter Jennifer.

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INTRODUCTION

Dry beans are nutritionally important in developing countries, as they are the primary source of proteins and other nutrients. However, the quality of the final product is diminished by the storage and the processing conditions, which affects the consumer acceptance.

Beans stored in adverse conditions develop the hard-to-cook (HTC) phenomenon. When soaked in high levels of divalent ion solutions, they fail to achieve the proper softness required for a good palatability. This leads to economic losses, and in developing countries where the source of energy is primarily the firewood, it can result in an increased deforestation.

The objectives of this research were to study the effect of cooking and soaking water composition, to study the effect of soaking methods on the firmness of the final product, and to investigate the contribution of the pectin methylesterase activity to the texture of processed beans.

Thus, the following hypotheses were tested:

- Ho_1: There is a difference in cooking time, soak weight and drained weight among Navy bean samples of diverse production or crop year;
- Ho_2: The calcium and the phosphate ions in cooking /soaking water or in brine affect the cooking time and the firmness of beans;
- Ho_3: The soaking methods affect differently the texture of beans.

- Ho_4: The interaction between calcium and phosphate ions affects the texture of canned beans.
- Ho_5: The soaking treatment conditions (time and temperature) affect the hydration, the pectin methylesterase activity and the texture of beans.
- Ho_6: The pectin methylesterase activity affects the texture of processed beans.

A broad base of experimental material within the range of commonly used beans in commercial practice and diverse cultivars of Navy beans (*Phaseolus vulgaris L.*) were used in this research.

The research was conducted in a series of three studies: 1) the cooking time was measured using the pin drop cooker with different levels of calcium and phosphate ions; 2) beans were soaked in specified conditions, filled into cans with specified brines, processed, stored and assessed for texture with a Kramer shear press texturometer; 3) the PME activity was measured on non-treated beans and beans soaked in specified conditions. The relationship between the PME activity and the texture was investigated.

LITERATURE REVIEW

Description of Bean Seed

The dry edible bean (*Phaseolus vulgaris L.*) is composed of a broad spectrum of seed types of varying size, shape and color. The commercial classes include white seeds (Navy and Great Northern) and various colored seeds (Pinto, Kidney and black beans).

The two major structural parts of the legume seeds (Mauseth 2003) are the seed coat and the cotyledons or embryonic leaves, but other features include the epicotyl (embryonic stem), the radicle (embryonic root) and the hypocotyl (root/shoot junction). Further, on the surface of the seed, may be seen the hilum, which is the scar left where it separated from the stalk within the pod, and the micropyle, a minute opening in the seed coat (figure 1).

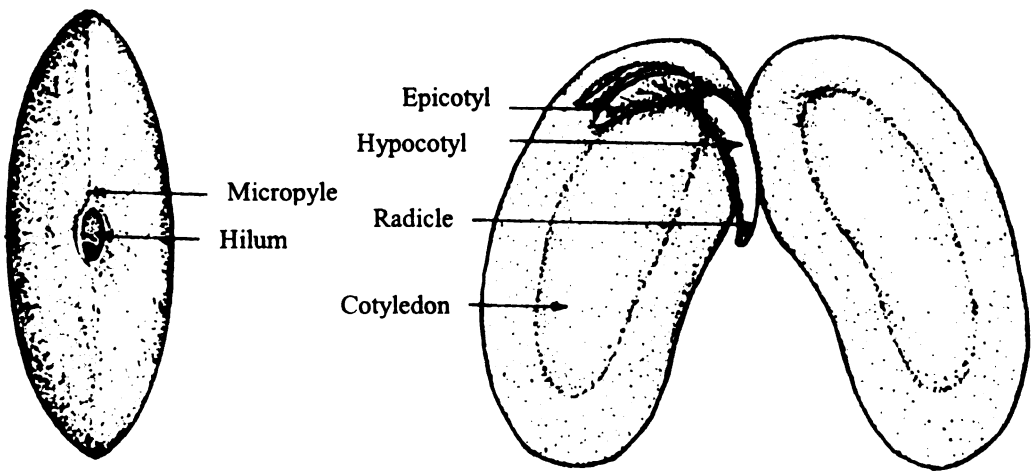


Figure 1.Structure of typical legume seed.
Left: external view. Right: internal view. Source: Northen 1958.

The physical structure and the chemical composition of primary components of the seed influence the hydration and the softening during cooking. The attributes of the

seed coat, cotyledons and the inherent cell wall structure, which influence the cooking quality, are presented:

Seed coat (testa). The legume testa is composed of an outer layer of sclereids and inner parenchymatous layers (figure 2). The outermost layer is composed of a thin cuticle that overlies a layer of prismatic, thick-walled contiguous cells called palisade cells. As the seed ripens, the walls of these cells contract and cause the hardness and impermeability of the testa. Hourglass cells are situated between the palisade and the stellate mesophyll (Sefa-Dedeh and Stanley 1979). The testa contributes to determining the water absorption rate and thus, the final cooking quality of the seeds.

Cotyledons. Form the major part of the seed and contain parenchymatous cells bounded by a distinct cell wall. The adjacent intercellular region, termed "middle lamella", is rich in pectin and water holding capacity. Cotyledon cells differ from typical parenchyma plant cells in that the desiccation during the maturation reduces cell organelles as storage proteins and starch accumulate. These cells contain elliptical starch granules embedded in a protein matrix consisting of protein bodies. Protein bodies are generally spherical and relatively smaller than starch granules. The starch and the protein contribute a significant influence to the rate and the potential of water permeation and water binding.

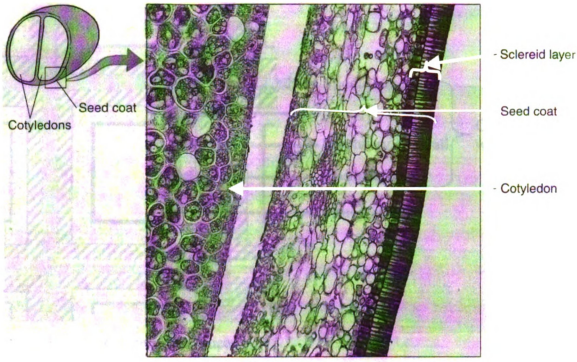


Figure 2. Seed coat structure of the bean. Source: Mauseth 2003.

The cotyledons of leguminous oilseeds such as soybean have a subcellular structure consisting of protein bodies surrounded by a cytoplasmic protein network in which are embedded lipid bodies or spherosomes (Stanley and Aguilera 1985).

Cell walls. The cotyledon cell walls are of particular importance from the standpoint of textural defects in legumes. The plant cell wall (figure 3) is that structure surrounding the protoplasm and exterior to the plasmalemma, a thin bilayer that functions in transport reactions. In mature cells a secondary cell wall, and exterior to this, a primary cell wall lie outside the plasma membrane. These structures consist of cellulose microfibrils, hemicellulose and lignin (Srisuma *et al* 1991).

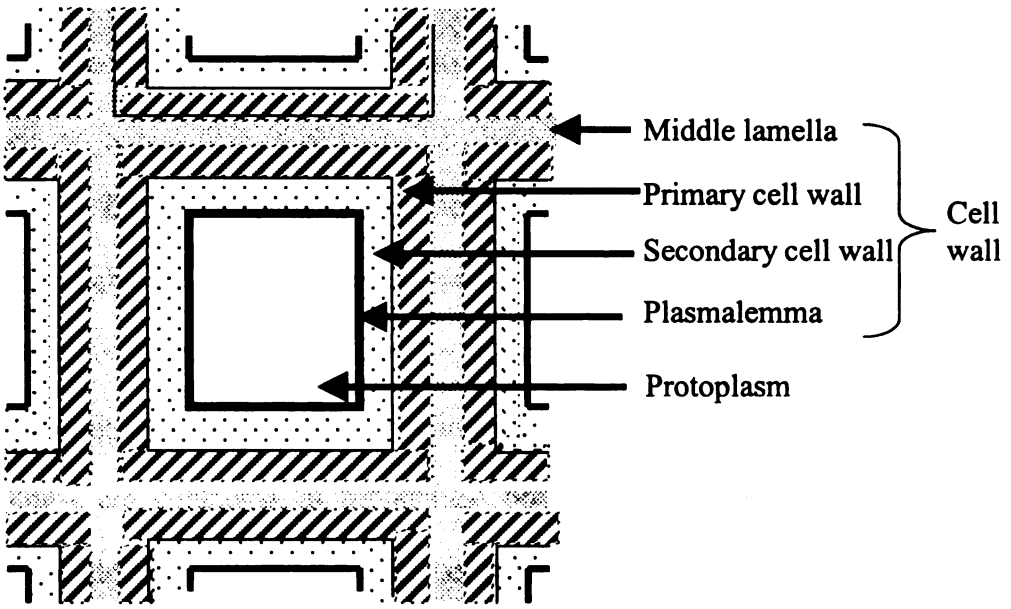


Figure 3. Cotyledon cell wall structure

The middle lamella region separates the primary walls of two cells. Pectic substances constitute a major part of the middle lamella, which provides the principal adhesion that holds plant cells together and dictates physical strength of the tissue.

The purpose of cooking is primarily to achieve the disruption of the middle lamella and the cell separation, which results in a softer texture and a better palatability.

Chemical Composition of seed legumes

Proteins. Physiologically, the storage proteins of legume seeds serve to supply amino acids and a pool of nitrogen compounds to the young seedling. They are primarily located in protein bodies within the cotyledon (Kashiwaba *et al* 1998). Legume proteins are nutritionally important because of their major contribution to total protein in the

human diet .Therefore, efforts must be made during storage and /or processing of legume seeds to preserve the protein quality.

Lipids. Legumes have total lipids determined as the total quantity of material extractable in organic solvents. Neutral triglycerides represent the major fraction of the compounds present, where they are stored as a compact source of energy for the growing seedling. The phospholipids are integral components of biological membranes. Storage lipids are contained in oil storage bodies (spherosomes) found in cotyledon cells. Oilseeds are an important source of polyunsaturated fatty acids, including essential fatty acids in the diet. Unsaturated lipids have a high oxidation potential, which can result in loss of quality (off-flavor and odors) particularly when held for extended periods of time and specifically when held under adverse storage conditions such as high temperature and high moisture content (Stanley and Aguilera 1985).

Carbohydrates. Legumes (pulses) contain 60-70% total carbohydrate on a dry basis of which starch is the major fraction (30-40%). Carbohydrates also comprise the primary composition of cell walls and testa which greatly influence the textural integrity of the seed. While the major constituents are pectic substances, hemicellulose, lignin and cellulose, cell walls also contain about 5-10% of polyphenolic compounds in the form of polysaccharide-protein-polyphenol complexes. The pectic substances comprise an intricate mixture of colloidal polysaccharides formed from pectin, de-esterified pectic acid and its salts (pectates) and certain neutral polysaccharides constituting the galacturonan structure (Stanley and Aguilera 1985).

These constituents provide differential hydration capacity with potential for crosslinking and further chemical and enzymatic action. The net result impacts cellular disruption and thus palatability of the cooked seed.

Several microcomponents within the seed structure greatly influence processed product palatability:

Phytic acid is the myo-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate (Figure 4). Its salts are generally refered to as "phytates". Both forms occur simultaneously in many seeds and researchers often do not make a distinction between these species.

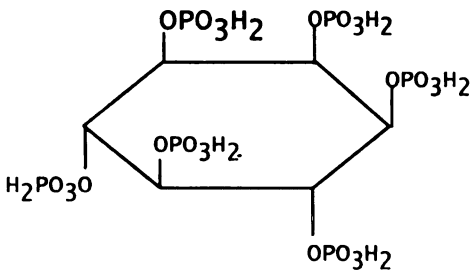


Figure 4. Chemical structure of phytic acid

Phytates are the primary storage form of both inositol and phosphate in all seeds and grains. Phytic acid is a strong chelator of divalent minerals (copper, calcium, magnesium, zinc and iron). This is a concern for animal and human mineral nutrition because it decreases the mineral availability. Phytates react with charged groups of proteins, which adversely influence the protein digestion and bioavailability, because they are ionic in nature (Reddy and Sathe 2002).

Phytates are also an energy store, a competitor for ATP during its biosynthesis near maturity, an inhibitor of metabolism and thus inducing dormancy, and a regulator of the level of inorganic phosphate (Plaami 1997).

Phytate content ranges from 0.17 to 9.15% in whole beans, 0.58 to 4.20% in bean flours and bean protein products, 0.05 to 5.20% in bean based foods (Reddy and Sathe 2002).

Phytate hydrolysis by phytases releases divalent cations (calcium and magnesium) which cross-link pectate chains. This reaction was believed to play a key role in the development of the HTC defect in legume seeds.

The polyphenolic compounds. The most numerous and widely distributed in the plant kingdom are phenolic acids and derivatives, tannins and flavonoids (Reyes-Moreno and Paredes-lopez, 1993). The changes occurring during post harvest storage of legumes have been associated with reactions of polyphenolic substances. They embrace a large array of chemical compounds that have at least one or more hydroxyl groups together with a number of minor constituents. The common phenolic constituents of plants may be divided into two groups: (1) phenolic acid and coumarins, and (2) flavonoid compounds, including anthocyanidins. Further, polymeric phenols can be characterized into two broad groups: tannins and lignins.

Tannins comprise a heterogeneous group of plant polyphenols, all of which are able to combine with proteins. Tannins are high molecular weight compounds containing sufficient phenolic hydroxyl groups to permit the formation of the stable cross-links with proteins. The stability of the complex is assured by hydrogen bonds, mainly from the

dihydroxyphenols and groups of the protein. Hydrophobic binding may also play an important role.Tannins have been classified into hydrolyzable and condensed tannins. Most hydrolyzable tannins contain a central core of glucose or other polyhydric alcohol esterified with gallic acid (figure 5) or hexahydroxy diphenyl acid. The condensed tannins (procyanidins) are mostly flavolans or polymers of flavan-3-ols (catechins) and/or flavan-3: 4-diols (leucoanthocyanidins). It appears that tannin content varies with the color of the seed coat. Low or negligible amounts are found in the cotyledons.

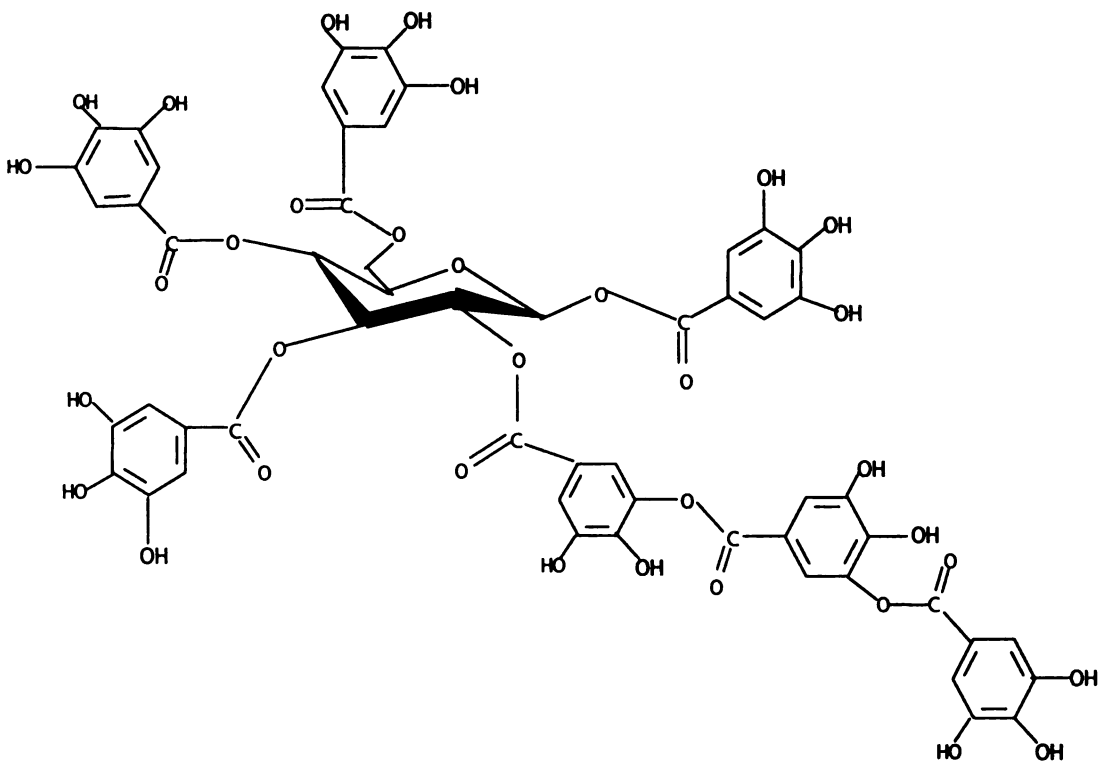
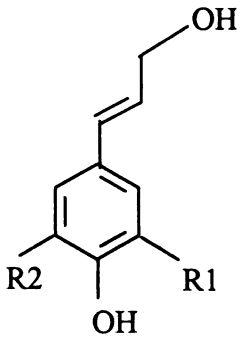


Figure 5. Example of tannin structure illustrating the central core of glucose with phenolic acid side chains.

Lignins are complex, three-dimensional polymers of phenylpropanoid units. The three-dimensional structure consists of short linear chains cross-linked by a variety of inter-chain covalent bonds. They are often described as dehydrogenated polymers of *p*-

coumaryl, coniferyl and sinapyl alcohols (Figure 6) (Lewis and Paice 1989). The function of lignins is to decrease the permeation of water across cell walls and to impart rigidity.



p-Coumary alcohol: R1,R2 = H

Coniferyl alcohol: R1 = OCH3, R2 = H

Sinapyl alcohol: R1,R2 = OCH3

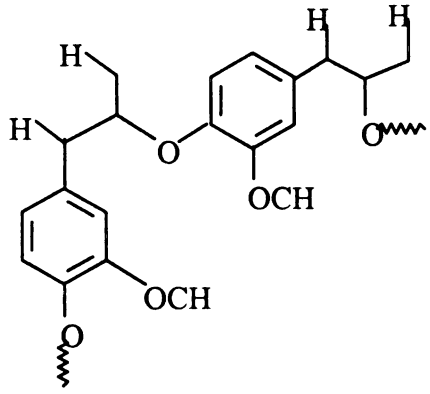


Figure 6. Structure of Lignin

Hardness of Beans

Hardening of legume seeds, also known as the Hard-To-Cook (HTC) phenomenon, is the failure of improperly stored seeds to soften enough to be eaten after cooking for reasonable time (Aguilera and Rivera 1992). Beans are susceptible to this phenomenon, which causes an increase in the cooking time needed to achieve an acceptable texture. This limits the utilization in consumption of beans because hardened cooked beans show an undesirable firm texture and adverse changes in color and flavor.

It is a major constraint to the consumption of *Phaseolus* and *Vigna* cultivars common in tropical countries, where high ambient temperatures and relative humidity are prevalent.

It is useful to divide the textural defects of legume seeds into two classes: 1)Hard-Shell and 2)Hard-To-Cook phenomenon (HTC).

Hard Shell Defect. This is a defect of legumes that do not soften sufficiently because of a failure to imbibe a sufficient quantity of water during soaking. Low relative humidities and moisture contents but high temperatures during storage favor the formation of hard shell in beans. It appears that storage conditions may be an important factor in this defect. The testa appears to be responsible for this defect, more specifically the palisade layer contained within the seed coat, the hilum the strophiose and various non-polar substances. These latter materials are enzymatically formed from oxidized monophenols, which can produce pigmented polyphenol complexes that may interact with proteins. Further, it cannot be excluded that this reaction leads to complex lignification (Stanley and Aguilera 1985).

Hard-To-Cook Phenomenon (HTC). Defect of legumes that do not soften because the soaked seeds do not become tender during reasonable cooking time. The mechanism differs significantly from hardshell because it is chemical rather than physical in nature. A number of hypotheses have been developed to explain the hard-to-cook defect (Stanley and Aguilera 1985).

The most advanced theory, the "middle lamella-cation-phytate-phytase theory", holds that insoluble pectates in the middle lamella, are rendered insoluble upon cooking

by replacement of their monovalent cations by calcium (Ca^{2+}) and magnesium (Mg^{2+}). Legume seeds stored at high temperatures and humidities develop the hard-to-cook defect because the phytase hydrolyzes its substrate, and release the divalent cations, which diffuse to the middle lamella and combine to form the complex pectates (figure 7).

Other proposed mechanisms include phenolic-protein complexes, starch swelling, gelatinization and retrogradation, lipid oxidation and polymerization. Many of the possible mechanisms are enzymatic in nature and implicate the role of specific enzymes. The role of selected enzymes include: 1) phytase (Ching and Schoolcraft 1968; Bernal-lugo *et al* 1990; Mafuleka *et al* 1993; Kilmer *et al* 1994), 2) pectin methylesterase (Gulati and Sood 1998; Liu, Phillips and Hung 1992; Mafuleka *et al* 1991), 3) lipoxygenase (Sambudi 1994), 4) protease (Ching and Schoolcraft 1968), 5) peroxidase (Rivera *et al* 1989) in the hard-to-cook phenomenon has been investigated by researchers.

Beans undergo changes not only during the development of the HTC in storage but also during subsequent processing.

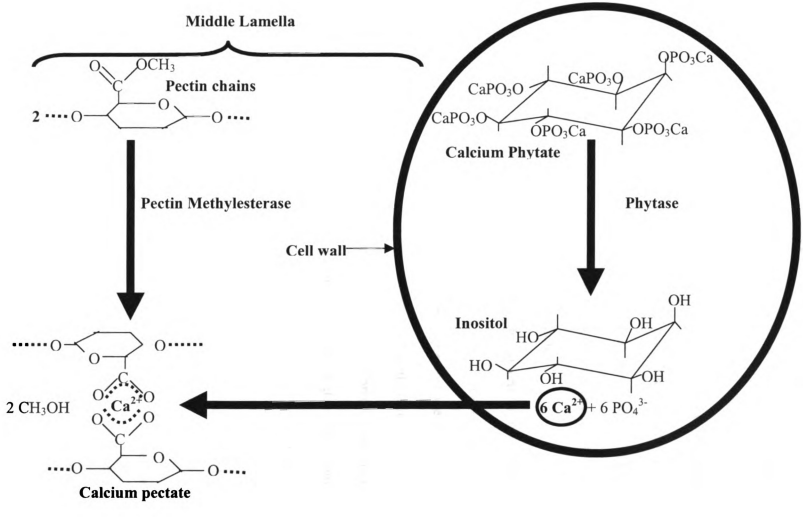


Figure 7. Proposed phytase/methylesterase mechanism of the HTC phenomenon in legumes. (Rendered after Kilmer *et al* 1994).

Physicochemical and biochemical changes of beans during storage

Much research has been conducted to establish decisively the effect of storage conditions on the development of hardening. Particularly the effects of temperature, relative humidity and the time of storage have been intensively investigated. The general feature of prior research has been the storage of legumes in known environmental conditions for extended time, or a physicochemical treatment of samples, and the measurement of hardening indicators (cookability, water uptake, texture) at known intervals. The results were compared to a control sample.

Jones and Boulter (1983) showed that reduced imbibition value and reduced pectin solubility could cause a reduction in the rate of cell separation during cooking and that these two factors acted synergistically. The solute leakage during soaking due to membrane breakdown, phytin catabolism and pectin demethylation were found to be key factors in the development of hard beans. Burr, Kon and Morris (1968) used an objective method and found that a high temperature, high moisture content and a long storage time contributed to impaired cookability in Pinto, large Lima and Navy beans. Aguilera and Rivera (1990) stored black beans for one year with 10% moisture content in simulated tropical conditions. Beans stored in impermeable bags and underground, hardened at a lower rate compared to unpackaged control samples.

Investigating the changes in textural properties of stored legumes, Garruti and Bourne (1985) used both the Texture Profile Analysis (TPA) by instruments and a trained sensory panel. These researchers stored red kidney beans at constant moisture, and high and low temperatures for six months. Their findings indicated that the hardness parameters (fracturability, gumminess, chewiness, springiness and cohesiveness) were

higher in samples stored at elevated temperature. Barron *et al* (1996) stored pinto beans for a long-term under adverse conditions before growing them in experimental plots. Newly harvested beans were tested for water absorption capacity, cookability, germination and texture. The HTC affected the germination and the development of normal plants, with apparent effect on yield. The water absorption capacity was reduced, however the cookability was not affected in the newly harvested beans.

Adverse storage conditions cause a dramatic change in the physicochemical profile of the legume seeds. Both the high temperature and the relative humidity result in solids loss and electrolyte leaching when the legumes are soaked, and manifest a high percentage of hardshell and HTC. Moreover, the percentage of seed water absorption and the germination rate are reduced (Berrios *et al* 1999). However, Hernández-Undón and Ortega-Delgado (1989) found that the water absorption of five to six-years-old beans were significantly higher than that of beans aged one to four years.

As the cooking time increases with the storage temperature and the relative humidity, phytate, phytase activity, amylase solubility, high methoxyl pectin and protein solubility decrease, whereas solids leaching and low methoxyl pectin increase (Hentges *et al* 1991). This is consistent with the theory that the HTC involves interactions between phytate, minerals and pectin.

Phytase activity. Hernández-Undón and Ortega-Delgado (1989) observed that the phytic acid decreased in hard beans as much as 94% to 98% during storage. They concluded that the disappearance of phytic acid resulted in a diminution of the chelation. Calcium (Ca^{2+}) and magnesium (Mg^{2+}) become free and associate with pectic substances

or proteinaceous materials, causing the HTC phenomena. As phytic acid is a good antioxidant, the seed is no longer protected against the oxidative degradation during storage.

Beans develop the HTC defect during soaking at 41°C in acetate buffer (pH 4.8), but not in fluoride ions (Kilmer *et al* 1994). Soaking fluoride-impregnated beans in an excess of tris-buffer reverses the inhibition by the fluoride. This is consistent with an inhibition of hardening through a competitive inhibition of phytase, as opposed to its interference in the formation of the magnesium and calcium ions as insoluble fluoride salts. Crean and Haisman (1963) studied the interaction between phytate and divalent cations during the cooking of dried beans. As cooking in extremely hard water complexes only a small fraction of the total phytate, insoluble phytates accounting only for a proportion of the absorbed ions, thus they concluded that the influence of phytate ions on the texture of beans was relatively small.

Finally, Bernal-Lugo (1990) and coworkers observed that the disappearance rate of phytic acid during storage does not correlate with the hardening extent. They suggested that the loss of phytic acid does not participate in an important way to the development of the HTC defect. However Mafuleka *et al* (1993) found a positive correlation between phytase activity and cooked bean hardness. Hinks and Stanley (1986) suggested that, in the multiple mechanism of bean hardening, phytate loss was a minimal contributor during initial storage while the lignification-like mechanism was a major contributor on extended storage. The authors showed the evidence of the role of the lignification in the HTC, with its implication for hydration during cooking, cell separation and texture.

Pectin methylesterase. The development of the HTC is accompanied by an increase in pectin insolubilization (Jones and Boulter 1983) and an increase in low methoxyl pectin (EDTA soluble) fractions (Hentges *et al* 1991). The decrease in pectin esterification would allow increased opportunity for binding divalent cations, which results in increased hardness.

Liu *et al* (1993) observed that pectin losses of aged cowpea seeds increased with the temperature in soaking, but decreased significantly in cooking. Theses losses were affected by medium pH and temperature, suggesting that pectin ß–elimination reaction occurs during heating (figure 8).

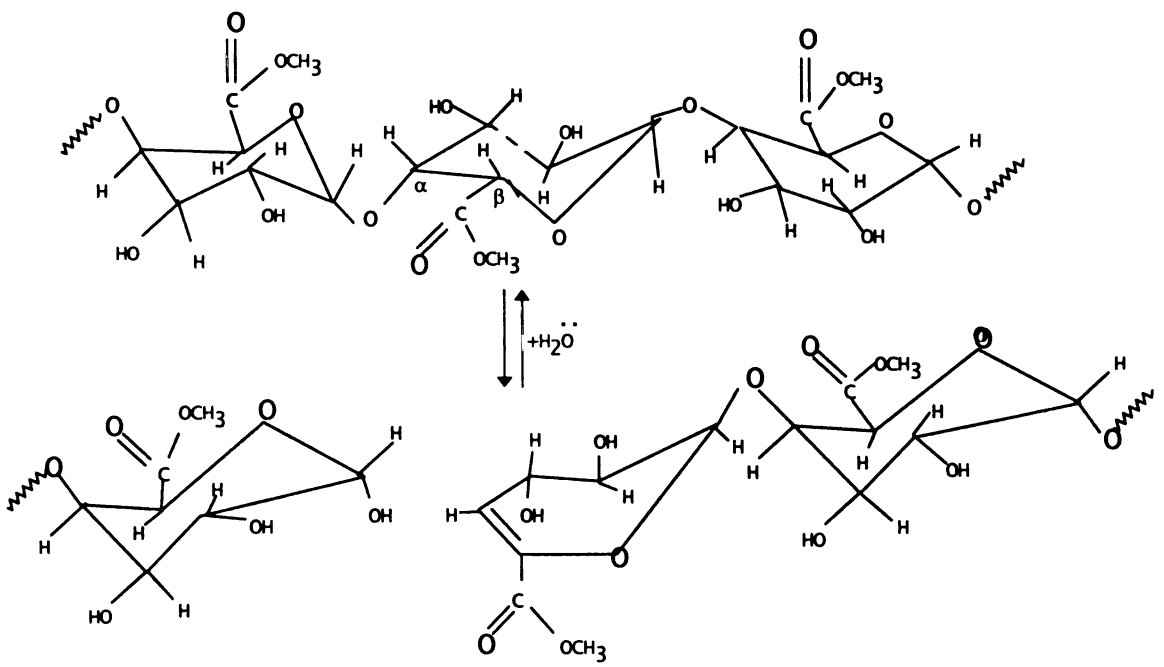


Figure 8.Pectin ß–Eliminative Reaction

This raises the concern of the role of the pectin methylesterase often invoked as a possible explanation of the HTC development. Mafuleka and coworkers (1990) observed

an increase of PME activity with storage time of decorticated Malawi bean varieties, but failed to demonstrate its influence on the hardness of the beans. They suggested that many factors could contribute in the hardness of the beans (time, water activity, phytase activity, lignin, phenolic compounds).

Proteins. During storage the proteins undergo changes suitable to contribute to the hardness of the beans. A decreased solubility proteins observed during storage may be attributable to the pH drop and this latter correlates with the HTC phenomenon (Liu *et al* 1992). In addition, assays conducted on common black beans revealed that as little as 34% of proteins denatured in HTC beans as compared to over 85% in soft cooking beans (Garcia-Vela *et al* 1989). This suggests that protein denaturation is one of the features of the HTC defect.

Carbohydrates. The starch undergoes changes during the development of the HTC phenomenon. Compared to soft beans, starch granules of hard beans are more resistant to the amyloglycosidase attack. These researchers observed more birefringence under polarized light microscopy, and higher gelatinization temperature for starches derived from HTC beans (Garcia-Vela *et al* 1994,1989).

Lipids. Studying the changes occurring in the aging of soybeans, Stewart and Bewley (1980) reported a high level of malondialdehyde, a product of the peroxidation of unsaturated fatty acids. They suggested that these changes could contribute to the loss of viability.

Phenolic compounds: tannins, lignin. Several researchers have investigated the involvement of the phenolic compounds in the development of the HTC. Srisuma *et al* (1989) and Garcia *et al* (1998) analyzed the extract of phenolic acids from HTC beans. HTC beans had more phenolic acids (hydroxycinnamic acids, especially ferulic acid) associated to the soluble pectic fraction. It is believed that the presence of more ferulic acid bound to soluble pectic, if involved in cross-links, contribute to changes in cell adherence, which leads to a textural defect. High levels of tannins, lignin and lignified proteins were found to be higher in HTC beans than the control (Martin-Cabrejas et al 1997). Stanley (1992) suggested that, under high temperature and relative humidity conditions, tannins migrate from the seed coat to the cotyledon where they condense and bind with macromolecular components of the cell wall and the middle lamella, leading to the HTC.

Physicochemical and biochemical changes of beans during processing

Typically, the thermal processing of beans involves the following steps:

- Soaking and blanching;
- Cooling and draining;
- Brining and exhausting;
- Sealing and processing;
- Storage and quality evaluation.

Soaking dry beans prior to cooking is beneficial to the final cooked product. It serves to eliminate the toxic factors (Kakade and Evans, 1966, qtd in Molina 1975), to remove foreign material, facilitate cleaning of beans, aid in can filling through uniform

expansion, ensure product tenderness and improve color (Cain, 1950; Crafts 1944; Hoff and Nelson 1966).

During soaking the beans imbibe water as free entrained water and hydrate by absorption and adsorption phenomena. The water status is a key element for the subsequent softening by cooking as shown by Burr, Kon and Morris (1968). Increasing the level of calcium in the soak water decreases the imbibition rate but increases the firmness of canned beans, by formation of calcium pectin complexes (Uebersax and Bedford, 1980). The high level of calcium in soak water decreases the drained weight of kidney beans, but improves the appearance by decreasing the percentage of split seeds. The temperature affects the imbibition rate, final weight after soaking, cooking time and loss of solids and nutrients (Kon, 1979). Many studies agree that the seed cookability is determined by the rate of cell separation that is attributed to improved solubilization of pectates and pectin β- elimination degradation (Liu, 1995).

However, Del Valle *et al* (1992) observed that the softening correlated with increased protein denaturation of isolated protein bodies and not with pectin solubilization during cooking. They suggested that softening was not totally attributable to thermal degradation of macromolecular components or middle lamella dissolution. A mechanism based on protein destabilization also contributes to bean softening. Liu, Hung and Phillips (1993) observed that, unlike the control, soaked aged cowpea seeds exhibited a coarse protein matrix with tightly embedded starch granules and resistance to fracture. Treated seeds also showed tight embedment of starch granules. This indicates that storage proteins had coagulated or aggregated during ageing or treatments, as evidenced also by their very low extractability.

Léon *et al* (1992) found that increasing the ratio of monovalent ions (Na^+ and K^+) to divalent ions (Ca^{2+} and Mg^{2+}) in the soak solution decreased significantly the cooking time of both fresh and HTC beans. Kyriakidis *et al* (1997) measured the hardness of beans boiled for 60 minutes in water with graded content of calcium and magnesium ions. Lower divalent cation content resulted in lower hardness and inversely. They tested the results by absorption experiments with divalent ions and concluded that the divalent cation content of boiling water and the phytic acid content of the beans were the crucial factors in the HTC.

Garcia-Vela *et al* (1991) observed that soaking in anion solutions prior to cooking induced softness as follows: $CO_3^{2-} > EDTA^{2-} = NO_3^- > SO_4^{2-} = Cl^-$. Softening was also promoted by increasing the ionic strength of the soaking solution. They demonstrated that storage conditions contribute more to the degree of cell separation after cooking than salt treatment. These results bring a mechanism based on chelation and ion exchange into question. The authors suggest that aqueous salts influence storage proteins by solubilization and rendering them thermally labile.

Liu *et al* (1993), studying the effect of freeze-thaw (FT) cycles and calcium chloride soaking of cowpeas on the HTC state, observed a relationship in control samples between electrolyte leakage after FT and HTC state induced by subsequent calcium (Ca^{2+}) ion soaking treatments. This relationship was not observed in aged seeds because of initial high electrolyte leakage during hydration. They suggested that one part of HTC inducement during cation soaking occurs through loss of cell membrane integrity that allows cations to bind intracellular components.

A loss of total solids during soaking has been reported to be higher in HTC beans than in control beans by numerous researchers (Jones and Boulter, 1983; Liu *et al* 1993; Liu *et al* 1992c; Richardson and Stanley, 1991; Hincks *et al* 1987). With aging the increased metabolic activity leads to phytin hydrolysis and membrane deterioration that result in leakage of calcium (Ca^{2+}) and magnesium (Mg^{2+}), pectin desolubilization and textural deterioration (Jones and Boulter 1983). Liu *et al* (1992b) observed that during incubation control seeds showed a dramatic increase in solid losses at greater than 45°C while aged seeds exhibited an initial high level of loss at 25°C. They suggested that a loss of membrane integrity occurred as a result of thermal stress or aging. Liu *et al* (1993c), observed that the pectin loss after cowpeas soaking increased as aging progresses, but decreased significantly after cooking. With heating temperature, pectin loss was lower below 60°C, higher at 85°C, and maximum at 100°C. These changes in pectin loss from cowpeas suggested that pectin beta-elimination reaction occurs during heating of cowpeas.

Paredes-Lopez *et al* (1991) claim having succeeded to eliminate the adverse effects of HTC condition by soaking seeds in salt solutions.

Water Absorption. Garcia-Vela and Stanley (1989) found that the water holding capacity (WHC) was significantly reduced in the pH range 2.5 to 5.1 in control but the effect was not as pronounced with HTC samples which had a lower WHC at each pH. WHC values in control beans tended to increase with higher ionic strength, although this effect was not as apparent for HTC beans. Solutions prepared with NaCl produced lower WHC values than $CaCl_2$ solutions in control, but not the HTC beans. WHC values in

control beans tended to increase with the ionic strength, this effect being not apparent for HTC beans.

In their kinetics study, Del Valle, Stanley and Bourne (1992) found that a modified first-order model, composed of an initial linear phase followed by a diffusion controlled phase, predicted the water absorption and swelling in several varieties of fresh and stored beans. Dehulling resulted in increased rate of water absorption, but equilibrium values for both water absorption and swelling were reduced as a result of elimination of free water held between the seed coat and cotyledons. Swelling of dehulled seeds were reduced initially because the seed coat swells faster than the cotyledon in the initial phase. The additions of carbonate salts to the soaking solution reduced water absorption and swelling. The water absorption was negatively correlated with cooked bean hardness.

It appears that, unlike water absorption during soaking, water absorption during cooking is related to hardness of cooked seeds. HTC seeds generally exhibit restricted water absorption during cooking compared with soft control seeds. In a study with cowpeas Liu *et al* (1993) have shown that only at a temperature above 60°C, the temperature at which starch granules start to swell, does the amount of water affect the texture.

Enzymatic activity. Soaking, as well as germination and fermentation, reduces the phytate content by activation of intrinsic phytase (Plaami, 1997) and it has been generally hypothesized that this hydrolysis liberates divalent cations, which contribute to hardening.

Loss of viability and vigor of seeds while aging have been shown to be related to the activity of proteases, phytase, and phosphatases since an increase of permeability, amino acids, and inorganic phosphate was observed in the aged material (Ching and Schoolcraft, 1968).

Soaking beans prior to cooking is also important because it enables further enzymes synthesis and greater enzyme accessibility to substrates. Vindiola *et al* (1986) observed a sharp decrease of cookability as pH of soaking water was decreased from 5.5 to 4.2. They attributed the decrease to the possible dissociation of a ternary complex among phytate, magnesium and protein. As this pH range corresponds to the normal optimum activity of plant phytase this bean hardening may be due to an increased accessibility of phytate to phytase. Using the Scanning Electron Microscopy (SEM) Sefa-Dedeh *et al* (1979) demonstrated a loss of some protein bodies after soaking HTC cowpeas in water and suggested that it may be due to the action of proteases stimulated during storage.

Effect of Heat Treatment. It is expected that thermal treatment of beans prior to storage inactivates phytase and methylesterase enzymes and leads to a texture improvement. However phytic acid degrades during boiling and steaming, but the degradation is greater in processes in which phytase is previously activated (Plaami, 1997). The rate of phytase destruction is low when it is associated with proteins and /or cations.

Molina *et al* (1976) treated whole black beans at 121°C and by steam at 98°C prior to controlled seed storage. After nine months storage, no significant difference was

detected between the hardness of cooked beans subjected to heat treatment and that of control beans. Aguilera *et al* (1986) and Dhahir (1988) reported the same result on roasted beans after 212 days and five months storage, respectively. Liu, Hung and Phillips (1993) showed that, unlike 60°C, heating at 85°C dramatically decreased protein extractability and led starch granules to swell partially in control seeds and with little swelling observed in HTC seeds. When cooked, HTC seeds showed lack of cell separation and restricted starch swelling, all of which were in sharp contrast with the control. Results support the role of cell middle lamella, thus, implying the involvement of multiple mechanisms in bean hardening.

The enzyme peroxidase has been suggested to have a role in the development of the HTC condition via lignification by catalyzing the polymerization of the phenolic subunits. However, Rivera *et al* (1989) showed that its inactivation by heating does not necessarily stop bean hardening. Higher temperatures above 105°C are required to control both the peroxidase activity and the development of the HTC condition.

Cooking is aimed at softening beans to achieve an acceptable palatability and at destroying anti-nutritional factors. Cooking is accompanied by a decline of essential amino amino acids and a decomposition and leaching out of tannins and phytic acid (Ziena *et al*, 1991).

The rate of cell separation and the pectin solubility determine the bean cookability (Jones and Boulter, 1983). Using light and scanning electron microscopy, Carabez-Trejo *et al* (1989) reported a remarkable difference in some of the changes generated by native and chemically induced hardening procedures. Uncooked samples hardened by storage showed smooth starch granules without any apparent deformation, whereas by the

chemical procedure, these granules were clearly deformed. Cotyledon cell separation was produced when fresh samples were cooked. Cells were smaller and separation was not as evident in HTC samples from the two procedures, compared to previous fresh samples. The chemical hardening technique appeared to make the cotyledon cell structure more rigid. Hincks *et al* (1987) showed that water absorption, solids loss and electrolyte leakage during the soaking and cooking processes increased in hard beans presumably due to the membrane damage. During cooking, hard beans lost fewer solids and minerals and did not continue to hydrate to the same degree as the controls. They suggested that it was a result of restricted cell separation. Their microscopic study indicated a reduced starch gelatinization in hard beans due to a reduced water absorption.

MATERIALS AND METHODS

Bean Source and Handling

Ten defined cultivars (Seafarer "aged", Seafarer "fresh", Mackinac, N9774, Ensign, Mayflower, Navigator, Vista-MI, Schooner, Vista-MN, Norstar) and seven commercial samples (NB-1, NB-2, NB-3, NB-4, NB-5, Bayside Best, Arkansas) of Navy beans with various harvest times (1998 – 2002) were used in this study. The source and description of the physical attributes are given in Table 1. All beans were delivered in paper or propylene bags with the exception of Arkansas, which was shipped in a closed plastic bucket. All beans were kept refrigerated (3-5°C) until use.

Experiments conducted

The experiments conducted and the samples used are shown in Table 2. The research focused on three studies:

- Study 1: Cooking Time and Cooking Characteristics of Selected Navy Beans (*Phaseolus vulgaris L*);
    - 1.1.Measurement of Cooking Time of selected Navy bean cultivars (*Phaseolus vulgaris L.*)
    - 1.2.Effect of Calcium and Phosphate ions on the Cooking time of Selected Navy beans.
- 1.3.Effect of Calcium and Phosphate ions on the Firmness of Cooked Navy beans (Mackinac).

- Study 2: Effect of Selected Bean Processing Techniques on the Texture of Canned Navy Beans (*Phaseolus vulgaris L*).
    - 2.1.Processing characteristics of selected beans (*Phaseolus vulgaris L)* cultivars and commercial samples.
    - 2.2.Effect of Phosphate (PO_4^{3-}) in brine on the firmness of canned Navy beans (Mackinac).
    - 2.3.Effect of selected soaking methods on the texture of canned Navy beans (*Phaseolus vulgaris*) .
    - 2.4. Interaction of Phosphate and Calcium ions in the soak water and their effect on the texture of canned Navy beans.
- Study 3: Measurement of Pectin Methylesterase Activity in Selected Commercial Samples and Cultivars of Navy Bean (*Phaseolus vulgaris L*).

  3.1.Effect of soak pre-treatment (time/temperature) on Pectin methylesterase activity of Navy beans (*Phaseolus vulgaris L*).

  3.2.Effect of soak temperature on the hydration, the Pectin Methylesterase activity and the texture of cooked Navy beans (*Phaseolus vulgaris L*) .

  Effect of cooking time on the firmness of cooked Navy beans (*Phaseolus vulgaris L*).

The cooking and soaking conditions used in this research are outlined in Table 3.

Table 1.Inventory of Navy beans samples used in these studies

Sample	Source	Percent						Hunter Lab coordinates			50 seeds weight (n=3)	
		H_2O	Skin	Split	Off Color	Damaged	Foreign material	L	a_L	b_L	(g)	Std
Seafarer, "aged"	Michigan Foundation Seed (MFS).1998 crop.	14.5	-	-	-	-	-	64.9	0.3	12.0	10.9	0.1
Seafarer, "fresh"	Michigan State University Breeding Program.2002 crop	13.7	-	-	-	-	-	64.0	0.3	7.6	10.3	0.3
Mackinac	Michigan Foundation Seed (MFS).1999 crop.	14.6	-	-	-	-	-	61.3	0.0	13.7	10.2	0.2
N9774	Michigan Foundation Seed (MFS).1999 crop.	12.3	-	-	-	-	-	65.8	0.4	9.8	12.3	0.2
Ensign	Casselton, ND 2002 Crop	15.9	-	-	-	-	-	58.0	1.0	10.7	10.4	0.2
Mayflower	Northwood, ND, 2002 Crop	15.6	-	-	-	-	-	61.7	0.8	10.9	10.6	0.2
Navigator	Casselton, ND, 2002 Crop	14.1	-	-	-	-	-	58.3	1.1	11.2	10.9	0.4
Vista-MI	Fairgrove, MI 2002 Crop	15.3	-	-	-	-	-	61.4	0.9	11.0	10.4	0.2
Schooner	Fairgrove, MI 2002 Crop	15.6	-	-	-	-	-	60.0	0.9	9.8	9.3	0.3

Table 1.Inventory of Navy beans samples used in these studies (continued)

Sample	Source	Percent						Hunter Lab coordinates			50 seeds weight (n=3)	
		H_2O	Skin	Split	Off Color	Damaged	Foreign material	L	a_L	b_L	(g)	Std
Vista-MN	Olivia, MN 2002 Crop	16.4	-	-	-	-	-	58.4	1.0	10.6	11.8	0.4
Norstar	Galesburg, ND 2002 Crop	16.5	-	-	-	-	-	59.2	1.3	10.5	10.8	0.3
NB-1	Commercial sample, DM, Reese Elevator, Michigan, 2000 Crop.	16.0	6.0	0.1	2.5	-	0.2	61.4	0.4	11.6	9.9	0.1
NB- 2	Commercial sample, ADM, Reese Elevator, Michigan, 2000 Crop.	15.4	6.0	0.1	2.0	-	0.1	61.6	0.1	11.4	9.6	0.1

Table 1.Inventory of Navy beans samples used in these studies (continued)

Sample	Description	Percent						Hunter Lab coordinates			50 seeds weight (n=3)	
		H_2O	Skin	Split	Off Color	Damaged	Foreign material	L	a_L	b_L	(g)	Std
NB- 3	Commercial sample, ADM, Reese Elevator, Michigan, 2001 Crop.	18.2	5.0	4.0	38.0	0	2.0	59.1	0.9	13.2	9.1	0.5
NB- 4	Commercial sample, ADM, Reese Elevator, Michigan, 2001 Crop.	16.1	5.0	0.7	22.0	0	0.5	62.0	0.1	11.9	9.8	0.3
NB- 5	Commercial sample, ADM, Reese Elevator, Michigan, 2001 Crop.	18.0	9.0	0.2	8.1	0.8	0.2	58.9	0.6	12.4	8.6	0.9

Table 1.Inventory of Navy beans samples used in these studies (continued)

Sample	Description	Percent						Hunter Lab coordinates			50 seeds weight (n=3)	
		H_2O	Skin	Split	Off Color	Damaged	Foreign material	L	a_L	b_L	(g)	Std
Arkansas	Commercial sample, received from University of Arkansas Laboratory, summer 2002, reported to be HTC	15.2	-	-	-	-	-	60.2	0.5	14.7	9.2	0.5
Bayside Best	Commercial retail, LLC SABEWAING, Michigan	16.6	-	-	-	-	-	61.8	0.2	12.6	9.3	0.1

Table 2.Summary matrix of experimental studies and experiments illustrating bean sample materials and general processing conditions.

		Study 1				Study 2				Study 3							
		Cooking Time				Canning Quality				Pectin methylesterase (PME) activity							
		Cook time			Open Kettle Ca^{2+}/PO_4^{3-}	Processing Characteristics	PO_4^{3-} In brine	Four Soak methods	Interaction Ca^{2+}/PO_4^{3-}	No Trt	Cold soak			MSU 30/30	Warm Soak 3h/46 °C	Hot Soak 3h/63 °C	T° Effect
Sample Material		Curve	CT_{13} Ca^{2+}	CT_{13} PO_4^{3-}	(60min/ 100°C						1h/ 21°C	3h/ 21°C	8h/ 21°C				
Cultivars	Seafarer "aged"	X	X	X		X			X	X	X	X	X	X	X	X	X
	Seafarer "fresh"	X	X	X		X				X	X	X	X	X	X	X	X
	Mackinac	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	N97774	X	X	X		X				X	X	X	X	X	X	X	
	Ensign		X	X		X											
	Mayflower		X	X		X											
	Navigator		X	X		X											
	Vista-MI		X	X		X											
	Schooner		X	X		X											
	Vista-MN		X	X		X											
	Norstar		X	X		X											
Commercial samples	NB-1		X	X		X				X	X	X	X	X	X	X	
	NB-2		X	X		X				X	X	X	X	X	X	X	
	NB-3		X	X		X				X	X	X	X	X	X	X	
	NB-4		X	X		X				X	X	X	X	X	X	X	
	NB-5		X	X		X				X	X	X	X	X	X	X	
	Arkansas	X	X	X		X			X	X	X	X	X	X	X	X	X
	Bayside Best		X	X		X				X	X	X	X	X	X	X	
	Experiment #	**1.1**	**1.2**		**1.3**	**2.1**	**2.2**	**2.3**	**2.4**	**3.1**							**3.2**

CT_{13}: Time required for cooking 13 beans on a total of 25 in a bean cooker. No Trt: no treatment

T° effect: temperature effect. X: experimental material used in study

Table 3. Cooking and soaking conditions (temperature/time) used in selected experiments

Cooking/Soak Method Terminology	**Conditions (Temperature/Time)**
Experiments 1.1-1.3.	
• Open kettle	100°C (boiling water)
Experiments 2.1-2.4	
• Cold soak	4 hours at 25.6°C+ 5 min at 93.3°C
• MSU soak	30 min at 25.6°C + 30 min at 87.8°C
• ComSoak 1 (commercial soak method)	3 hours at 54.5°C+ 6 min at 79.4°C
• ComSoak 2 (commercial soak method)	3 hours at 54.4°C+2.5min at 71.1°C + 3.5 min at 78.9°C
Experiments 3.1,3.2	
• Cold soak	21.1°C/ 1-8hours
• Warm soak	46.1°C/3 hours
• Hot soak	62.8°C/3 hours
• Variable temperature study	21.1-71.1°C/3 hours

Study 1: Cooking Time and Canning Characteristics of Selected Navy Beans (*Phaseolus vulgaris L*)

Experiment 1.1: Measurement of cooking time of selected Navy bean cultivars (*Phaseolus vulgaris L.*) in distilled water

Five bean samples used for this experiment included: Seafarer "aged", Seafarer "fresh", Mackinac, N9774 and Arkansas. The choice was dictated by the need of investigating the effect different levels of aging and by the samples availability.

The cooking time (CT_{13}) was determined using a modified Mattson bean cooker (Burr *et al* 1968; Jackson and Varriano-Marston 1981; Chhinnan 1985; Hentges *et al* 1991; Downie *et al* 1997) shown in Figure 9. Beans were positioned into each of the 25 holes of the cooker so that the piercing tip of the 90 grams rod was in contact with the surface of the bean.

During an experimental run the cooker loaded with 25 beans was plunged in a 5L pot containing 4L of boiling distilled water (100°C). The quantity of water was maintained by addition of boiling water. The cook cycle was maintained at constant temperature and timed observations made

Beans were judged "cooked" when the tip of the rod passed through the bean. The cooking time (minutes) of each bean at penetration was recorded. The data used were the mean of two replicated cooks. The number of cooked beans was plotted versus the cooking time. CT_{13} was the time when 13 beans of a total of 25 were cooked and it was used as a comparison parameter among beans.

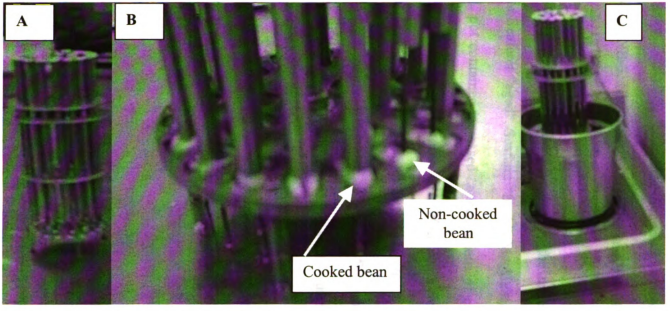


Figure 9. Bean cooker. A: Bean cooker loaded with beans. B: Details showing cooked and non-cooked beans. C: Bean cooker plunged in boiling water contained in a pot.

Experiment 1.2: Effect of Calcium and Phosphate ions on the Cooking time of Selected Navy beans

Bean cultivars (Seafarer "aged", Seafarer "fresh", Mackinac, N9774, Ensign, Mayflower, Navigator, Vista-MI, Vista-MN, Schooner and Norstar) and commercial samples (NB-1, NB-2, NB-3, NB-4, NB-5, Arkansas and Bayside Best) were used in this experiment.

Beans (25 individual seeds) were positioned in the same cooker described in the experiment 1.1. For Seafarer "aged", Seafarer "fresh", Mackinac, and N9774 the cooking water (4L) in a 5L pot contained increased concentrations of calcium ($CaCl_2$ from EM Science, Gibbstown, NJ) or phosphate ions ($Na_3\ PO_4.12H_2O$ from Sigma Chemical Co, P.O.Box 14508, St Louis, MO 63178 USA) as follows: (0, 5, 25, 50, 200 ppm). For Ensign, Mayflower, Navigator, Vista-MI, Vista-MN, Schooner and Norstar which belonged to a different lot, measurements were done only in distilled water, 100 ppm of phosphate and 50 ppm of calcium because of time and material constraints. The cooker was heated and the volume of boiling water maintained by addition of hot distilled water. The cooking time of the thirteenth bean (CT_{13}), calculated as the mean of two replicates, was plotted versus the concentration of calcium or phosphate ions.

Experiment 1.3: Effect of Calcium and Phosphate ions on the Firmness of Cooked Navy beans (Mackinac)

Navy beans Mackinac were used in this study. Approximately 100g of dry beans were cooked for 60 minutes in a 5L kettle containing 4 liters of boiling water with 0, 5,

25, 50,100, or 200 ppm of calcium or phosphate ions. Cooked beans were drained for two minutes on an eight inches diameter U.S. Standard No.8 Screen (0.094 inch openings).

After cooling at room temperature the texture was analyzed in triplicate (25 and 12.5g samples) on the Texture Analyser TA-XT2^{i} ® of Stable Micro Systems, England. The probe was the ten bladed Kramer Shear cell HDP/KS 10. The data were collected and analyzed on a computer using the software Texture Expert for Windows ™ Operating System of Texture Technologies Corp., New York.

The instrumental settings for data collection were as follows:

Test mode and option

Measure Force in

Compression

Return to Start

Parameters

Pre-test speed: 2.0 mm/s

Test speed: 2.0mm/s

Post-test speed: 10.0mm/s

Distance: 135.0 mm

Trigger

Type: Button

Stop Plot at: Target

Auto tare: (checked)

Break

Detect: Off

Units

Force: Newton

Distance: Millimetres

The Texture Analyzer displayed a graph (Figure 10) where the shear force was plotted versus the distance (mm) of the probe travel. The total shear work (obtained by integration of the curve between the beginning and the end of shearing) and the maximum shear force were recorded.

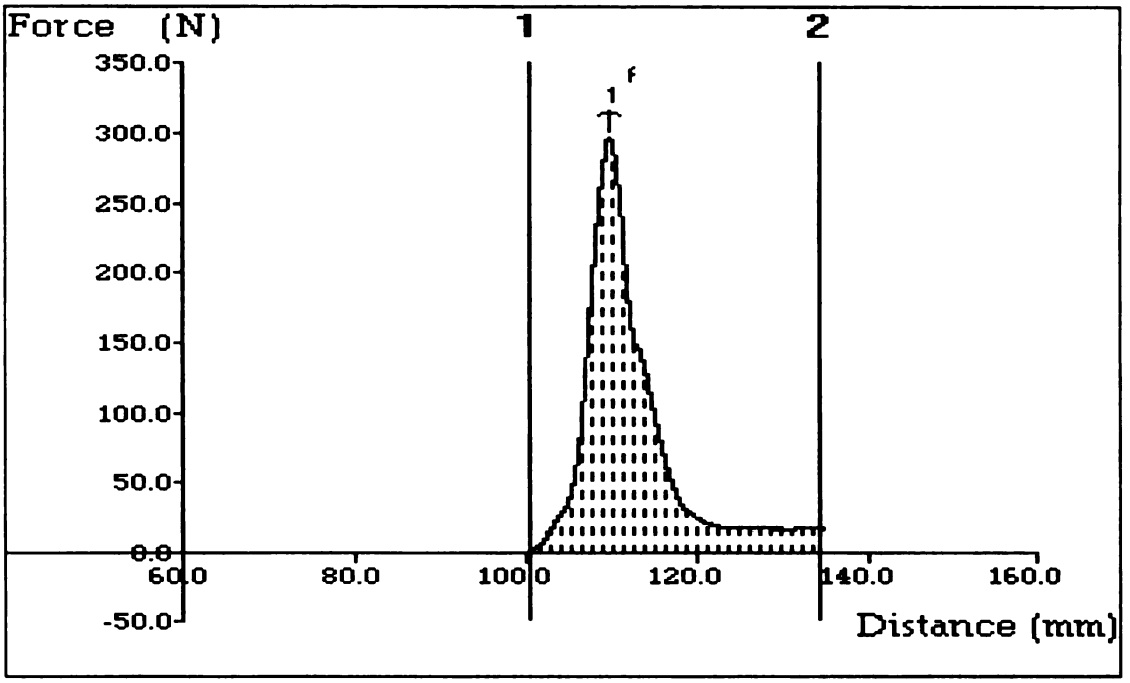


Figure 10. Model of graph displayed by the TA-XT2^{i} ®. The anchors 1 and 2 represent the beginning and the end of shearing by Kramer shear probe. The top f is the maximum shear force and the shaded area is the total shear work.

Study 2: Effect of Selected Bean Processing Techniques on the Texture of Canned Navy beans (*Phaseolus vulgaris L.*)

Experiment 2.1: Processing Characteristics of Selected Beans (*Phaseolus vulgaris L)* Cultivars and Commercial Samples

Bean cultivars (Seafarer "aged", Seafarer "fresh", Mackinac, N9774, Ensign, Mayflower, Navigator, Vista-MI, Schooner, Vista-MN, Norstar) and commercial samples (NB-1, NB-2, NB-3, NB-4, NB-5, Arkansas and Bayside Best) were used in this experiment.

The initial moisture content of beans was measured in duplicate using a Moisture Analyzer IR-200 of Denver Instrument Company. For moisture measurements dry beans were pre-ground for approximately two minutes using a household coffee mill KRUPS Type 203.

The weight of beans equivalent to 100 g of total solids was calculated using the initial moisture content (Equation 1).

$$\text{Weight of beans equivalent to 100g total solids} = \frac{10^4}{(100 - \text{Initial moisture \%})}$$

Equation 1:Calculation of bean weight equivalent to 100 g total solids

This quantity of beans was weighed in two replicates, put in nylon mesh bags, closed and soaked following the MSU soak method as reported in Table 3 (30 min at 21°C and 30 min blanching at 87.8°C). The soak water contained 100 ppm of calcium ions.

Soaked beans were cooled in tap water, drained, weighed (soak weight), put in 303 cans. The percent water uptake was calculated using the equation 2.

$$\% \text{ Water uptake} = \frac{(\text{Soak weight} - \text{Initial weight}) \times 100}{\text{Initial weight}}$$

Equation 2: calculation of percent water uptake

A standard sugar/salt brine was used for canning. This brine contained 100 ppm Ca^{2+}, 1.249% NaCl, 1.562% sugar and was prepared by dissolving 0.28 g $CaCl_2$ anhydrous, 12.5g NaCl 1.25% and 15.63 g of sugar 1.56% in 1000 g of distilled water (Uebersax and Hosfield 1996). Boiling brine was added and cans were exhausted and sealed. Sealed cans were retorted for 45 min at 115.6°C (240°F) in a still retort, cooled 15 min with cold water and stored at room temperature for at least two weeks prior to quality evaluation.

The quality evaluation consisted in the measurement of wash drained weight and the texture. Cans were opened and the content poured and distributed evenly on an 8 inch diameter, U.S. Standard N° 8 Screen (0.094 inch openings). The screen and contents were immersed in 21°C water with agitation in a swirling motion to wash the sauce off the beans. After two final immersions the product was drained for two minutes at an angle, transferred to a tared bottom plate and weighed on a top loading Mettler balance. The wash drained weight (grams) was recorded.

The texture was measured on two replicates for each can (i.e. four replicates for each bean) using the method described in experiment 1.3 on 50g-samples. The total shear work (Newtons.millimeters) and the maximum shear force (Newtons) were recorded.

Experiment 2.2: Effect of Phosphate (PO_4^{3-}) in Brine on the Firmness of Canned Navy Beans (Mackinac)

The cultivar Mackinac was used in this experiment. The quantity of beans equivalent to 100 g of total solids were weighed as lots, each lot was put in individual nylon mesh bags, soaked and blanched following the MSU soak method (Table 3) in 100 ppm Ca^{2+}. Beans were assigned cans intended to contain respectively 0, 5, 25, 50, 100 and 200 ppm PO_4^{3-} in the brine (two replicates for each concentration). These concentrations were obtained by dissolving the required quantity of $Na_3PO_4.12H_2O$ in the standard brine. Cans were filled, brined, exhausted, processed for 45 minutes at 115.6°C (240°F) in a still retort, cooled 15 minutes with cold water and stored at room temperature for at least two weeks before quality evaluation.

The wash drained weight and the texture were measured in two replicates for each can (four replicates for each phosphate concentration) on 50 g samples, following the method previously described in experiment 2.1.

Experiment 2.3: Effect of Selected Soaking Methods on the Texture of Canned Navy Beans (*Phaseolus vulgaris*)

The cultivar Mackinac was tested in this experiment using the following four soaking methods: 1) Cold soak (25.6° C/ 4 hours), 2) MSU soak (25.6 ° C / 30 min + 87.8 ° C /30 min), and 3) two commercial soaks: a) ComSoak1 (54.4° C / 3 hours + 79.4 °C /6 min) and b) ComSoak2 (54.4 °C / 3 hours + 71.1 °C /2.5 min + 78.9 °C /3.5 min). The practical conditions of these methods are given in Table 3.

The quantity of beans equivalent to 100g total solids was weighed in two replicates for each soak method, put into nylon mesh bags, soaked and blanched in 100 ppm of Ca^{2+} using the defined protocol of the four soaking methods. After soaking, beans were cooled, drained, filled in cans, covered with the standard brine and retorted at 115.6°C (240°F) for 45 min, cooled for 15 min in cold water and stored at least two weeks at room temperature prior to quality evaluation.

Experiment 2.4: Interaction of Phosphate and Calcium Ions in the Soak Water and their Effect on the Texture of Canned Navy Beans

Two cultivars: Seafarer "aged" and Mackinac, and one commercial bean: Arkansas, chosen for their high firmness proven by the previous experiments, were used.

Four soak methods were used: Cold soak, MSU soak, ComSoak1 and ComSok2. Their practical conditions (time and temperature) are given in Table 3.

Four ratios of calcium and phosphate ions were used:

$[Ca^{2+}]$ (ppm)	$[PO_4^{3-}]$ (ppm)
0	0
100	0
0	100
0	200
100	100
200	200

The quantity containing 100g total solids was weighed in two replicates for each combination bean sample x soak method x calcium / phosphate ratio.

After soaking beans were cooled, drained, weighed, put into 303 cans, filled with the standard brine, exhausted, sealed, retorted and stored at least two weeks before quality evaluation.

The wash drained weight was measured following the method described in experiment 2.1. The texture was analyzed on a 50 g sample in two measures for each replicated can (four measures for each treatment combination), using the method described in experiment 1.3.

Study 3: Pectin Methylesterase (PME) Activity

Experiment 3.1: Effect of Soak Pre-treatment (time/temperature) on Pectin Methylesterase Activity of Navy Beans (*Phaseolus vulgaris L*)

Sample preparation

The pectin methylesterase (PME) activity was assayed on dry beans and soaked beans. Cultivars (Seafarer "aged", Seafarer "fresh", Mackinac, N9774) and commercial beans (NB-1, NB-2, NB-3, NB-4, NB-5, Bayside Best, Arkansas) were used in this study. Dry beans were fine ground (± 2 min) with a household coffee mill KRUPS Type 203. The PME was then assayed on a 5.0 g sample in three replicates.

Five differential soaking conditions were used:

- "Cold soak: 21.1°C/ 1h": one hour of soaking in water with Ca^{2+} .50 ppm at 21.1°C (70°F).
- "Cold soak 21.1°C / 3h": 3 hours of soaking in water with Ca^{2+}. 50 ppm at 21.1°C (70°F).
- "Cold soak 21.1°C / 8h": 8 hours of soaking in water with Ca^{2+}. 50 ppm at 21.1°C (70°F).
- "MSU soak method": 21.1°C / 30 minutes + 87.8°C / 30 minutes in water with Ca^{2+}. 50 ppm.
- "Warm soak 46.1°C /3h": 3 hours at 46.1°C (115°F) in water with Ca^{2+} .50 ppm.
- "Hot soak 62.8°C /3h": 3 hours at 62.8°C (145°F) in water with Ca^{2+} 50 ppm.

After soaking beans were quickly cooled in tap water, dried on a towel paper and frozen at –18°C until use.

For PME assay soaked beans were thawed and ground (±2 minutes) with Cuisinart ® Miniprep® Plus grinder. The moisture content was determined in duplicate using the IR-200 Moisture Analyzer of Denver Instrument Company, on a 3.5-4 g sample, according to the recommendations of the manufacturer. The PME activity was assayed in three replicates on a 5.0 g sample of ground soaked beans.

Pectin Methylesterase Activity

Pectin slurry preparation

A volume of 20.0 ml NaCl 2 M was poured into a blender (model Waring). The blender was filled with de-ionized water to two liters. 20.0 g citrus pectin (from Sigma Chemical Co, P.O.Box 14508 St Louis, MO, 6317 USA) were added and the mixture blended on the lowest setting for 2 min. The mixture was then heated in a water bath at 50°C until the solution was completely dissolved. It was refrigerated while not in use.

Titration of pectic acid

PME assays were done in three replicates on a 5.0 g sample. 50 ml of pectin (30-33°C) was added to a beaker containing a stir bar. Then 5g of sample was added and the pH adjusted to 7.0 with NaOH 0.1N and NaOH 0.02N while the stirrer was turned on. The pH was controlled using a digital pH meter (Corning No.610A, Corning Co., Medfield, MA). When the pH dropped under 7.0 one to 3 ml of 0.02N NaOH were added from the manual titrator and the timer was started. The volume of NaOH used and the time for the pH to drop back under 7 were recorded. To maintain the same conditions of enzyme activity the temperature was not allowed to exceed 3 degrees of deflection from 30°C.

The pectin methylesterase units (PEU) for each sample/treatment, were calculated using the equation 3:

$$PEU = \frac{10^4 \times 0.02N \times \text{Volume NaOH (ml)}}{5\text{ g} \times \frac{(100 - \%\text{ Moisture})}{100} \times \text{Time (min)}} \quad [\text{meq/g x min}]$$

Equation 3: calculation of pectin methylesterase unit.

Experiment 3.2: Effect of Soak Temperature on the Hydration, the Pectin Methylesterase Activity and the Texture of Cooked Navy Beans (*Phaseolus vulgaris L*). Effect of Cooking Time on the Firmness of Cooked Navy Beans (*Phaseolus vulgaris L*)

Two beans, Seafarer "aged" and Seafarer "fresh" were used in this study. 100 ±0.1g samples in labeled nylon mesh bags were soaked for three hours in distilled water in kettles maintained respectively at 21.1°C (70 °F), 46.1 °C (115 °F), 54.4 °C (130 °F), 62.8 °C (145 °F) and 71.1 °C (160°F). Samples were drained, put in plastic zip bags and rapidly cooled on ice to room temperature, dried on towel paper and weighed. The percent water uptake was calculated using the equation 2.

The soaked beans were tested for PME activity in three replicates on a 5g sample of ground beans using the method previously described in experiment 3.1. The pectin methylesterase units were calculated using the equation 3.

Soaked beans were then cooked in distilled water or 50 ppm of Ca^{2+} for 60, 90 and 120 min and the texture was measured in three replicates using the method described

in experiment 1.3 on a 25 g sample. The total shear work and the maximum shear force were recorded.

Statistical Analysis

To ascertain the difference among treatments, data were subjected to analyses of variance (ANOVA) using the Analysis ToolPak of Microsoft Excel 2000. The level of significant difference was expressed as the probability levels of $p<0.05$. An additional t-test was run by calculating the least significant difference (LSD) as explain by Snedecor and Cochran (1967). The correlation between data series was tested by calculating the correlation coefficient r using the Analysis ToolPak. Data were presented with a standard error (SD) calculated with Microsoft Excel 2000 using the formula:

$$SD = \sqrt{\frac{\sum_{s=1}^{m}\sum_{i=1}^{n}(y_{is} - M)^2}{(n_y - 1)}}$$

Where:

s = series number

i = point number in series s

m = number of series for point y in chart

n = number of points in each series

y_{is} = data value of series s and the i^{th} point

n_y = total number of data values in all series

M = arithmetic mean

RESULTS AND DISCUSSION

Study 1: Cooking Time and Canning Characteristics of Selected Navy Beans (*Phaseolus vulgaris L*)

Measurement of cooking times of selected Navy bean cultivars (*Phaseolus vulgaris L.*)

The cooking times of the five beans studied are shown in Table 4. The number of cooked beans is plotted versus the time (Figure 11). The curves possess the characteristic sigmoidal "S-shape" previously found by several researchers (Jackson and Varriano-Marston 1981; Chhinnan 1985; Hentges *et al* 1991; Downie *et al* 1997).

The range of difference among cooking times (CT_R) between the first and the 25th bean gives an indication of the homogeneity of the bean sample. The smaller the CT_R, the more homogeneous the bean sample. CT_R is 44, 50, 60, 63, and 70 min, respectively, for Seafarer "fresh", Mackinac, Seafarer "aged", N9774 and Arkansas. Thus, the homogeneity in these samples decreases in the same order.

The median cooking time (CT_{13} in this study) is 58, 65, 76, 81 and 96 min respectively for N9774, Seafarer "fresh", Seafarer "aged", Mackinac and Arkansas and the hardness increases in the same order.

Establishing the S-shape curve can be time consuming and the determination of CT_{13} is frequently deemed sufficient to compare the hardness among bean samples. CT_{13} determined for all the bean samples (Table 5) shows three samples as "hard beans" with a value higher than 80 minutes: including Arkansas (104 min), Seafarer "aged" (81.5 min)

and Mackinac (80.5 min). The "soft beans" with CT_{13} less than 70 minutes include: N9774 (58 min), NB-1 (57 min), NB-2 (57.5 min), NB-3 (58.5 min), NB-4 (59.5 min) and NB-5 (51.5 min), Ensign (62 min), Mayflower (59.5 min), Navigator (49.5 min) Vista-MI (49 min), Schooner (54.5 min), Vista-MN (40.5 min), Norstar (50.5 min) and Bayside Best (61 min).

Table 4.Cooking times of five Navy bean cultivars (*Phaseolus vulgaris L.*) in distilled water

	Cooking Time (min)									
Bean#	Seafarer "aged"	SD	Seafarer "fresh"	SD	Mackinac	SD	N9774	SD	Arkansas	SD
1	45	14	37	6	52	1	35	1	59	26
2	53	4	43	12	63	2	38	1	63	28
3	57	2	51	2	67	4	44	7	76	18
4	59	1	54	1	68	4	49	3	80	13
5	61	1	54	0	71	6	51	2	82	16
6	61	1	57	1	72	6	51	2	83	16
7	69	2	60	4	74	6	53	0	86	12
8	71	5	61	4	74	7	54	1	88	10
9	73	4	61	3	76	8	55	1	92	5
10	74	5	61	3	79	6	56	1	93	4
11	74	5	63	4	79	6	56	0	94	4
12	75	6	64	2	80	5	56	0	94	4
13	76	5	65	4	81	5	58	2	96	4
14	76	4	67	6	81	5	59	2	97	4
15	79	5	68	5	82	5	61	4	101	0
16	80	7	69	4	83	6	62	4	102	0
17	82	7	7	5	85	4	65	1	106	4
18	85	6	71	5	87	3	65	1	109	2
19	85	6	71	5	89	1	67	3	109	3
20	89	10	71	4	91	4	70	7	112	5
21	94	10	73	5	92	3	71	8	115	7
22	96	8	73	4	94	4	73	6	123	1
23	97	6	75	6	96	4	79	12	127	0
24	101	6	77	6	100	1	86	14	128	1
25	105	6	81	6	102	4	98	4	129	1

n=2

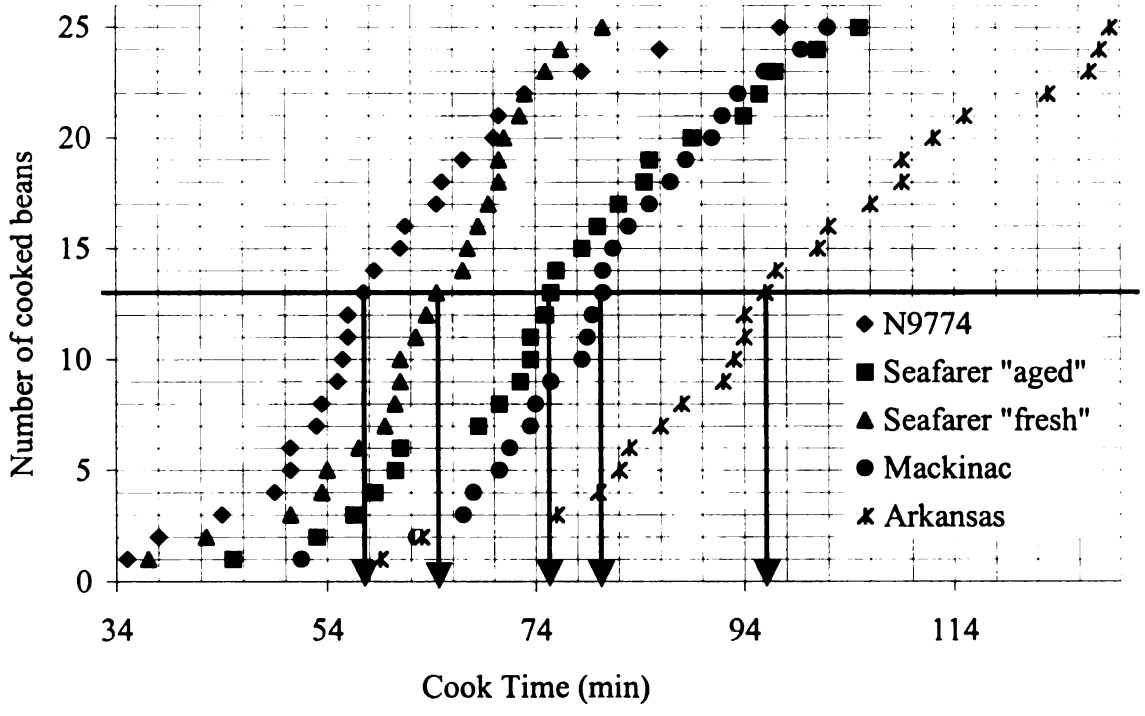


Figure 11. Cooking plots of five selected Navy beans (*Phaseolus vulgaris L.*) ; n=2

Effect of calcium and phosphate ions on the cooking time of selected Navy beans.

The effect of increased concentrations of calcium ions on the cooking time is shown in Table 5 and the corresponding plot presented in Figure 12. For all the beans CT_{13} increased significantly with the concentration of calcium ions in the cooking water before reaching a plateau. The existence of the plateau suggests that the beans have binding sites for calcium ions, susceptible of saturation with increased concentration. Liu *et al* (1992c) presented this conceptual model for calcium site saturation.

The binding sites are believed to be free carboxylic groups released by demethylation of pectin. The result is consistent with the findings of Hentges *et al* (1991) that, as cooking time increased high methoxyl pectin decreased and low methoxyl pectin increased. However, in another experiment intended to test the validity of this concept, Liu *et al* (1992 b) reported that the degree of pectin methylation of the cell wall in cowpeas remained unchanged during HTC development. They suggested that this model of increased cation-uptake capacity during HTC development due to PME action or pectin de-methylation was inadequate to explain the phenomenon.

Table 5. Cooking time (CT_{13}) of Navy bean samples (*Phaseolus vulgaris L.*) in increased concentration of calcium ion (Ca^{2+})

	Samples	0 ppm (min)	SD	5 ppm (min)	SD	25 ppm (min)	SD	50 ppm (min)	SD	LSD (0.05)
Cultivars	Seafarer,"aged"	81.5	7.8	140.0	17.0	213.0	10.6	228.0	0 .0	37.3
	Seafarer,"fresh"	64.5	3.5	76.0	1.4	117.5	0.7	124.5	3.5	7.3
	Mackinac	80.5	4.9	133	10.6	-		-		35.6
	N9774	60.0	3.7	65.5	3.5	94.5	7.8	120.0	0.0	16.2
	Ensign	62.0	1.4	-		-		162.5	17.7	54.0
	Mayflower	59.5	0.7	-		-		121.5	29.0	88.2
	Navigator	49.5	0.7	-		-		95.9	16.3	49.5
	Vista-MI	49.0	1.4	-		-		105.0	5.7	17.7
	Schooner	54.5	2.1	-		-		122.0	4.2	14.4
	Vista-MN	40.5	0.7	-		-		128.0	11.3	34.5
	Norstar	50.5	0.7	-		-		104.5	2.1	6.8
Commercial samples	NB-1	57.5	0.7	80.0	14.1	103.0	3.5	109.0	7.1	31.9
	NB- 2	57.5	0.7	73.5	3.5	117.0	2.8	113.0	3.5	8.0
	NB- 3	58.5	2.1	71.0	7.1	116.0	10.6	143.0	7.1	20.4
	NB- 4	59.5	2.1	86.5	4.9	125.0	14.1	135.0	11.3	26.2
	NB- 5	51.5	4.9	91.0	1.4	103.0	14.1	109.0	0.7	20.9
	Arkansas	104.0	5.6	213	9.9	-		-		34.7
	Bayside Best	61.0	4.2	95.0	7.1	139.0	26.9	148.0	7.1	40.2
	LSD (0.05)	10.1		19.4		28.5		25.0		

n=2

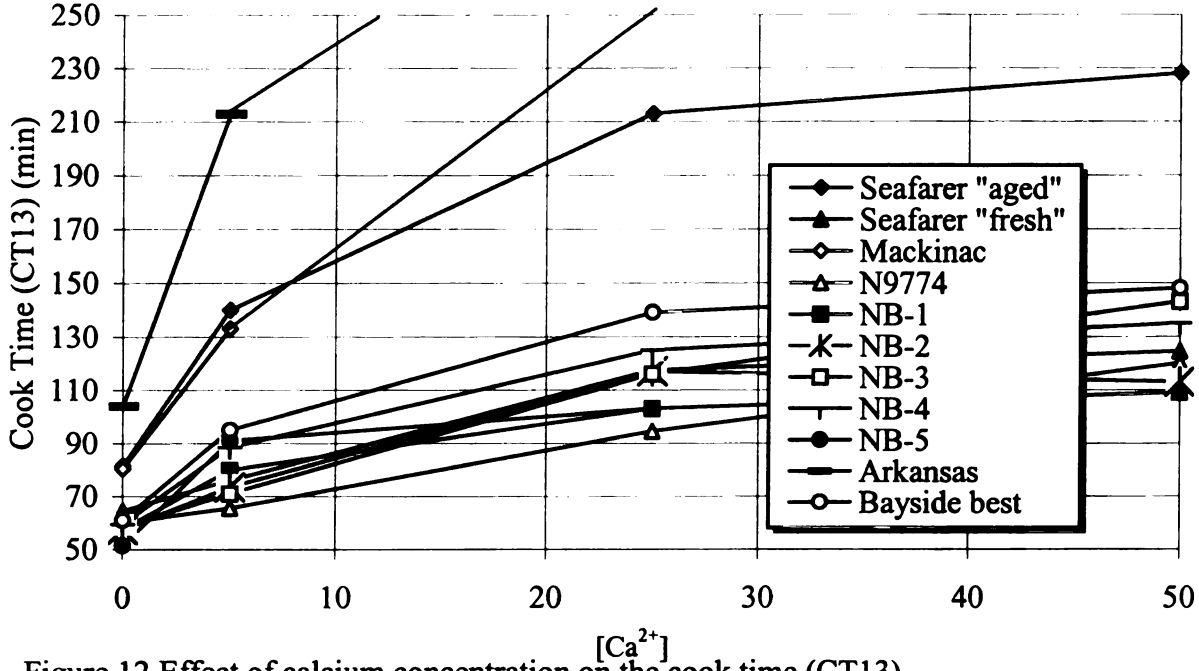


Figure 12.Effect of calcium concentration on the cook time (CT13) (min)

The effect of increased concentrations of phosphate ions on the cooking time is shown in Table 6 and the corresponding plot presented in Figure 13. The behavior of beans in increased concentrations of phosphate ions vary among samples. For Seafarer "aged", Mackinac and NB-4 there was an initial increase in CT_{13} at low concentrations. After reaching a maximum CT_{13} dropped to a plateau below the initial value. For N9774, NB-1, NB-2, NB-3, Arkansas and Bayside Best there was no initial hardening effect at low concentrations: the CT_{13} dropped to a plateau with an increase in phosphate concentration.

The softening effect of phosphate is already known. Rockland (Aguilera and Stanley 1985) outlined the technology termed "quick cooking". It consists basically in a vacuum infiltration of monovalent phosphate and other salts followed by drying. The

Table 6. Cooking time (CT_{13}) of Navy beans cultivars (*Phaseolus vulgaris L.*) in increased concentration of PO_4^{3-} (minutes).

	Sample	0 ppm		5 ppm		25 ppm		50 ppm		100 ppm		200 ppm		
		(min)	SD	(min)	SD	(min)	SD	(min)	SD	(min)	SD	(min)	SD	LSD
Cultivars	Seafarer,"aged"	81.5	7.8	94.0	8.5	92.5	3.5	70.0	0.0	-		62.5	10.6	14.3
	Seafarer,"fresh"	63.5	3.5	56.5	4.9	53.0	1.4	50.5	2.1	-		44.5	0.7	7.6
	Mackinac	80.5	4.9	112.0	12.1	108.0	24.7	88.0	5.7	-		75.0	14.1	17.1
	N9774	60.0	3.7	58.0	2.4	55.0	7.1	49.0	4.2	-		53.5	2.1	10.4
	Ensign	62.0	1.4	-		-		-		58	2.8	-		9.5
	Mayflower	59.5	0.7	-		-		-		50	2.8	-		8.8
	Navigator	49.5	0.7	-		-		-		49	0.7	-		3.0
	Vista-MI	49.0	1.4	-		-		-		42	4.0	-		12.9
	Schooner	54.5	2.1	-		-		-		50	0.7	-		6.8
	Vista-MN	40.5	0.7	-		-		-		38	0.7	-		3.0
	Norstar	50.5	0.7	-		-		-		46	0.7	-		3.0
Commercial samples	NB- 1	57.5	0.7	52.5	0.7	51.0	2.8	48.0	2.8	-		42.0	4.2	19.9
	NB- 2	57.5	0.7	56.0	7.1	48.5	3.5	-				46.0	1.4	10.1
	NB- 3	58.5	2.1	50.5	0.7	51.0	4.2	46.5	4.9	-		44.5	3.5	8.9
	NB- 4	59.5	2.1	61.5	0.7	48.0	4.2	48.5	2.1	-		44.0	7.1	10.1
	NB- 5	51.5	4.9	53.0	0.0	48.0	2.8	49.0	2.8	-		36.5	0.7	6.7
	Arkansas	104.0	5.7	97.5	3.5	83.0	5.7	76.5	3.5	-		71.0	5.7	12.6
	Bayside Best	61.0	4.2	57.5	3.5	51.0	2.8	53.0	4.2	-		53.0	5.7	10.8
	LSD(0.05)	10.1		9.3		8.9		6.7		5.2		12.9		

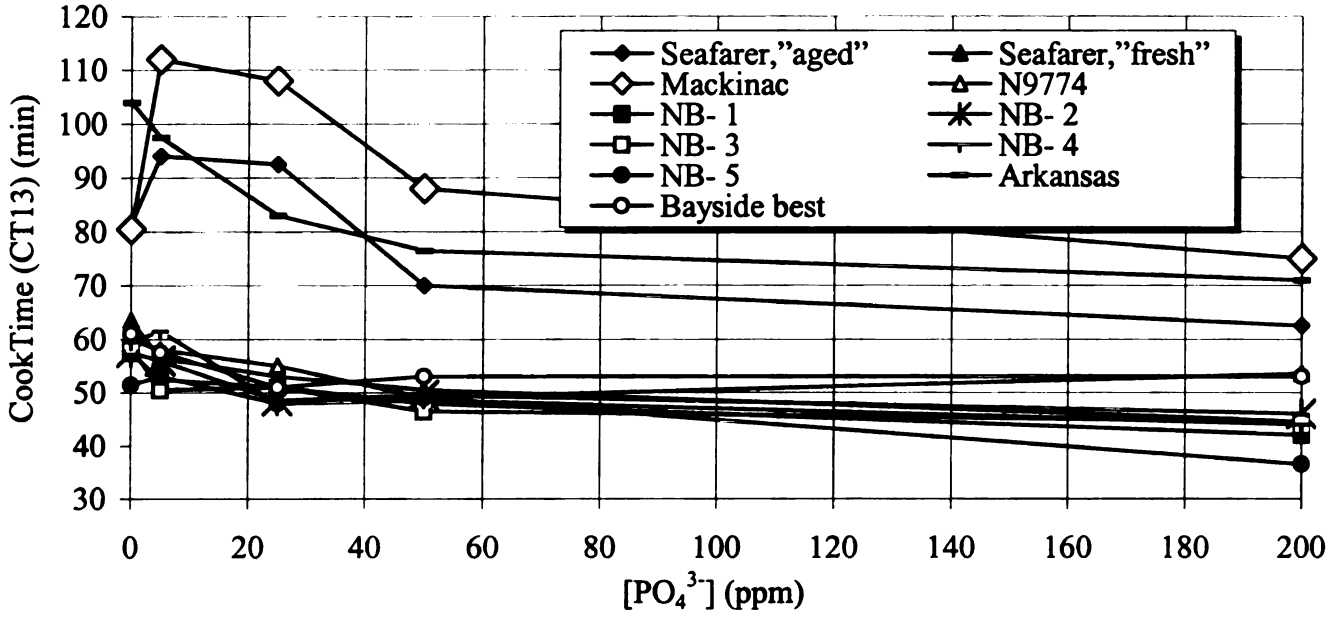


Figure 13. Effect of phosphate on the cook time of selected Navy bean commercial samples and cultivars (*Phaseolus vulgaris L.*)

HTC reversion was also reported by Kilmer *et al* (1994) and Vindiola *et al* (1986). The former authors suggested a mechanism based on calcium and magnesium chelation.

To our knowledge the hardening effect of phosphate ions, observed in some samples at low concentrations, has not yet been reported. The curves suggest the existence of two competitive types of phosphate bindings both with limited sites.

The first type of binding has a few sites that end at low phosphate concentrations. They are only found in some beans that show the hardening at low concentrations. This type may be due to the chelation of calcium and magnesium by phosphate ions. The resulting complex should then contribute to the firmness of the beans. That should be true if it could be demonstrated that beans with the initial hardening had significant amounts of free magnesium and calcium ions, released by phytases for example, as opposed to beans without initial hardening characterized by no or negligible levels of free divalent ions.

The second type of binding has higher but limited sites. One of the possible mechanisms should be the insertion of phosphate ions between negatively charged carboxylic groups borne by demethylated pectin chains, thus preventing the crosslinking reactions. This should result in increased bean softness.

Further research is necessary to test these hypotheses. The first experimental design can be the verification of a difference in the concentration of free divalent ions in beans with different behavior once cooked in phosphate ions. The second can be the monitoring of effective insertion of phosphate ions between negatively charged pectates.

The effects of 50 ppm of calcium ions and 100 ppm of phosphate ions are shown separately in Figure 14 for the cultivars Ensign, Mayflower, Navigator, Vista-MI,

Schooner, Vista-MN and Norstar. The calcium at 50 ppm expectedly increased significantly ($p < 0.05$) the firmness, compared to the control. This is consistent with previous findings on the other bean samples. However, the softening effect of phosphate ions (100 ppm) was significant ($p < 0.05$) only for Norstar.

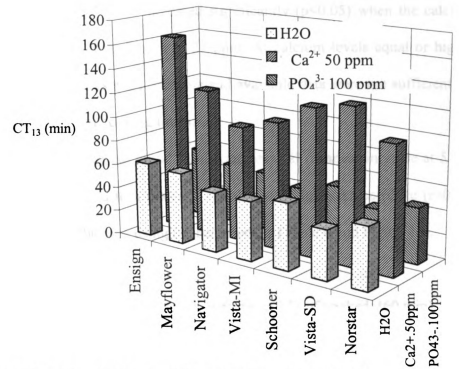


Figure 14.Effect of calcium and phosphate on the cooking time of selected Navy bean cultivars

Effect of calcium and phosphate ions on the firmness of cooked Navy beans (Mackinac).

The effects of calcium and phosphate ions on the firmness of cooked beans are shown in Tables 7 and 8 and the corresponding graphs represented in Figures 15 and 16. No significant effect ($p>0.5$)was detected at 5 ppm Ca^{2+} but at 25 ppm, the total shear work and the maximum shear force increased significantly ($p<0.05$) when the calcium concentration was increased from 0 ppm to 25 ppm. At calcium levels equal or higher than 50 ppm the two parameters were off-scale and available data were not sufficient for a comparison with results from cooking times.

For phosphate ions, after an initial significant textural firmness increase at 5 and 25 ppm, the firmness decreased to a plateau. The values of firmness correlate ($r=0.82$) with those of cooking time obtained using the bean cooker (Table 6 and Figure 13).

Table 7. Specific shear work and maximum shear force on 25g of cooked (60 min) Navy beans (*Phaseolus vulgaris L.)* (Mackinac) in Calcium solutions (Ca^{2+})

	$[Ca^{2+}]$						
	0 ppm	5 ppm	25 ppm	50 ppm	100 ppm	200 ppm	LSD (0.05)
SW	2002	1897	3983	OS*	OS	OS	
SD	50	134	88	OS	OS	OS	137
F_{max}	230	211	520	OS	OS	OS	
SD	11	5	19	OS	OS	OS	18

n=3 *Off-scale

Table 8.Total shear work and maximum shear force on 25 g of cooked (60 min at 100°C) Navy beans (*Phaseolus vulgaris L.)* (Mackinac)) in Phosphate solution (PO_4^{3-})

	$[PO_4^{3-}]$						
	0 ppm	5 ppm	25 ppm	50 ppm	100 ppm	200 ppm	LSD (0.05)
SW	2002	2381	2310	1627	1446	762	
SD	50	16	85	100	61	76	88.3
F_{max}	230	303	299	195	179	104	
SD	11	9	2	17	7	5	12.3

n=3

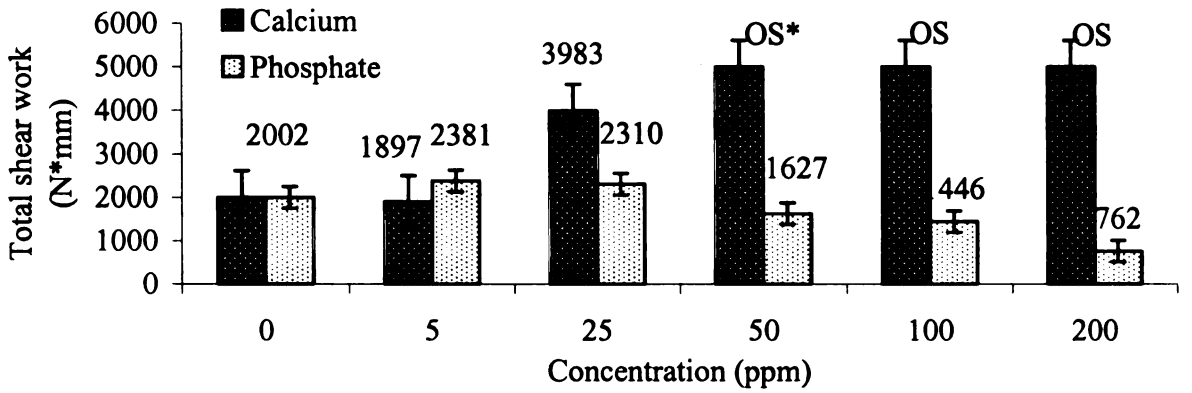


Figure 15. Effect of calcium and phosphate ions on the firmness of cooked Navy beans (Mackinac)(25g sample)

* off-scale

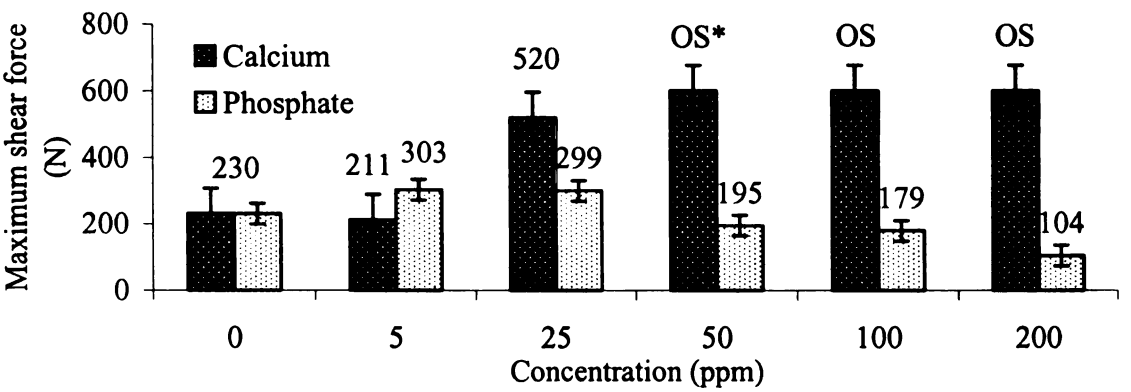


Figure 16. Effect of calcium and phosphate ions on the firmness of cooked Navy beans (Mackinac) (25g sample)

* Off-scale

Study 2: Effect of Selected Bean Processing Techniques on the Texture of canned Beans (*Phaseolus vulgaris L.*)

Processing characteristics of selected bean samples (*Phaseolus vulgaris L*)

Table 9 and Figure 17 present the mean values of soak weight and wash drained weight of bean cultivars and commercial samples used in this research. Both parameters are negatively correlated (r = -0.93 for the soak weight and -0.63 for the wash drained weight) with the cooking times in distilled water (Tables 5 and 6). Thus, "hard beans" are resistant to hydration during soaking and subsequent cooking. This is consistent with previous findings of many researchers (Berrios *et al* 1999, Hincks and Stanley 1986, Shehata et *al* 1987, Shehata et *al* 1988, Youssef *et al* 1982).

Table 9. Soaked weight and wash drained weight of selected processed Navy beans (initial weight = 116.3g)

	Navy beans	Soak weight (g)	SD	Wash drained weight (g)	SD
Cultivars	Seafarer, "aged"	207.8	0.8	271.9	0.9
	Seafarer, "fresh"	213.2	1.2	294.3	0.4
	Mackinac	208.2	0.4	284.1	3.4
	N97774	214.0	1.4	293.5	6.2
	Ensign	209.4	0.5	280.1	4.0
	Mayflower	210.7	0.8	287.4	0.6
	Navigator	203.9	1.0	283.0	1.6
	Vista-MI	215.3	0.3	293.6	2.9
	Schooner	216.7	0.3	300.8	4.7
	Vista-MN	212.5	0.2	300.3	4.7
	Norstar	206.8	0.4	298.9	9.1
Commercial samples	NB-1	214.0	1.4	285.1	1.6
	NB-2	212.3	0.6	291.6	1.4
	NB-3	210.7	2.7	272.0	2.3
	NB-4	209.9	0.7	280.7	2.5
	NB-5	214.6	2.1	284.4	3.4
	Arkansas	198.9	0.1	263.7	6.1
	Bayside Best	211.9	0.6	278.3	2.7
	LSD (0.05)	2.0		7.3	

n=2

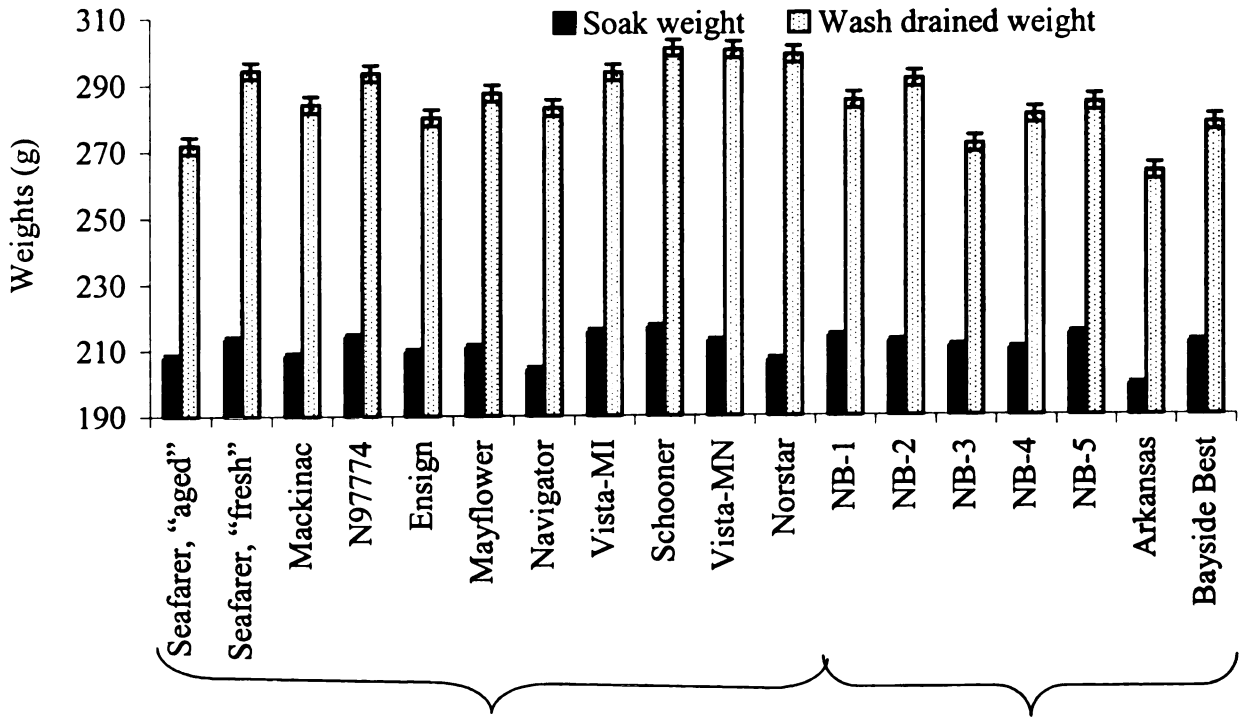


Figure 17. Soak weight and drained weight of selected canned Navy beans

The Table 10 and the Figures 18 and 19 show the total shear work and the maximum shear force on 50 g samples of beans. Both parameters are highly correlated (r = 0.86 and 0.82, respectively) to the cooking times. However the texture measurement is more indicative of palatability because it gives the average of firmness of an aggregate sample. The results of cooking time measurement are influenced by the characteristics of individual beans. For example NB-3, which was classified as "soft beans" is classified as "hard beans" based on the aggregate texture measurement. In addition the shear work is negatively correlated to the soak weight (r = -0.91) and to the wash drained weight (r = -0.80). These results confirm that "hard beans" possess resistance to water imbibition during both soaking and cooking, and the resultant beans show an increased shear resistance compared to "soft beans".

Table 10. Total shear work and maximum shear force of selected processed Navy beans (50 g sample)

		Shear Work		Maximum Shear Force	
		(N.mm)	SD	(N)	SD
Cultivars	Seafarer,"aged"	2382.4	73.2	199.8	8.2
	Seafarer,"fresh"	1844.4	96.1	149.6	8.7
	Mackinac	2619.0	204.7	196.8	2.8
	N97774	1491.6	48.9	131.4	2.8
	Ensign	2068.0	220.1	177.8	18.1
	Mayflower	1811.7	129.3	161.1	10.7
	Navigator	1703.2	96.5	157.9	8.6
	Vista-MI	1581.2	47.5	143.1	3.4
	Schooner	1614.1	53.0	135.1	5.5
	Vista-MN	1232.5	70.3	112.8	6.3
	Norstar	1608.2	114.5	139.0	7.6
Commercial sample	NB-1	1468.3	63.2	127.8	8.0
	NB-2	1596.3	87.1	128.8	3.4
	NB-3	2654.4	194.0	231.6	19.1
	NB-4	1788.8	71.2	152.1	5.1
	NB-5	1571.0	161.9	141.3	11.2
	Arkansas	3447.2	187.6	303.4	16.5
	Bayside Best	1913.7	25.3	166.9	8.5
LSD (0.05)		36.2		10.6	

n = 4

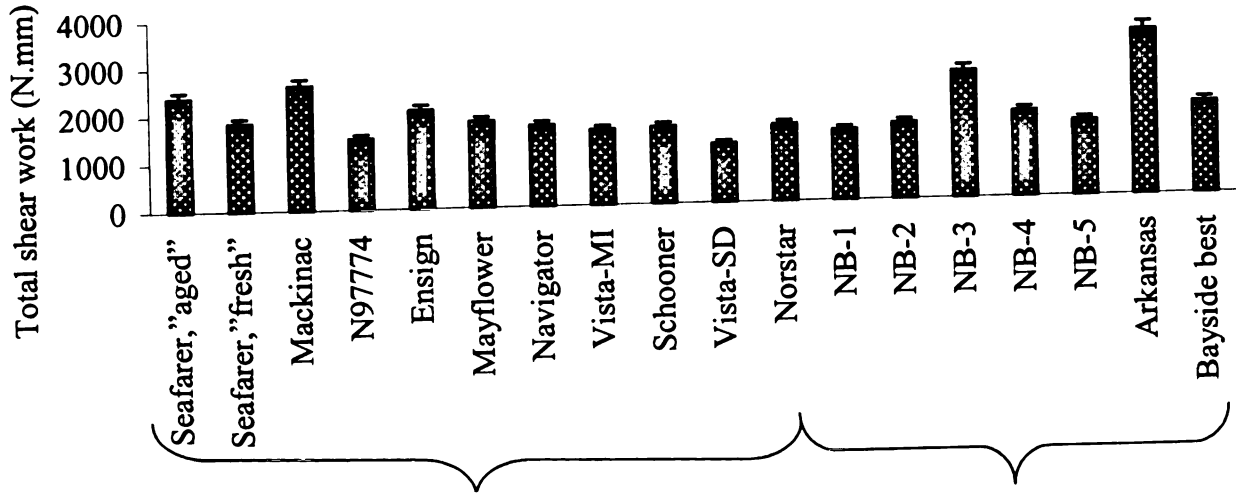


Figure 18. Total shear work of 50g of selected canned Navy beans (MSU soak method)

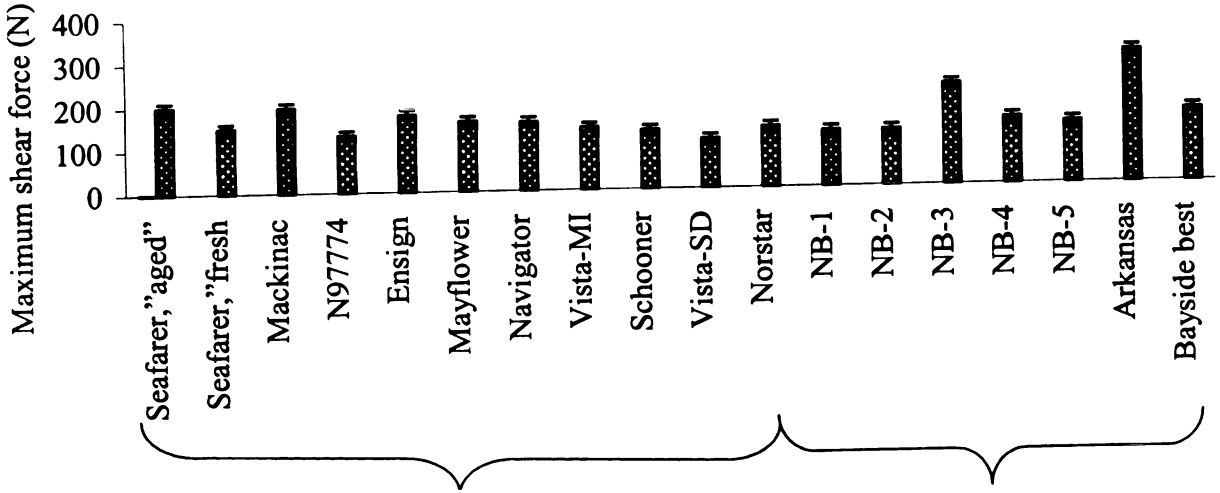


Figure 19. Maximum shear force of 50g of selected canned Navy beans (MSU soak method)

Effect of Phosphate ions (PO_4^{3-}) in Brine on the Firmness of Canned Navy beans (Mackinac).

The data of the wash drained weight, the shear work and the maximum shear force are reported in Table 11.

The phosphate in the cover brine had no significant effect ($p>0.05$) on the wash drained weight and the texture of canned beans. This demonstrates that the softening effect occurs during soaking.

Table 11. Effect of phosphate in brine on the firmness of canned Navy Beans (Mackinac) Initial weight =116.3g; Mean percent water gain = 83.1% (n=12)

$[PO_4^{3-}]$	Wash drained weight		Shear work		Maximum shear force	
(ppm)	(g)	SD	(N.mm)	SD	(N)	SD
0	286.9	1.6	2281	112	175	8
5	287.7	0.5	2322	115	181	5
25	285.3	1.3	2231	82	171	2
50	287.5	1.5	2361	121	177	4
100	287.4	1.6	2159	125	171	5
200	289.0	1.7	2139	43	174	3
LSD (0.05)	3.5		248.4		14.9	
	n=2		n=4		n=4	

Effect of Selected Soaking Methods on the Texture of Canned Navy Beans (*Phaseolus vulgaris L.*)

The percent water gain and the wash drained weight are presented in the Table 12. The corresponding plots are illustrated in Figures 20 and 21.

Compared to the phosphate, the calcium in the soaking water resulted in a significantly lower percent water uptake ($p<0.01$) except for the MSU soak method where the difference was insignificant ($p > 0.05$). The comparison among the methods after soaking in calcium showed that the following hydration order:

MSU soak (30 min at 25.6°C + 30 min at 87.8°C)

< ComSoak1 (3 hours at 54.5°C+ 6 min at 79.4°C)

and ComSoak2 (3 hours at 54.4°C+2.5min at 71.1°C + 3.5 min at 78.9°C)

< Cold soak (4 hours at 25.6°C+ 5 min at 93.3°C).

However, there was no significant difference between the two commercial soaking methods at $p=0.05$. Uebersax and Bedford (1980) reported the inverse relationship between the water uptake and the concentration of calcium ion.

After soaking in phosphate the only significant difference ($p<0.05$) in percent water gain was detected between the cold soak and the two commercial soak methods.

The analysis of the wash drained weight demonstrates that the calcium and phosphate in soaking water resulted in significantly different values ($p<0.05$). When the calcium was used in soaking water the drained weights values were significantly different from each other in the following order:

(ComSoak1, ComSoak2) < MSU < Cold soak

The difference between the two commercial soak methods was insignificant ($p > 0.05$).

Table 12. Water gain and drained weight of canned Navy beans (Mackinac) with selected soaking methods

	Soak Method	% H2O Gain	SD	Drained weight	SD
Ca^{2+} 100 ppm	Cold Soak	81.2	0.9	272.4	1.0
	MSU Soak	74.5	0.8	268.4	1.2
	ComSoak 1	79.2	0.5	246.4	1.3
	ComSoak 2	78.7	1.0	245.5	1.9
PO_4^{3+} 100 ppm	Cold Soak	88.0	0.9	290.9	0.4
	MSU Soak	78.1	8.4	283.7	10.9
	ComSoak 1	82.5	1.0	277.1	1.0
	ComSoak 2	84.1	0.9	276.0	1.6
LSD (0.05)		3.8		4.9	

n=3
Initial weight per can = 116.28g. Moisture: 14%
%H_2O Gain= (Soak eight - 116.28*100)/116.28

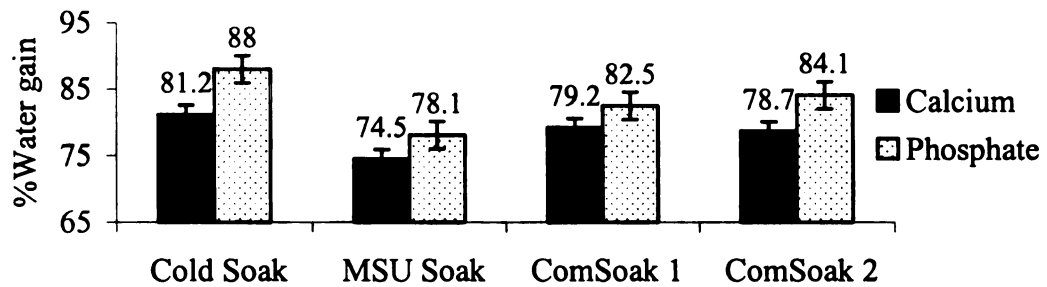


Figure 20. Percent water gain of Navy beans (Mackinac) after soaking with selected methods

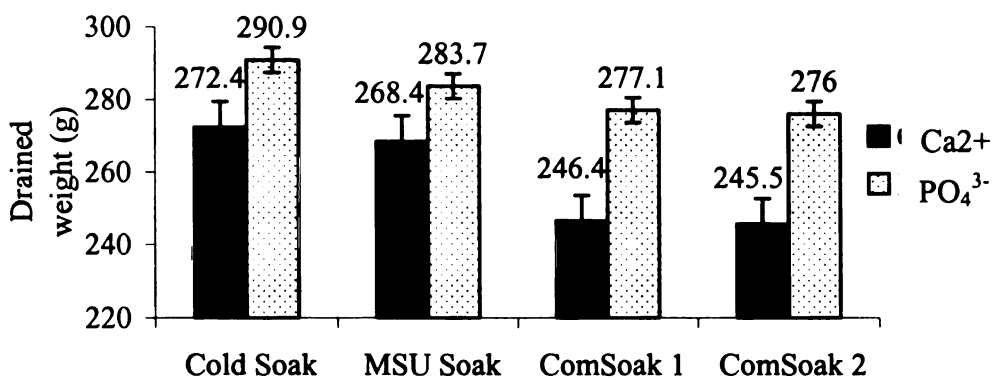


Figure 21. Drained weight of canned Navy beans (Mackinac) following selected soaking methods

When the phosphate ion was used in the soak water there was no significant difference between the two commercial methods. The drained weights were significantly ($p < 0.05$) different from each other in the following order:

(ComSoak1 and ComSoak2) < MSU soak < Cold soak

The data for texture measurement are shown in Table 13 and Figure 22. Compared to the calcium, the phosphate ion in the soaking water resulted in a significantly softer product ($p<<0.05$) in all methods. The firmness follows the order:

Cold soak < MSU soak < ComSoak1 < ComSoak 2

The two commercial soak methods yielded practically the same texture ($p > 0.05$) when the calcium was used in the soak water, but the firmness in ComSoak1 was significantly lower ($p < 0.05$) when the phosphate was used.

When calcium was used the following order of firmness was found;

Cold soak < MSU soak < ComSoak2 < Comsoak1.

When the phosphate was used in the soak water the four methods resulted in significantly different firmness values ($p < 0.05$). The order of firmness was as follows:

Cold soak < MSU soak < ComSoak1 < ComSoak2

The firming effect of calcium ions and the softening effect of phosphate ions were consistent with results of cooking time presented in 1.2. The cold soak and the MSU soak gave a relatively softer product than the two commercial soak methods. One possible explanation is the seed hydration potential and the inherent enzymatic activity. The longer soaking time at low temperature in the cold soak favors the cotyledon hydration, which is a key factor of the final softness, but reduces the pectin methylesterase activity because of the relatively low temperature. The short soaking time in the MSU method

Table 13. Total shear work and maximum shear force on 50g samples of canned Navy beans (Mackinac) with selected soaking methods in 100 ppm of Ca^{2+} and 100 ppm of PO_4^{3-}

		Shear Work (N*mm)	SD	Maximum Shear force (N)	SD
Ca^{2+}	Cold Soak	4031	183	308	16
	MSU Soak	4165	143	319	10
	ComSoak 1	6823	111	543	9
	ComSoak 2	6727	137	541	18
PO_4^{3-}	Cold Soak	1855	143	161	8
	MSU Soak	2180	112	168	4
	ComSoak 1	2670	88	208	5
	ComSoak 2	2857	122	217	12
LSD (0.05)		81.9		6.2	

n=6

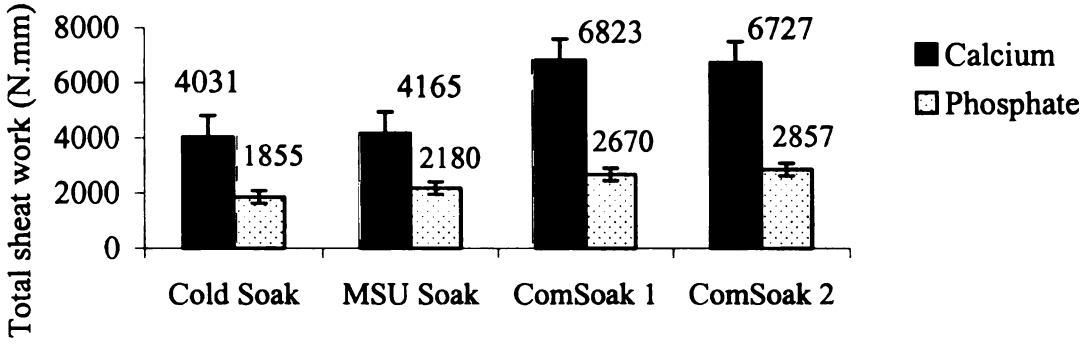


Figure 22. Shear work on 50g sample of canned navy bean (Mackinac) following soaking in 100 ppm Ca^{2+} or PO_4^{3-}

was not optimal for hydration but the texture of the final product was improved because the 30 minutes blanching at 190°F inactivated the pectin methylesterase. Finally, the long soaking time (3 hours) in the commercial soak methods resulted in a high hydration, but the soaking temperature of 54.4°C (130°F) was within the optimal range of pectin methylesterase activity 45-55°C as previously reported (Collins 1970). Consequently the two commercial soak methods gave a firmer end product than the laboratory method.

Interaction of Phosphate and Calcium Ions in the Soak Water and their Effect on theTexture of Canned Navy Beans

The interaction of calcium and phosphate was investigated to assess the effects on the hydration rates and the texture of canned beans.

The parameters of hydration, percent water gain and wash drained weight, are presented in Table 14 and the corresponding graphs in Figures 23 and 24. The texture data are presented in Table 15 and the corresponding graphs in Figures 25 and 26. The effect of calcium and phosphate on the hydration rate and the texture was analyzed in three stages: 1) the independent effect of calcium ion, 2) the independent effect of phosphate and 3) the effect on combined calcium phosphate within the soak water.

Compared to the control (distilled water) the calcium ion in soaking water decreased significantly ($p<0.05$) the soak weight in the three bean samples although the decrease was insignificant for Mackinac treated with Cold soak and ComSoak2, Seafarer in ComSoak 1 and Arkansas in ComSoak2. The calcium had no significant effect on the wash drained weight, except Arkansas in the Cold soak, MSU soak and ComSoak1, and Seafarer "aged" in MSU soak where it decreased significantly ($p<0.05$). The calcium in soak water resulted in a significantly firmer product.

The effect of phosphate ion in the soak water on the water uptake depended on the soaking methods and the bean samples.

- In the Cold soaking method the soak weight increased significantly when the concentration of phosphate was raised to 100 and 200 ppm. However there was no significant difference between the corresponding soak weights, due probably to the

saturation of the binding sites. The softening effect of phosphate is insignificant at 200 ppm level.

- The phosphate soak treatment did not change significantly the soak weight of beans in the MSU soak method in all bean samples.
- For the two commercial soaking methods ComSoak1 and ComSoak2 the only significant increase in soak weight was observed in Mackinac and Seafarer "aged" when soaked in phosphate at 200 ppm level in ComSoak1 and at 100 ppm level in ComSoak2.
- At the levels used in the soaking water the phosphate had limited impact and no significant effect on the wash drained weight was observed, except Mackinac in Cold Soak.
- The phosphate had no significant effect on the texture in the cold soak.
- In the MSU soaking method Arkansas and Mackinac were significantly softer at100 and 200 ppm of phosphate than the control.
- In ComSoak1 no significant softening effect was observed at 200 ppm of phosphate for all bean samples.
- In ComSoak2 only Mackinac softened significantly at 200 ppm phosphate.

To investigate the possible interaction calcium/phosphate, the mean of values of percent water gain, wash drained weight and shear force at concentration ratio Ca^{2+}/PO_4^{3-} 100 ppm/ 0 ppm and 0 ppm/100 ppm were compared to the mean at the concentration ratio Ca^{2+}/PO_4^{3-} = 100 ppm/100 ppm.

- No significant differences were detected at $p = 0.05$ and thus it was concluded that the calcium and phosphate ions exert respectively the demonstrated firming and softening effect independently of each other. When the calcium and the phosphate are combined in the soak water (concentration ratio Ca^{2+}/PO_4^{3-} = 100 ppm/100 ppm) the firming effect of 100 ppm of calcium alone is added to the softening effect of 100 ppm of phosphate alone. No synergy or competition was evident. This suggests that the binding sites of calcium and phosphate ions may be different.
- Finally, little to insignificant difference was detected in the percent water gain, the wash drained weight and the texture between beans soaked in Ca^{2+}/PO_4^{3-} = 100 ppm/100 ppm and Ca^{2+}/PO_4^{3-} = 200 ppm/200 ppm. This supports the hypothesis of the existence of saturable binding sites.

Table 14. Percent water gain and wash drained weight (g) of Navy beans (*Phaseolus vulgaris L.*) following selected soaking methods in selected calcium/phosphate concentration ratio and canning.

			Arkansas		Mackinac		Seafarer aged	
			(g)	SD	(g)	SD	(g)	SD
Cold soak (4h/21.1°C+5min/93.3°C)	0/0	%H2O	87	0.1	86.8	0.2	87.2	0.1
		Drained weight	274.8	3.2	286.3	2.3	292.5	3.1
	100/0	%H2O	84.7	0.2	85.3	1	86.5	0.1
		Drained weight	259.1	2.4	280.5	3	282	5
	0/100	%H2O	90.4	0.1	88.6	1.1	88.5	0.6
		Drained weight	274.7	0.7	285.2	2.1	296.7	
	0/200	%H2O	91.2	0.7	90.6	0.6	92	0.4
		Drained weight	277.2	3.5	281.2	0.2	294.5	0.8
	100/100	%H2O	85.9	0.4	86	0.1	86.5	0.4
		Drained weight	265.3	0.4	280.7	0.7	290.4	5.4
	200/200	%H2O	85.8	0.3	85.9	0.6	86	0.1
		Drained weight	265.8	1.5	281.2	0.2	285.4	6.2
MSU soak (30min/25.6°C+30min/87.8°C)	0/0	%H2O	85.6	0.4	88	1.3	87.4	1.1
		Drained weight	268.1	1.8	278.1	8.8	287.4	1.8
	100/0	%H2O	74.7	0.2	79.1	0.6	79.8	0.8
		Drained weight	256.2	1.1	277.6		271.4	5.2
	0/100	%H2O	81.3	2.2	84.4	1.7	83.9	0.5
		Drained weight	271.4	1.2	284	0.6	286.4	3.1
	0/200	%H2O	83.9	0.5	86	0	85.3	1.1
		Drained weight	272.6	0.8	282.7	0.6	293.5	2.3
	100/100	%H2O	78.4	2.9	82.5	0.3	81.6	0.7
		Drained weight	262.6	2.5	282.3	0	283.7	2.1
	200/200	%H2O	75.2	0.4	80.5	1.8	80.5	2.2
		Drained weight	264.8	1.8	282.6	2.8	285.3	4.8

Table 14. Percent water gain and wash drained weight (g) of Navy beans (*Phaseolus vulgaris L.*) following selected soaking methods in selected calcium/phosphate concentration ratio and canning (Continued).

			Arkansas		Mackinac		Seafarer aged	
			(g)	SD	(g)	SD	(g)	SD
		Drained weight	264.8	1.8	282.6	2.8	285.3	4.8
ComSoak 1(3h/54.4°C+6min/79.4°C)	0/0	%H2O	80.1	0.2	81	0.2	80.4	0.7
		Drained weight	256.7	1.5	273.2	2.7	276.5	6.4
	100/0	%H2O	77.7	0.6	79	0.1	79.9	0.5
		Drained weight	248.9	2	267.7	3.2	266.9	4.5
	0/100	%H2O	79.6	1	81.4	0.1	81.4	0.5
		Drained weight	259	2.1	274.9	2.1	271.9	3.4
	0/200	%H2O	80.2	0.9	83.1	0.1	84.8	0.8
		Drained weight	256	3.7	276.8	0.2	277.4	5.9
	100/100	%H2O	78.6	0.1	80.6	0.4	79.9	0.3
		Drained weight	252.3	0.6	268.9	2.8	261.6	2.9
	200/200	%H2O	78.2	0.9	79	0.4	80.4	0.8
		Drained weight	249.4	1.9	267	1.3	261.1	3.9
ComSoak 2 (3h/54.4°C+2.5 min/71.1°C+3.5 min/79.4°C)	0/0	%H2O	79.6	0.5	79.7	0.5	80.3	0.1
		Drained weight	254.2	1.1	270.7	3	266.3	
	100/0	%H2O	77.3	1.9	79	1.9	78.7	0.3
		Drained weight	250.4	7	267.9	7.4	257.3	11
	0/100	%H2O	78.6	1.6	81.2	0.1	80.9	0.4
		Drained weight	259.8	5.7	270.9	4.6	280.4	7.1
	0/200	%H2O	80.1	1.3	82.2	2.1	83.4	2.2
		Drained weight	261.3	6	277.2	2	284.5	19.2
	100/100	%H2O	79.6	0.4	81.3	0.1	81.3	0.7
		Drained weight	254.5	1.6	272.9	0.1	273.5	0.3
	200/200	%H2O	77.6	0.4	79.5	0.1	79.5	0.2
		Drained weight	251.4	1.6	264.9	4.1	259.7	3

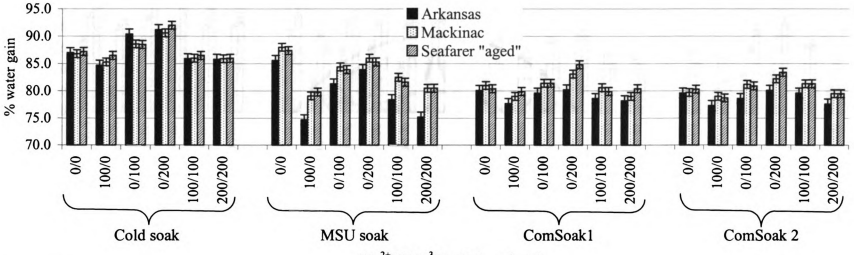


Figure 23.Water gain of Navy beans (*Phaseolus vulgaris L.*) after selected soaking methods in selected Ca^{2+}/PO_4^{3-} ratio

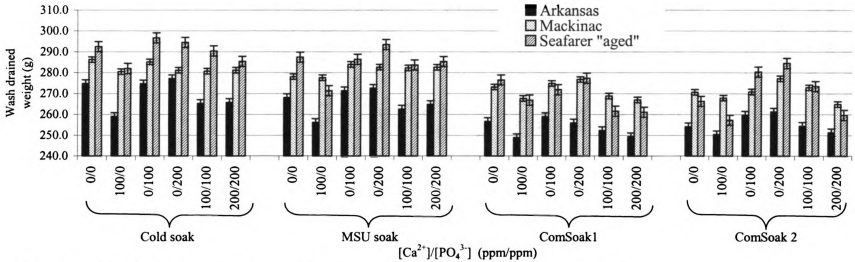


Figure 24. Wash drained weight of canned Navy beans (*Phaseolus vulgaris L.*) following selected soaking in selected calcium / phosphate ratio

Table 15. Total shear work SW (N.mm) and maximum shear force Fmax (N) on 50g samples of canned Navy beans (*Phaseolus vulgaris L.*) following selected soaking methods in selected calcium/phosphate concentration ratio

			Arkansas		Mackinac		Seafarer aged	
			(g)	SD	(g)	SD	(g)	SD
Cold soak (4h/21.1°C+5min/93.3°C	0/0	SW	2208	97	1987	135	1614	91
		Fmax	232	11	190	11	157	8
	100/0	SW	4677	123	3026	49	2545	180
		Fmax	428	14	246	9	228	22
	0/100	SW	2233	47	1980	84	1622	89
		Fmax	231	8	189	3	152	6
	0/200	SW	2024	267	1728	129	1529	156
		Fmax	211	18	169	14	149	9
	100/100	SW	3612	196	2746	121	2025	103
		Fmax	346	17	234	11	182	6
	200/200	SW	3812	41	2894	161	2260	274
		Fmax	347	11	242	10	197	22
MSU soak (30min/25.6°C+30min/87.8°C)	0/0	SW	3099	216	2334	110	2106	160
		Fmax	312	16	196	8	180	5
	100/0	SW	4722	158	3556	39	3220	232
		Fmax	440	10	269	10	280	15
	0/100	SW	2622	80	2303	110	2012	44
		Fmax	263	7	199	7	177	4
	0/200	SW	2469	93	2010	64	1828	65
		Fmax	240	5	188	7	164	6
	100/100	SW	3819	156	2644	134	2309	96
		Fmax	348	9	215	9	192	4
	200/200	SW	4019	109	2741	100	2446	57
		Fmax	365	18	220	9	209	15

Table 15. Total shear work SW (N.mm) and maximum shear force Fmax (N) on 50g samples of canned Navy beans (*Phaseolus vulgaris L.*) following selected soaking methods in selected calcium/phosphate concentration ratio (Continued).

			Arkansas		Mackinac		Seafarer aged	
			(g)	SD	(g)	SD	(g)	SD
ComSoak 1(3h/54.4°C+6min/79.4°C)	0/0	SW	4519	240	3149	114	2920	444
		Fmax	398	30	247	5	241	41
	100/0	SW	5864	101	4187	227	3640	141
		Fmax	530	14	330	17	323	18
	0/100	SW	4585	181	3166	277	3078	366
		Fmax	397	20	249	16	272	50
	0/200	SW	4456	185	2785	123	2793	316
		Fmax	406	9	220	7	235	33
	100/100	SW	5689	261	3868	267	4037	168
		Fmax	513	10	304	22	363	9
	200/200	SW	5999	225	4026	235	3682	217
		Fmax	529	26	321	13	321	20
ComSoak 2 (3h/54.4°C+2.5 min/71.1°C+3.5 min/79.4°C)	0/0	SW	4717	254	3673	171	3301	81
		Fmax	420	20	275	19	283	8
	100/0	SW	5214	413	3981	457	4658	881
		Fmax	269	42	310	31	416	67
	0/100	SW	4152	787	3049	201	2502	256
		Fmax	371	26	234	18	211	28
	0/200	SW	4069	614	2770	187	2327	549
		Fmax	357	56	217	14	199	47
	100/100	SW	5437	195	3693	194	3208	132
		Fmax	474	17	283	12	266	7
	200/200	SW	5578	107	4343	635	3993	141
		Fmax	480	6	343	52	339	14

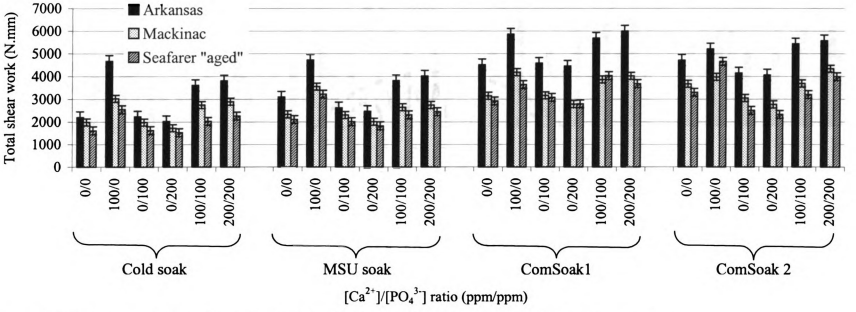


Figure 25. Total shear work on 50 g of Navy beans (*Phaseolus vulgaris L.*) with selected soak methods in selected calcium / phosphate ratio

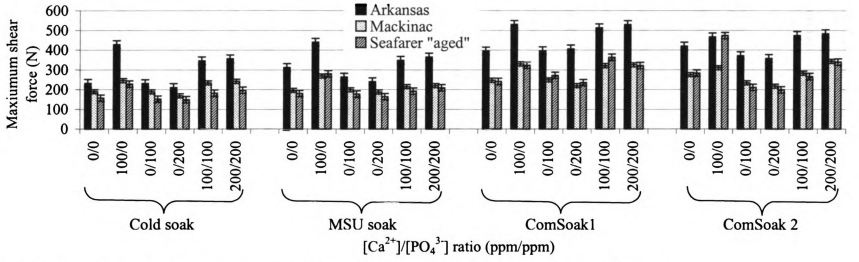


Figure 26. Maximum shear force on 50g samples of Navy beans (*Phaseolus vulgaris L.*) with selected soaking methods in selected Calcium/Phosphate ratio

Study 3: Measurement of Pectin Methylesterase Activity in Selected Cultivars and Commercial Samples of Beans (*Phaseolus vulgaris L.*)

Effect of soak treatment (time/temperature) on pectin methylesterase activity of Navy beans (*Phaseolus vulgaris L*)

The results of pectin methylesterase (PME) measurements are shown in Table 16. The results of PME activity in non-treated samples (Figure 27) allow to classify beans into three distinct groups:

- Beans with low PME activity: Seafarer "fresh", Mackinac, N9774, NB-5 and N97774 (17.6-27.7Meq/(g*min));
- Beans with intermediate PME activity: NB-1, NB-2, NB-4, (37.4-45.6 Meq/(g*min));
- Beans with high PME activity: Seafarer "aged", NB-3, Arkansas and Bayside Best (>53.97Meq/(g*min)).

Except Mackinac, the hard beans (Seafarer "aged", Arkansas and NB-3) had a high PME activity. The high availability of pectin methylesterase in hard beans was probably due to cell wall degradation and the resulting solid leaching, which characterizes the development of the HTC phenomenon (Jackson and Varriano-Marston 1981; Jones and Boulter 1983a and 1983b; Hincks 1987, Shomer *et al* 1990; Liu *et al* (1992c); Liu *et al* 1993).

The effect of soaking time at 21.1°C (70°F) is shown in Figure 28. Generally, the PME measured in soaked beans is significantly higher than in non-soaked beans ($p<0.05$). For Seafarer "aged" no significant change in PME activity was detected after eight hours soaking at 21.1°C (70°F). For the other beans the PME activity was limited

after one hour, but increased significantly ($p<0.05$) after three hours. Generally, the longer the soak time, the higher the PME activity. This suggests that the cell wall permeability is a leading factor of the PME expression. The enzyme has to cross the cell wall to gain access with the substrate. This is more or less difficult depending on the state of the cell wall integrity. Aged beans will express higher PME activity than fresh beans because aged bean cell walls are typically damaged, therefore the enzyme will easily leach out to bind the substrate.

The Figure 29 gives a comparison of PME activity after the MSU soak method, the warm soak and the hot soak, relative to the non-soaked beans. The behavior varies among samples.

- Compared to non-treated beans, the MSU soak method did not significantly ($p>0.05$) change the PME activity of Seafarer "aged", N97774, NB-4 and NB-5, whereas the increased activity demonstrated in Seafarer "fresh", Mackinac, NB-1, NB-2, Arkansas and Bayside Best was statistically significant ($p<0.05$). The PME in NB-3 was significantly decreased.
- After the warm soak there was no significant change in PME compared to the non-soaked beans in Seafarer "aged", NB-5 and Arkansas, whereas the increased PME activity was significant ($p<0.05$) in Seafarer "fresh", Mackinac, N9774 and NB-1. The PME decreased significantly for NB-1, NB-2, NB-3, NB-4, and Bayside Best.
- The hot soak had no significant effect on the PME activity for Seafarer "aged", NB-1, NB-2, NB-4 and Arkansas but the increase was significant for Seafarer "fresh", N97774, Mackinac, NB-1 and NB-5, and the decrease significantly for NB-3 and Bayside Best.

For some bean samples the PME activity was better expressed by the MSU method (Seafarer "fresh", NB-1, NB-2). In Seafarer "fresh" and N97774 the higher activity was expressed by the cold soak (3h). In NB-5 the highest PME activity was expressed by the hot soak. In NB-4, none of the three soak methods (MSU soak, warm soak and hot soak) caused a significant activation of PME activity.

This difference of PME response to the soaking methods could be due to a couple of phenomena involved in the expression of the enzymatic activity: 1) the cell wall permeability which enables the enzyme to come in contact with the substrate, and 2) the enzyme thermal deactivation. The equilibrium of the two opposing phenomena determines the resultant extent of the measured enzyme activity. Further research is needed to verify the value of this hypothesis.

Table 16. Pectin methylesterase (PME) activity in dry Navy beans (non soaked) and Navy beans subjected to selected soak treatments

	Sample	No Treatment		Cold soak (21.1°C)(1h)		Cold soak (21.1°C)(3h)		Cold soak (21.1°C)(8h)		MSU soak 30/30		Warm soak (46.1°C)(3h)		Hot soak (62.8°C)(3h)		**LSD (0.05)**
		PME	SD	PME	SD	PME	SD	PME	SD	PME	SD	PME	SD	PME	SD	
Cultivars	Seafarer,"aged"	75.7	11.3	77.0	16.5	97.5	5.4	92.5	4.8	82.3	37.1	53.8	24.3	80.4	12.9	**23.8**
	Seafarer,"fresh"	17.6	2.6	63.71	12.1	27.43	3.4	72.4	7.1	109.0	12.7	127.0	21.7	61.8	13.2	**15.0**
	Mackinac	24.1	1.6	71.8	6.8	96.7	8.0	119.5	24.5	89.9	26.7	110.5	19.2	83.8	19.8	**21.9**
	N97774	27.7	6.8	34.4	7.7	60.5	3.1	45.3	5.6	29.8	10.8	124.1	5.6	83.7	24.8	**14.1**
Commercial Samples	NB-1	37.4	3.7	33.4	5.5	51.3	7.8	65.8	17.9	93.3	19.8	23.1	2.6	58.2	9.3	**14.1**
	NB-2	43.1	17.6	29.2	4.8	61.6	2.7	55.4	14.7	74.4	11.8	15.4	2.0	43.1	3.9	**12.5**
	NB-3	81.1	41.5	65.8	5.6	102.5	6.9	109.9	18.3	52.8	18.7	27.1	1.8	48.9	20	**25.5**
	NB-4	45.6	9.7	64.3	5.6	44.4	4.1	58.1	1.2	38.9	9.5	18.0	1.3	45.9	8.9	**8.3**
	NB-5	18.2	3.8	57.0	5.7	108.6	20.6	75.0	10.1	14.6	1.2	26.4	3.5	53.9	9.9	**12.3**
	Arkansas	62.1	9.9	47.3	8.3	101.8	31.0	131.2	58.7	93.1	25.7	42.0	15.1	50.9	21.3	**36.0**
	Bayside Best	53.9	2.0	39.1	7.5	77.0	9.4	81.2	7.2	66.1	0.3	22.3	0.3	36.3	5.8	**7.2**
	LSD (0.05)	**17.8**		**10.2**		**15.0**		**26.0**		**22.8**		**15**		**18.8**		

n=3

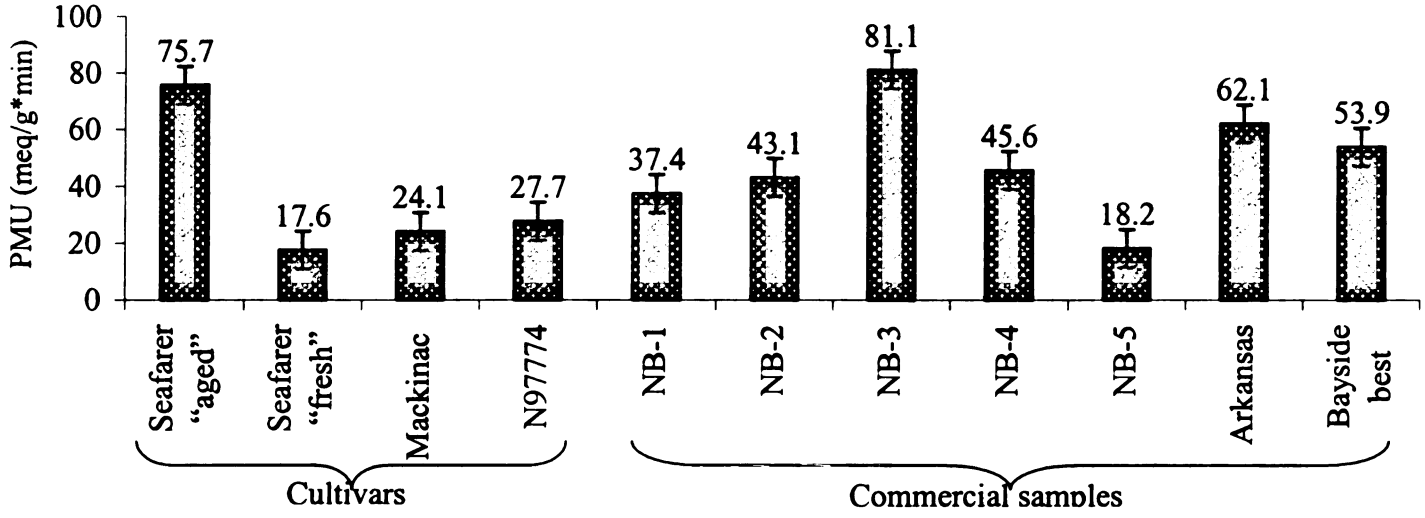


Figure 27.Pectin methylesterase activity (PME) of selected dry Navy beans (*Phaseolus vulgaris L* .), commercial samples and cultivars, prior to soak treatment

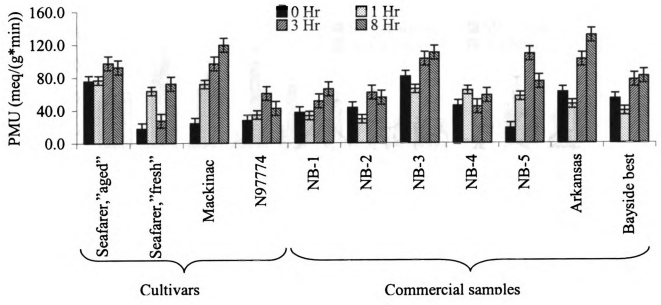


Figure 28.Effect of soaking time at 21°C (70°F) on the pectin methylesterase (PME) activity of selected Navy beans (*Phaseolus vulgaris L.*)

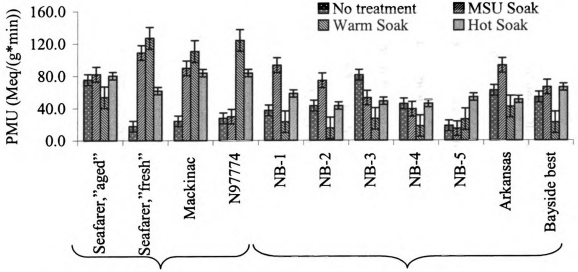


Figure 29. Effect of various soak treatments on the pectin methylesterase (PME) activity of selected Navy beans (*Phaseolus vulgaris L.*)

Effect of Soak Temperature on the Hydration, the Pectin Methylesterase Activity and the Texture of Cooked Navy Beans (*Phaseolus vulgaris L*)

Effect of Cooking Time on the Firmness of Cooked Navy Beans (*Phaseolus vulgaris L*)

Table 17 and Figure 30 show the percent water gain of selected beans soaked for three hours at various temperatures. "Hard beans" (Seafarer "aged", Arkansas, Mackinac) had numerically a lower water gain percent than "soft beans" (Seafarer "fresh"), although the difference was not statistically significant ($p>0.05$) (Garcia-Vela and Stanley 1989b; Plhak *et al* 1989; Richardson and Stanley 1991; Sambudi 1994). The starch and the proteins are responsible for the water holding capacity of the beans. The HTC development causes the starch alteration and, as a consequence, raises the temperature of gelatinization, and a protein denaturation. Both phenomena affect the water holding capacity and the bean hydration (Sefa Dedah 1979; Youssef *et al* 1982; Vindiola *et al* 1986; Hincks *et al* 1987; Hohlberg and Stanley 1987; Paredes *et al* 1988; Henteges *et al* 1991; Paredes *et al* 1991; Del Valle *et al* 1992a and 1992b; Liu *et al* 1992; Liu *et al* 1993; Garcia and Lajolo 1994; Reyes Moreno *et al* 1994; Hung *et al* 1995; Martin Cabrejas *et al* 1995).

Results demonstrated that the highest level of hydration during soaking was achieved in the temperature range 46.1-54.4°C.

The effect of the temperature on the PME activity is shown in Table 18 and Figure 31. Fresh beans are generally recognized to have a lower PME activity than "aged beans" (Mafuleka *et al* 1991). The PME activity was higher in Seafarer "aged" soaked at

21.1°C than in Seafarer "fresh". However, the differences between Arkansas and Seafarer "fresh" and between Mackinac and Seafarer "fresh", were not significant.

The soak temperature had no significant effect on the PME activity of Mackinac. Temperatures from 21.1 to 46.1°C decreased significantly the PME in Seafarer "aged" and Seafarer "fresh". In Arkansas the decrease became significant from 46.1 to 54.4°C. From 54.4 to 62.8°C the PME increased significantly in Seafarer "aged" and Arkansas, but the change in Seafarer "fresh" was insignificant. Temperatures from 62.8 to 71.1°C significantly decreased the PME in Arkansas whereas no change was observed in the other beans.

Unlike the water gain percent, the PME activity was inhibited by soaking in the temperature range 46.1-54.4°C.

The data presented in Table 19 and the Figure 32 demonstrate that the soaking temperature range 46.1-54.4°C produced beans of higher firmness.

- The shear work decreased significantly ($p<0.05$) when the cooking time was increased from 60 to 90 minutes, and also from 90 to 120 minutes.
- Compared to distilled water, the calcium (50 ppm) in cooking water significantly increased the shear work ($p<0.05$).
- The shear work was significantly higher ($p<0.05$) for beans pre-soaked at the temperature range 46.1-54.4°C than those pre-soaked at 21.1, 62.8 and 71.1°C.

However the cause-effect relationship between the PME activity and the texture of processed beans was not readily apparent. Indeed, contrary to the expectation, the response to a change of temperature of the PME measured in Seafarer "aged" (Table 18) was negatively correlated to that of firmness of cooked beans (Table 19), and this

response became more evident as the cooking time increased (r = -0.6 for 60 min, -0.7 for 90 min and -0.8 for 120 min). Therefore the hypothesis that the HTC develops via a pectin de-methylation during aging is not valid because the higher PME activity did not lead to a firmer product. This corroborates the findings of Liu *et al* (1993c) in cowpeas that, with aging time and heating temperature, there was a negative correlation (r = -0.926) between seed texture and pectin loss during soaking or heating. They suggested that the hard-to-cook defect was caused in part by reduced pectin beta-degradation during cooking.

Table 17. Percent water gain of selected Navy beans cultivars (*Phaseolus vulgaris L.*) soaked for three hours at various temperatures.

	Seafarer,"aged"		Seafarer,"fresh"		Arkansas		Mackinac		**LSD**
Soaking T° (°C)	% H_2O Gain Mean	SD	% H_2O Gain Mean	SD	% H_2O Gain Mean	SD	% H_2O Gain Mean	SD	**(0.05)**
21.1	77.5	1.6	83.0	0.5	79.2	1.2	77.8	0.2	**2.9**
46.1	85.0	0.0	93.8	0.2	78.8	0.2	81.5	0.2	**0.5**
54.4	78.8	0.2	87.0	0.5	80.8	1.2	81.8	0.2	**1.8**
62.8	77.8	0.2	84.8	0.7	77.8	0.2	78.8	1.2	**2.0**
71.1	78.7	0.5	84.0	1.4	78.0	0.0	79.0	1.9	**3.3**
LSD (0.05)	**2.0**		**2.0**		**2.0**		**2.6**		

n=2

Table 18. Pectin methylesterase (PME) activity in aged and fresh Navy beans (*Phaseolus vulgaris L.*) (Seafarer) soaked three hours at various temperatures

	Seafarer,"aged"		Seafarer,"fresh"		Arkansas		Mackinac		**LSD**
Soak Temperature (°C)	PME (PMU)	SD	PME (PMU)	SD	PME (PMU)	SD	PME (PMU)	SD	
21.1	38.32	7.72	13.41	1.49	23.71	6.66	16.12	0.85	**8.4**
46.1	9.52	1.28	7.96	0.19	15.72	4.01	14.21	2.91	**4.2**
54.4	7.69	1.2	6.66	2.47	8.03	1.46	13.76	7.12	**6.3**
62.8	17.32	2.51	6.9	0.82	21.52	4.89	13.54	2.14	**4.9**
71.1	27.99	8.71	10.68	4.96	10.39	3.2	15.13	6.11	**9.9**
LSD (0.05)	**8.5**		**4.1**		**6.9**		**7.1**		

n=3

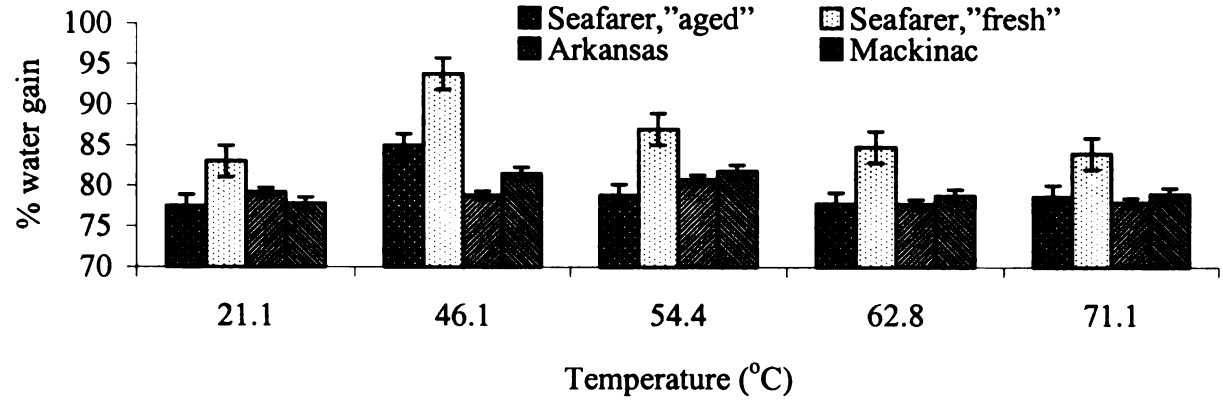


Figure 30.Percent water gain of selected Navy beans (*Phaseolus vulgaris L.*) after a three hours soaking in distilled water at selected temperatures

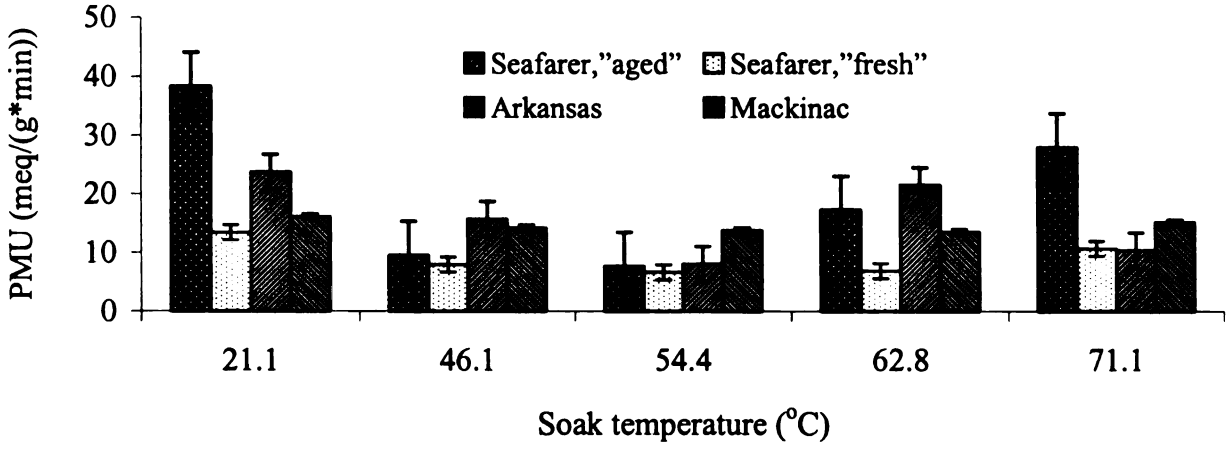


Figure 31. PME activity of selected Navy beans cultivars (*Phaseolus vulgaris L.*) after a three hours soaking in distilled water at selected temperatures

Table 19. Total Shear work on 25g sample of Navy beans (*Phaseolus vulgaris L.*) (Seafarer "aged") soaked 3 hours at various temperatures and Cooked (100°C) for Different Times

	Cooked in distilled water						Cooked in Ca^{2+}. 50 ppm						**LSD**
	60min		90min		120min		60min		90min		120min		**(0.05)**
T°	(N.mm)	SD	(N.mm)	SD	(N.mm)	SD	(N.mm)	SD	(N.mm)	SD	(N.mm)	SD	
21.1	1652	97	1392	30	1084	66	OS*	-	4824	201	4711	192	**174**
46.1	2156	142	1752	111	1397	60	OS	-	OS	-	OS	-	**155**
54.4	2700	392	1657	142	1315	55	OS	-	OS	-	OS	-	**343**
62.8	2889	70	1895	94	1304	57	OS	-	OS	-	OS	-	**106**
71.1	2610	216	1669	116	1341	39	OS	-	OS	-	OS	-	**202**
LSD (0.05)	**279**		**136**		**72**				-		-		-

n= 3 * Off-scale

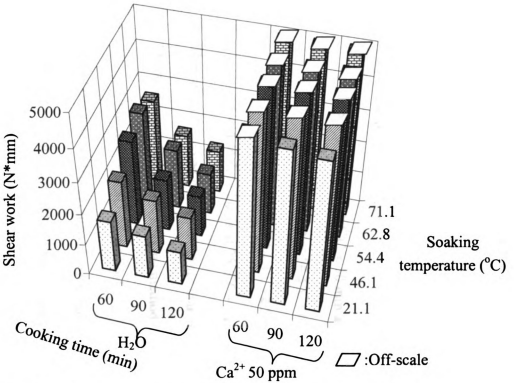


Figure 32. Effect of soaking temperature and cooking time on the firmness of cooked Navy beans (Phaseolus vulgaris L.) (Seafarer "aged")

CONCLUSION

The following conclusions from this thesis research are presented:

- HTC beans have a longer cooking time than soft beans. The calcium increases the cooking time up to a plateau whereas the phosphate decreases it up to a plateau. The existence of the plateau supports the existence of saturable binding sites in the bean cotyledons.
- HTC beans resist hydration during soaking and cooking, and require a higher shear work than soft beans. The soak weight and the wash drained weights were lower in calcium soaked than in phosphate soaked beans. The two parameters vary in the following order:

  MSU (30 min at 25.6°C + 30 min at 87.8°C)

  < ComSoak1 (3 hours at 54.5°C+ 6 min at 79.4°C),

  ComSoak2 (3 hours at 54.4°C+2.5min at 71.1°C + 3.5 min at 78.9°C)

  < Cold soak (4 hours at 25.6°C+ 5 min at 93.3°C).
- The calcium soaked beans are firmer than the phosphate soaked ones, the order of firmness depending on the soaking method:

  Cold soak < MSU < ComSoak1, 2
- The phosphate added exclusively in brine (0-200 ppm) had no significant effect on the wash drained weight and the texture of canned beans.
- The addition of calcium decreased the soak weight and increased the firmness of canned beans, but had no significant effect on the wash drained weight. This may be attributable to a net effect of decreased hydration and increased retention of solids within the bean.

- The phosphate in soaking water increased the soak weight in the Cold soak and the two ComSoak, but had no significant effect on the soak weight in the MSU soak method. It had no significant effect on the wash drained weight but decreased significantly the firmness.
- The calcium and phosphate in soaking water independently exert their effect on the texture of canned beans.
- "Aged beans" have a higher PME activity than "fresh beans". Soaking did not significantly affect the PME in aged beans. However, in the fresh beans, the longer the soaking time the higher the observed PME activity.
- The expression of the PME activity was affected by the state of the sample ("aged" versus "fresh") as previously found by Mafuleka *et al* (1991), and the soaking method (time and temperature).
- Limited activation of PME was observed in the temperature range 46.1-54.4°C (115-130°F) and the texture was firmer than alternative temperatures.
- There is a negative correlation between the response of the PME activity to a change of soaking temperature and that of firmness of cooked beans. This corroborates the conclusion of other researchers that the PME activity has a limited role in the development of the HTC phenomenon.

The hypotheses of this research are evaluated as follows:

- Ho_1 (there is a difference in cooking time, soak weight and drained weight among Navy bean samples of diverse production or crop year): accepted because it was demonstrated that there was a large variability of cooking time among bean samples.

- Ho_2 (the calcium and the phosphate ions in cooking /soaking water or in brine affect the cooking time and the firmness of beans): accepted because clear evidence was presented to demonstrate the firming effect of calcium ions and the softening effect of phosphate ions, and their effect on the cooking time.
- Ho_3 (the soaking methods affect differently the texture of beans): accepted because it was demonstrated that the cold soak and the MSU soak method lead to a significantly firmer product than the two commercial soak methods.
- Ho_4 (the interaction between calcium and phosphate ions affects the texture of canned beans): rejected because it was demonstrated that calcium and phosphate ions exert independently of each other, respectively their firming and softening effect.
- Ho_5 (the soaking treatment conditions (time and temperature) affect the hydration, the pectin methylesterase activity and the texture of beans): accepted because it was demonstrated that the different soak treatment resulted in significantly different bean hydration and firmness.
- Ho_6: (the pectin methylesterase activity affects the texture of processed beans): accepted because a negative correlation was found between the PME activity and the texture of processed beans. However, further research is needed to understand the exact role of the PME activity in the development of the HTC phenomenon.

Finally, the implications of this research for improving the welfare of rural and urban citizens of Rwanda are numerous and include:

- The perspectives of using phosphates ions in cooking and canning. Very hard water should be avoided for improving the cookability of beans. In addition the

MSU soaking method should be recommended to the food industry as it presents the advantages of rapidity (30 minutes soaking followed by 30 minutes blanching) and improved texture, compared to the others methods. The decreased cooking time for beans soaked and cooked in solutions of phosphate will result in decreased energy consumption through decreased firewood use.

- The pin drop cooker testing protocol may result in assessment of hard-to-cook beans in Rwanda and enables recommendations for phosphate treatments as needed.
- Canning procedures may be considered for enhanced preservation and convenience for high nutritive value for urbanized residents.
- Very close attention to regionally adapted varieties and appropriate storage conditions for dry beans is essential in Rwanda because of the diverse impact on cooking time attributable to variety and storage time demonstrated in this research.

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