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EFFECTS OF PROCESSING FACTORS ON THE QUALITY CHARACTERISTICS OF SOAKED AND PROCESSED NAVY BEANS

By

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ABSTRACT

EFFECTS OF PROCESSING FACTORS ON THE QUALITY CHARACTERISTICS OF SOAKED AND PROCESSED NAVY BEANS

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Effects of (1) the divalent ions, Ca^{+2} and Mg^{+2} (2) the additive, sodium hexametaphosphate (NaHMP), and (3) the combination of Ca^{+2} and NaHMP on qualities of soaked and processed beans were studied.

Effects of selected enzymes, α-amylase, gluco-amylase, pectinase, cellulase and protease on qualities of soaked beans were performed by soaking beans with and without seedcoats at 20, 40, 60 and 80°C for 5 hours. Activities of the enzymes in reducing whole ground bean flour viscosities were carried out using the Brabender Visco-Amylograph with temperature programmed from 45-95°C.

The divalent ions decreased drained weight and firmed the beans. NaHMP accelerated water uptake, softened soaked beans and increased soluble solids leaching. The combination treatment decreased drained weight and showed no Ca^{+2} effect on shear resistance.

The enzymes were ineffective on water uptake and shear resistance. The temperature and seedcoat affected

these two characteristics. α-Amylase and pectinase reduced the viscosity; while glucoamylase, cellulase and protease had no effects.

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INTRODUCTION

Edible dry beans are an important source of protein, having nutritional values higher than the cereal grains (Anonymous, 1975) but lower than the animal proteins. However, supplemented by a relatively small amount of methionine, the legumes may provide the most efficient and economical medium for the conversion of nitrogen into nutritional human food (Rockland et al., 1974). In addition, they also provide calories, minerals and vitamins to the diet (de Moraes and Angelucci, 1971; Rockland et al., 1977b).

Raw, dry legumes contain a variety of undesirable components, most of which are eliminated during soaking and cooking. The preparation of dry beans generally involves hydration in water to a moisture content of between 53 and 57%, followed by extended cooking. The wide spread utilization of dry legumes depends on proper handling, storage, and processing (Johnson, 1965; Thompson et al., 1962).

Information on the chemical composition of specific tissues,the structure of cells, and the localization of chemical constituents in the cells of the mature navy bean (Phaseolus vulgaris L.) seed is a prerequisite for providing an explanation of physical and chemical changes

in bean tissue which occur during mechanical, thermal, chemical and enzymatic treatments.

According to the report of Powrie et al. (1960), the seedcoats, cotyledons and embryonic axes constitute 7.7%, 90.5% and 1.8% respectively of the dry weight of mature navy beans. The two cotyledons are the most important components of the seeds by weight and volume. On dry weight basis, the cotyledons contain 27.5% protein, 1.65% ether extractables and 3.5% ash. Moreover, the starch content of the cotyledons is 39.3%. The protein content of the seedcoat is 4.8%, while the outer portions of the seedcoat contain 0.4% ether extractable wax-like material. The ash content of the seedcoat is 8.44%. The hypocotyl and epicotyl constitute the axis of the bean embryo; the embryonic axes contain 45.6% protein, 3.11% ether extractables and 3.58% ash.

The textural characteristics and nutritive value of processed seeds presumably are influenced to a large extent by the size and shape of cells, dimensions of cell walls, and the localization of chemical constituents in the cotyledons. The outermost layer of the cotyledon consists of epidermal cells. The inner and outer epidermal cells have been designated as those along the flat and curved surfaces of the cotyledon. From a three dimensional viewpoint, the inner epidermal cells are elongated cubes with the long axes in the transverse direction, while the outer epidermal cells are somewhat cubical. The cell contents of the epidermis appear

granular, containing protein but no starch. The layer of cells inward from the outer epidermis is the hypodermis with cells larger than the outer epidermal cells and elliptical in shape. The cell content appears to be a granular matrix containing tyrosine.

Parenchyma cells and vascular bundles constitute the remaining tissue of cotyledon. The parenchyma cells near the outer hypodermis are somewhat smaller than the remainder of the parenchyma cells. The thick walls in the longitudinal section of parenchyma cells are responsible for the rigidity of the soaked beans. In each parenchyma cell , starch granules are imbedded in a matrix composed chiefly of tyrosine and cysteine containing proteins. With breakdown of starch granules, the intact, rigid materials possess cavities which vary in size. Each cavity is enveloped by proteinaceous matter. The shapes of the liberated granules vary from round to elliptical and possess smooth surfaces.

The epidermal and hypodermal cells possess no secondary walls. However, in the parenchyma cells, the secondary walls are observed to be very thick compared to the primary walls and contain numerous small cavities called simple pits. Each pit is opposite another one in the adjacent secondary wall. The presence of pits in the secondary wall facilitates the diffusion of water into the protoplasm during the soaking period.

Dry beans have been traditionally soaked overnight (12-16 hours) in cold water. This initial soaking

treatment ensures uniform expansion and tenderization during subsequent thermal processing, increases product yield, and improves cleaning of the beans. Several methods have been proposed to improve soaking procedures by accelerating water uptake and tenderizing dry beans. These include heat treatment (Gloyer, 1928; Morris et al., 1950; Dawson et al., 1952), vacuum treatment (Hoff and Nelson, 1965), the use of additives (Reeve, 1947; Rockland, 1963; Rockland and Karl, 1966; Rockland and Metzler, 1967), and irradiation (Bedford, 1971). Several factors have been shown to influence water uptake. These include the age and composition of dry beans, storage conditions, moisture contents and production condition factors (Bedford, 1971). Pectic substances, hemicellulose and protein are functional components in absorbing water in plants (Ott and Ball, 1943). Smith and Nash (1961) concluded that the seedcoat is the principle factor controlling the rate of water uptake in soy beans. Hamad and Powers (1965) found rates of water imbibition were inversely related to the pectic content of dry peas and beans. Uebersax (1972) reported water uptake values decreased with high temperature and high humidity storage. Burr and Kon (1967) reported decrease in water absorption rate for sanilac beans with increased bean moisture content and storage time. Nordstrom and Sistrunk (1979) reported low original moisture levels before soaking resulted in high hydration ratios. Acidity of soak water reduced the rate of water

uptake (Snyder, 1936). Luh et al. (1975) postulated that at low pH, starch and protein swelling are inhibited, causing a reduction in hygroscopicity. Cholinesterase activity, phosphorous content and phytin content were suggested to have some influence on the imbibition and tenderness of rehydrated dry beans (Andus, 1955; Fowler, 1956, 1957). The effect of soak water additives have also been studied. Such additives include sodium bicarbonate (Greenwood, 1935), hexametaphosphate (HMP), sulfite, oxalic acid, ammonium oxalate and hydrochloric acid (Reeve, 1947), and ethylene diamine tetracetic acid (EDTA) (Elbert, 1961; Luh et al., 1975). In general, results of these studies have shown that HMP increased the rate of imbibition, by serving as a chelating agent for calcium ion, solubilizing the bean cotyledon proteins as well as aiding in dissociating metal-salt-protein complexes which could interfere with water absorption (Holmquist et al., 1948; Rockland, 1963). EDTA and the mixture of alkaline carbonates increased water uptake apparently by softening the seedcoat. Sodium chloride may or may not increase water uptake depending on the quality of dry beans (Hoff and Nelson, 1965).

Most commercial canners use some type of heat treatment during soaking. Mattson (1946) stressed that heat was needed to promote precipitation of calcium and magnesium by phytin. According to Mattson, precipitation prevented divalent ions from interacting with pectin to form tough, pectin-metal ion complexes. Morris and

Seifert (1961) reported, however, that heat inactivated the enzymes phytase and pectinesterase. If these enzymes were allowed to act, phytin would be dephosphorylated and pectin would be demethylated causing a shift of divalent ions to form the tough pectin-metal ion complexes (Matz, 1962).

Cooking of dry beans is necessary to develop an acceptable flavor and texture, to destroy toxic material and to maximize the nutrient availability of bean protein (Kakade and Evans, 1963). The cooking rate for intact dry lima beans was estimated with an automatic recording Lee-Kramer shear press (Binder and Rockland, 1964). The shear peak is related to the shearing strength of the sample or cohesiveness as defined by Szczesniak (1963). Burr et al. (1968) studied the effect of storage temperature and time and moisture content on the cooking rate of dry beans. They concluded that the cooking quality of beans with a moisture content of 10% or less is not greatly affected by storage temperature and time. At moisture levels above 10%, bean cookability deteriorated in good correlation with increased storage temperature and time. Bean processors often find that the heat process required to sterilized canned beans is insufficient to make them tender (Bigelow and Fitzgerald, 1927). Several solutions have been proposed to resolve the hard to cook phenomenon of dry beans. Such approaches have included the development of precooked hydrated beans

(Esselen and Davis, 1942; Feldberg et al., 1956), quick cooking dry beans (Rockland and Metzler, 1967), and heat treated beans (Molina et al., 1976). The method used to prepare quick-cooking beans consists of (1) loosening the seedcoats by vacuum infiltration in a solution containing NaCl, $Na_5P_3O_{10}$, $NaHCO_3$ and Na_2CO_3; (2) soaking the beans in these same salt solutions; (3) rinsing; and (4) drying, cooking or freezing the beans depending on their ultimate utilization. The resulting product cooks in 15 minutes or less. Rockland and Jones (1974) indicated that this process did not affect the structure and appearance of the beans when compared with untreated samples. Rockland et al. (1977a) reported that the purified, free, dispersed lima bean starch granules deformed and gelatinized in pure water within the range of about 71-79°C. In a dilute mixed salt solution which was employed to produce quick cooking beans, the starch dispersions gelatinized within the temperature range of about 79-85°C. The increase in extracellular gelatinization temperature of lima bean starch granules in the mixed salt solution was consistent with the effect of salt solution on intracellular bean starch gelatinization observed by Hahn et al. (1977). Varriano-Marston and De Omana (1979) studied the effect of sodium salt on the chemical composition and morphology of quick cooking black beans. By means of atomic absorption and flame emission spectrophotometry, light

and scanning electron microscopy, and x-ray analysis, these researchers reported that the amount of sodium in the soak water did not significantly affect the amount of water absorbed by the beans; the pH was the critical determinant. However, the sodium salts affect the mineral content as well as the amount of pectic substances solubilized from the beans during the soaking and processing periods. These researchers suggested that the mechanism for the quick cooking of black beans presoaked in sodium salt solutions prior to cooking was an ion exchange and chelation mechanism. Ions which stabilized the structure of intercellular pectic substances were replaced by sodium ions or were removed by chelation. The result was a solubilization of pectic substances during soaking and cooking. The cooking process was thought to further facilitate the dissociation of intercellular cement by disruption of hydrogen bonding. The similarities between the presoaking treatments of black beans in $Na_5P_3O_{10}$ and combined salt solutions with respect to mineral losses, pectic substances solubilized, and cell separation, indicated that the $Na_5P_3O_{10}$ treatment may be a satisfactory alternative in the production of quick cooking black beans.

Color is another factor affecting the quality of canned dry beans. Addition of calcium chloride or citric acid to brine during canning improved the color of dry lima beans (Luh et al., 1975). The beans treated with calcium chloride had higher Rd values than the

control samples. Acidification of the brine with citric acid at 0.25% and 0.50% levels decreased the formation of gray color compounds. The lowering of pH of the brine improved color by increasing dissociation of metallic copper sulfite complexes.

The use of enzymes to improve the quality of canned beans and peas has been studied by Powers et al. (1961). Amylolytic and pectolytic enzymes were applied to reduce the gelling of canned black eyed peas and pinto beans. Insufficient catalytic activity made commercial use of the enzymes impractical. However enough hydrolytic activity did result to establish that both starch and pectin fractions were involved in gelling in canned peas and beans. α-Amylase (α-1,4-glucan 4-glucanohydrolase, EC 3.2.1.1) is an endosplitting enzyme which hydrolyzes the α-1,4-glycosidic bonds of the substrate in a random fashion. Calcium ion is necessary to keep the enzyme active and stable. The pH-reaction rate profile for the α-amylase is a bell shaped curve with the optimum activity varying from pH 4.8~7.0 depending upon the enzyme sources. Glucoamylase (α-1, 4-glucan glucohydrase, EC 3.2.1.3) is an exosplitting enzyme and removes successive glucose units from the non-reducing ends of the substrate chains. The action of the enzyme on a chain decreases markedly when an α-1, 6 linkage is encountered as in amylopectin and glycogen. The enzyme also hydrolyzes α-1,6 and α-1,3 linkages but more slowly than α-1,4 linkages are hydrolyzed. The

optimum pH is from 4.0 to 4.4 at 25°C. The pectic enzymes are generally classified as pectinesterases, polygalacturonases and pectate lyases dependent on the mode of degradation of the pectic substances. Pectinesterase (pectin pectyl-hydrolase EC 3.1.1.11) removes methoxyl groups from methylated pectic substances (pectin) and therefore belongs to the subdivision of enzymes which hydrolyze carboxylic acid esters. The polygalacturonases (poly-α-1,4-galacturonide glycanohydrolase, EC 3.2.1.15) hydrolyze glycosidic linkages in pectic substances by the use of water. The pH optima for most of the polymethylgalacturonase and polygalacturonases are in the range of 4.5 to 6.0. The pectate lyases (poly-α-1,4-D-galacturonide lyases, EC 4.2.99.3) split the glycosidic bond by trans elimination of hydrogen from the C-4 and C-5 positions of the aglycone portion of the substrate. A requirement for calcium ions is a characteristic of the trans-eliminases. The enzymes which hydrolyze cellulose and its derivatives can be divided into three groups (King and Vessal, 1969). C_1, an enzyme whose action is not well defined, is required for hydrolysis of highly oriented solid cellulose by the β-1,4 glucanases and has been referred to as "hydrogen bondase." There is no evidence that it hydrolyzes glycosidic bonds. The β-1,4 glucanases are of two types, the exo-β-1,4 and the endo β-1,4 glucanases. The β-glucosidases act most specifically on the small molecular products. The

β-glucosidases and exo-β-1,4 glucanases have in common the substrates cellobiose to cellohexaose. The β-glucosidase will hydrolyze cellobiose much more rapidly than cellohexaose while the reverse is true for the exo-β-1,4 glucanoses. Homogeneous Myrothecium verrucaria cellulase has a molecular weight of 63,000; a pH optimum of 5.5 to 6.0. The enzyme is inhibited by heavy metal ions, by sulfhydryl reagents, by oxidizing and reducing agents, and by glucoses. The proteolytic enzymes of fungi have usually been classified on the basis of their pH optima. The group named "acid protease" indicates that the pH optima of the enzymes are at a low pH and does not indicate the groups involved in the active site. A large number of microbial proteases belong in this group (Whitaker, 1972; Reed, 1975).

The purpose of this study was (1) to evaluate the firming effects of calcium and magnesium ions during soaking and processing of dry navy beans, (2) to evaluate the effect of NaHMP and combination of NaHMP and calcium ions on the quality of canned dry navy beans, and (3) to study selected enzymatic soak treatments on the softening of whole beans with and without seedcoats, as well as these enzymatic effects on the viscosity of whole ground bean flour during gelatinization.

MATERIALS AND METHODS

Raw Material

Samples of the 1977 harvest of Michigan-grown navy beans were obtained from B and W Cooperative, Breckenridge, MI. All beans were held at 20°C (68°F) until soaked and processed or milled into whole flour. The initial moisture content was approximately 13.6%. Whole beans and bean flour were used in the soak water and process brine additive studies and gelatinization experiments, respectively. Enzyme treatment soak studies were performed on whole beans with and without seedcoats.

Soak Water and Process Brine

Chemicals used in soak water and process brine additive studies were analytical reagent grade $CaCl_2$, $MgCl_2 \cdot 6H_2O$ (Fisher Scientific Company) and sodium hexametaphosphate (NaHMP) (Monsanto Company). All soak water was prepared in one gallon polyethylene containers and held for approximately 16 hours prior to use.

Five types of enzymes were used in the enzyme soak and gelatinization studies: α-amylase, glucoamylase, pectinase, cellulase and protease. The source and activity

of each enzyme are listed in Table 1. The enzyme solutions were prepared in distilled water adjusted with hydrochloric acid or sodium hydroxide to the specified stable pH. Each enzyme solution and its corresponding control solution contained 50 ppm calcium ion.

Study Design

Divalent ions, Ca^{+2} and Mg^{+2} treatments

Soak water and process brine cation levels were 0, 50, and 100 ppm.

Soaking -- Beans were soaked in each level of divalent ions. Water uptake and shear resistance of soaked beans were determined. Ash and calcium content determination were conducted on final soak water and beans soaked in different calcium ion levels.

Processing -- Soaked beans were processed in all possible level combinations of soak water and process brine. Drained weight, Hunter Lab color and shear resistance of processed beans were determined. Ash and calcium content determinations were conducted on final process brine and beans processed in different calcium ion levels.

NaHMP and combination of calcium ion and NaHMP treatments

The soak water and process brine additive levels are listed in Table 2.

Table 1. Source and activity of selected enzymes

Enzyme Type	Source	Activity
α-Amylase	Bacterial type II-4, Lot 86C-0071. Sigma Chemical Co.	1270 units/mg solid; one unit will liberate 1.0 mg of maltose from starch in 3 minutes at pH 6.9, 20°C
Glucoamylase	Rhizopus sp. mold, Grade II Lot 127C-0505. Sigma Chemical Co.	2200 units/gram solid; one unit will liberate 1.0 mg of glucose from soluble starch in 3 min at pH 4.5, 55°C
Pectinase	Fungal Tech Powder. Nutritional Biochemicals Corp.	partially purified concentrate; 1 gm reduce viscosity of 1500 gm of pectin by 50% in 15 min at pH 3.5, 40°C
Cellulase	Cellulase 4000, Control No. F9985. Miles Chemical Co.	
Protease	Fungal protease 31000, Control No. 1050. Miles Chemical Co.	

Table 2. Levels of NaHMP and combination of Ca^{+2} and NaHMP in soak water and process brine

Additives	Levels
NaHMP (%)	0, 0.25, 0.50, 0.75, 1.0, 1.5
Ca^{+2}(ppm)+NaHMP (%)	0+0, 50+0, 100+0, 0+1.0, 50+1.0 100+1.0

Soaking -- Beans were soaked in each level of soak water additives. The dependent measures included water uptake, shear resistance of soaked beans, as well as total nitrogen (%) in soaked beans and final soak water.

Processing -- Soaked beans were processed in the same level of brine additive as that of soak water. The dependent measures included drained weight, Hunter Lab color, and shear resistance of processed beans, as well as total nitrogen (%) in processed beans and final process brine.

Selected enzyme treatments

Soaking -- Soak studies with 5 selected enzymes were conducted on whole beans with and without seedcoats. The enzyme concentration and incubation pH are listed in Table 3. The water uptake and shear resistance of soaked beans were determined on enzyme treated and control samples.

Table 3. Concentration and incubation pH of selected enzymes

Enzyme Type	Concentration	pH
α-Amylase	127 unit/ml	6.9
Glucoamylase	0.22 unit/ml	4.5
Pectinase	0.1 mg/ml	3.5
Cellulase	0.1 mg/ml	6.5
Protease	0.1 mg/ml	3.5

Gelatinization -- Whole bean flour was used in this experiment. Concentration and incubation pH of each enzyme were identical to that in Table 3 except for α-amylase. The concentration of 12.7 unit/ml was used. The activity of each enzyme in reducing the viscosity of bean flour during gelatinization was measured by a Visco-Amylograph.

Soaking and Processing Procedure

One hundred grams of beans were soaked in solutions containing various levels of divalent ions, NaHMP or combination of calcium ion and NaHMP shown in the study design. One part of dry beans was immersed in four parts of soaking water in a 600 ml beaker. Beans were initially soaked 30 min at 20°C (68°F) and then were transferred to a steam kettle with water maintained at 87.8°C (190°F) for an additional 30 minute soak. The

temperature variation within the beaker was ± 1°C. Soaked beans of 100 g dry solids were drained and filled into 303x406 enameled cans, covered with 93.3°C (200°F) brine, double sealed and retorted at 115.6°C (240°F) for 45 minutes. Cans were cooled to 32.2°C (90°F) to 37.7°C (100°F) in cold running water and air dried. Cans were stored at 20°C (68°F) for at least two months to ensure complete equilibration prior to analyses of chemical and quality characteristics.

Enzyme Soak and Gelatinization Studies

Soak Studies

Whole beans with and without seedcoats were used in these studies. Beans were previously soaked in distilled water for 30 minutes to facilitate the removal of seedcoats. Fourty-five grams each of whole beans with seedcoats intact and with seedcoats removed were placed into beakers containing 180 ml of enzyme solutions or control solutions. The beans were soaked and incubated in the solutions for 5 hours at 20°C, 40°C, 60°C, and 80°C in a temperature controlled water bath. After soaking, the beans were drained on an 8 inch No. 8 mesh sieve, and water uptake and shear resistance were measured.

Gelatinization

The Brabender Visco-Amylograph (No. 888, Type Vavi, Volt 110, Amp 5, Cy. 60, C. W. Brabender Instruments, Inc., South Hackensack, N.J.) was used in this experiment. The Visco-Amylograph peak (viscosity) was obtained using the procedure of AACC (1962) with the modification that 15.03% (w/v) of bean flour was used and the temperature programmed from 45°C to 95°C at the rate of 1.5°C/minute. These modifications were required due to the consistency and gelling characteristics of the bean flour.

Analytical Methods

Moisture

Initial moisture content of dry beans were measured by a Motomco Moisture Meter (Model 919, Motomco Inc., Clark, N.J.) in conjunction with calibrated moisture chart no. B-5. All measurements were obtained following the procedure recommended by the manufacturer. Moisture contents of soaked and processed beans or bean flour were determined according to the AOAC (1975) air dried oven method. Twenty grams of well mixed soaked or processed bean flour were placed in a tared aluminum moisture dish. The dish and its contents were dried to a constant weight (overnight) in a forced convection oven, then transferred to a $CaSO_4$ desiccator and weighed soon after reaching room temperature. Residues were determined as total solids and loss in weight as moisture content.

Ash

Ash content was determined according to AOAC (1975) on beans soaked and processed in calcium ion solutions and on the final soak water and brine. Approximately 500 mg of well mixed dry samples were weighed into previously ashed and tared Coors crucibles. Samples were pre-ashed over a Fisher burner, incinerated in a muffle furnance set at 525°C until light gray ash was obtained (overnight). Residues were cooled over $CaSO_4$ in desiccators and weighed soon after reaching room temperature. Ash content was determined using the following formula:

$$\text{Ash (\%)} = \frac{\text{Ash Residue (g)}}{\text{Sample Weight (g)}} \times 100$$

Calcium content

Calcium content was determined on soaked beans, soak water, processed beans and brine using Perkin Elmer Model 303 Atomic Absorption Spectrophotometer (Norwalk, Connecticut) at wave-length 211 nm. Ashed samples were digested with 6N nitric acid for about 45 minutes, then diluted to 200 ml with 1N nitric acid. Lanthanum chloride (0.1% w/v) was added in sample solutions to avoid interference caused by phosphate, sulfate, silicon or alumin. Standard calcium (Sargent Welsh) for mineral analysis was diluted with 1N nitric acid to the linear working range of the instrument (5ug/ml Ca^{+2}). All the acid diluted standards contained equal levels of lanthanum. Data were reported on a dry weight basis.

Water uptake

The water uptake of soaked beans was measured by weight gain during the soak period. Beakers containing soaked beans were cooled under running tap water for 5 minutes prior to draining. The soaked beans were emptied onto an eight inch diameter, U.S. standard No. 8 sieve at a 30 degree incline and drained for 2 minutes. Beans plus screen were weighed. Soak water uptake was determined as total weight of soaked beans. The soaked beans treated with the enzymes were emptied onto the screen, and the water uptake was determined without cooling.

Drained weight

Processed beans were emptied onto an eight inch diameter, U.S. standard No. 8 screen and distributed evenly. The screen and contents were rinsed in 20°C (68°F) water for about 15 seconds to remove the gelling material. Beans were immersed and screen agitated in a slow swirling motion. The screen was drained at an angle for 2 minutes. Beans and tared screen were weighed for determination of washed drained weight. Canned beans treated with calcium or magnesium were emptied onto the screen, and the drained weight was determined without rinsing.

Color

Objectively reflectance color measures were determined by a Hunter Lab Model D_{25} Color and Color Difference

Meter (Hunter Associates, Fairfax, Virginia). The instrument was standardized using a standard white tile (L=94.5, a_L=-0.6, b_L=+0.4). Processed beans of 100 g were placed in an optically pure glass sample dish and covered to shield interferring light from activating photo cells. Coordinate values (L, $\pm a_L$, $\pm b_L$) were recorded for each replicate and total color differences from the standard (ΔE) were calculated by $\Delta E = \sqrt{(\Delta L)^2+(\Delta a_L)^2+(\Delta b_L)^2}$.

Shear resistance

The Lee-Kramer Recording Shear Press, Model TR-1 (Food Technology Coorporation, Reston, Virginia) was used for all shear resistance measurements. The 3,000 pound test ring and No. C-15 standard shear compression cell were used. The rate of shear compression blade travel was standardized to 0.52 cm/sec. A sample size of 50 g of soaked or 100 g of processed beans was placed in the cell, evenly distributed, and sheared. The entire cell was cleaned and rinsed between each measurement.

Soaked beans -- Shear resistance was measured directly after the soak period using range 50. Shear resistance was reported as peak pound force/50 g of soaked beans.

Processed beans -- Shear resistance was measured on rinsed or non-rinsed processed beans using range 5. Shear resistance was reported as peak pound force/100 g of processed beans.

Total nitrogen

Analyses were conducted on beans soaked and processed in different levels of NaHMP and the combination of calcium ion and NaHMP. The semi-micro Kjeldahl method for determination of total nitrogen was used according to AACC (1962). A 200-300 mg dry sample was digested with concentrated H_2SO_4 (12N) as oxidant with the addition of K_2SO_4 to elevate the temperature in the presence of a selenium catalyst. The clear sample solution, neutralized with concentrated NaOH (10N), was steam distilled into 10 ml of H_3BO_3--indicator solution until the final volume 40 ml was collected. The distillate was titrated with 0.1774N H_2SO_4. The total nitrogen, reported as % was calculated by the formula:

$$N\ (\%) = \{[\text{Eq. wt of N (14)} \times \text{ml of } H_2SO_4 \text{ used} \times \text{Normality of } H_2SO_4] \times 100\} / \text{Sample wt in mg}$$

Statistical Analysis

All dependent measures for the soaking and processing studies to determine the effect of soak water and process brine additives were analyzed using one way or two way ANOVA, fixed effects with replications (Neter and Wasserman, 1974). The selected enzyme soaking studies were analyzed using multiple factor (enzyme type, temperature, seedcoat, and group) ANOVA, fixed effects with replications. The Tukey method was utilized for multiple pairwise comparisons of means (Tukey, 1953). Like letters were used to denote no significant

differences among means. Linear regressions were calculated for selected dependent measures in soak water and process brine additive studies using the method of least squares. All correlations were calculated as the Pearson Product - Moment correlation coefficient (Sokal and Rohlf,1969). Coefficients of variation (CV) were calculated to express the standard deviation for each measure as a percent of the mean (Little and Hills, 1972).

RESULTS AND DISCUSSION

Effects of Divalent Ions, Ca^{+2} and Mg^{+2} on the Quality Characteristics of Soaked and Processed Navy Beans

Calcium ion

The mean values of calcium ion level effects on processed navy beans are reported in Table 4. The summary of statistical analyses of these data is presented in Table 5.

Soaked beans -- The calcium ion levels did not significantly ($P=0.05$) affect water uptake during soaking. The shear resistance of soaked beans increased significantly ($P=0.01$) as the soak water calcium ion levels increased. Results of analyses by atomic absorption spectrophotometry showed significant differences ($P=0.01$) in calcium content in the final soak water, but no significant ($P=0.05$) difference in calcium content in soaked beans among soak water calcium ion levels. This indicated limited calcium ions diffused into the beans from the soak water during the soaking peroid. However, this amount of calcium was sufficient enough to result in significant firming of soaked beans.

Table 4. Mean values of calcium ion level effects on the quality characteristics of soaked and processed beans

Soaked Beans				
Ca^{+2} (ppm)	Measures			
Soak water	Water uptake (g)	Shear resistance (lbs/50g)	Ca^{+2} (mg/10g) Soak water	Ca^{+2} (mg/10g) Soaked beans
0	229.69 a	862.5 a	14.84 a	16.40 a
50	226.95 a	918.75 a a	20.00 a	16.39 a
100	226.05 a	956.25 b b	23.02 b	16.64 a
CV(%)	4.06	1.96	9.46	5.25

Processed beans									
		Measures							
Ca^{+2} (ppm)				Hunter				Ca^{+2} (mg/10g)	
Soak water	Brine	Drained wt.	Shear resistance (lbs/100g)	L	$+a_L$	$+b_L$	ΔE	Brine	Beans
0	0	430.26	57.38	47.52	2.42	11.94	50.66	17.86	15.37
0	50	426.00	60.00	47.38	2.68	13.06	49.06	20.84	16.21
0	100	379.14	80.93	48.04	3.00	12.81	49.29	16.89	17.28
50	0	404.70	68.07	47.24	2.64	11.98	50.48	16.39	17.05
50	50	387.66	82.13	46.59	2.73	11.97	50.26	17.05	17.96
50	100	374.88	90.94	47.33	3.08	12.88	50.05	16.63	19.17
100	0	394.76	73.50	47.93	2.89	12.94	49.02	18.51	16.41
100	50	356.77	96.04	47.33	3.03	13.05	50.01	17.30	18.37
100	100	342.22	106.63	47.15	3.48	13.41	50.39	18.12	19.93
CV(%)		4.51	6.83	1.43	12.26	4.06	1.44	3.67	14.92

Table 5. Statistical summary of calcium ion level effects

Measures	Source of variation	D.F.	M.S.
Soaked beans			
Water uptake	Treatment	2	262.37
	Error	30	85.35
Shear resistance	Treatment	2	4452.89**
	Error	3	318.74
Soak water Ca^{+2}	Treatment	2	34.17*
	Error	3	3.33
Soaked beans Ca^{+2}	Treatment	2	3.74×10^{-2}
	Error	3	7.48×10^{-1}
Processed beans			
Drained wt.	Treatment	8	1715.81**
	Error	9	307.60
Shear resistance	Treatment	8	546.46**
	Error	9	29.51
Hunter L	Treatment	8	3.65×10^{-1}
	Error	9	5.69×10^{-1}
Hunter $+a_L$	Treatment	8	1.94×10^{-1}
	Error	9	1.25×10^{-1}
Hunter $+b_L$	Treatment	8	2.66×10^{-1}*
	Error	9	9.63×10^{-2}
ΔE	Treatment	8	5.07×10^{-1}
	Error	9	5.19×10^{-1}
Brine Ca^{+2}	Treatment	8	3.69**
	Error	9	4.24×10^{-1}
Beans Ca^{+2}	Treatment	8	44.18**
	Error	9	6.84

**denotes significant F, P=0.01

*denotes significant F, P=0.05

Processed beans -- The ANOVA showed significant ($P=0.01$) differences in drained weight, shear resistance, and processed bean and brine calcium contents among different calcium ion levels. The plots of drained weight and shear resistance on the calcium content of processed beans indicated apparent linear relationships. Therefore, linear regression analyses were performed to evaluate these relationships. Table 6 contains the linear regression equations and simple correlation coefficients for drained weight and shear resistance. These significant ($P=0.01$) correlation coefficients indicated that the drained weight and shear resistance of processed beans were associated with the calcium content of processed beans. The calcium content of processed beans directly increased by the addition of calcium ions to soak water and process brine. These results were in agreement with the theory that pectin and calcium ion retarded water uptake and increased firmness through the formation of tough pectin-metal complexes (Matz, 1962).

Table 6. Drained weight and shear resistance regression on processed bean calcium content

Dependent measures	Regression	$r_{Ca^{+2}}$	$SEr_{Ca^{+2}}$
Drained weight	Y=705.26-18.07X	-0.9065**	0.1595
Shear resistance	Y=-107.23+10.66X	0.9477**	0.1206

**denotes significant r, P=0.01

Luh et al (1975) reported that addition of calcium chloride affected the Rd values of processed dry lima beans. However the color measurements Hunter L,a,b and ΔE were not significantly (P=0.05) affected by calcium ion in this experiment. These may be due to the different genotypic pigments between the seedcoats of lima and navy beans.

Magnesium ion

The mean values of magnesium ion level effects are presented in Table 7. The summary of statistical analyses of these data is reported in Table 8.

Soaked beans -- The non-significant (P=0.05) difference in water uptake and the significant (P=0.01) difference in shear resistance of soaked beans among soak water magnesium ion levels were consistent with calcium ion treatment.

Processed beans -- Drained weight and shear resistance of processed beans were significantly (P=0.01) affected by soak water and process brine magnesium ion levels. As the magnesium ion levels increased, the drained weight decreased, but the shear resistance increased. The color measurements, Hunter L,a,b were significantly (P=0.05) affected by magnesium ion levels. Hunter L decreased, Hunter $+a_L$ and $+b_L$ increased as the soak water and process brine magnesium ion levels increased.

Table 7. Mean values of magnesium ion level effects on the quality characteristics of soaked and processed beans

Soaked beans		
Mg^{+2} (ppm)	Measures	
Soak water	Water uptake (g)	Shear resistance (lbs/50g)
0	230.51 a	862.50 a
50	234.27 a	915.00 a a
100	225.65 a	993.75 b b
CV(%)	5.56	1.66

Processed beans							
Mg^{+2} (ppm)		Measures					
Soak water	Brine	Drained wt. (g)	Shear resistance (lbs/100g)	Hunter L	Hunter $+a_L$	Hunter $+b_L$	Hunter ΔE
0	0	443.04	64.50	46.09	2.75	11.94	50.66
0	50	426.00	70.31	48.67	3.24	13.06	49.06
0	100	421.74	75.38	47.84	3.03	12.81	49.29
50	0	433.10	70.88	46.39	2.79	11.98	50.48
50	50	430.26	82.31	46.73	2.77	11.97	50.26
50	100	406.12	90.94	48.18	3.14	12.88	50.05
100	0	427.42	63.94	47.20	3.49	12.94	49.02
100	50	387.66	86.25	46.86	3.12	13.05	50.01
100	100	353.58	106.88	46.91	3.69	13.41	50.39
CV(%)		3.40	5.68	1.30	6.59	2.35	0.89

Table 8. Statistical summary of magnesium ion level effects

Measures	Source of variation	D.F.	M.S.
<u>Soaked beans</u>			
Water uptake	Treatment	2	458.07
	Error	30	163.74
Shear resistance	Treatment	2	8278.13^{**}
	Error	3	234.75
<u>Processed beans</u>			
Drained wt.	Treatment	8	1564.64^{**}
	Error	9	198.06
Shear resistance	Treatment	8	393.65^{**}
	Error	9	20.16
Hunter L	Treatment	8	1.45^{*}
	Error	9	3.78×10^{-1}
Hunter $+a_L$	Treatment	8	$2.14 \times 10^{-1*}$
	Error	9	4.21×10^{-2}
Hunter $+b_L$	Treatment	8	$6.20 \times 10^{-1**}$
	Error	9	8.89×10^{-2}
ΔE	Treatment	8	0.79^{*}
	Error	9	1.90×10^{-1}

**denotes significant F, P=0.01

*denotes significant F, P=0.05

Effect on NaHMP and Its Combination with Ca^{+2} on the Quality Characteristics of Soaked and Processed Navy Beans

NaHMP

The mean values of NaHMP level effects on processed beans are reported in Table 9. The summary of statistical analyses of these data is presented in Table 10.

Soaked beans -- The water uptake increased, the shear resistance decreased as the soak water NaHMP levels increased. These results are in agreement with previous findings (Holmquist et al., 1948; Hoff and Nelson, 1965). The regression equations as well as correlation coefficients of water uptake and shear resistance of soaked beans are presented in Table 11. The water uptake was directly correlated with NaHMP levels; the shear resistance was negatively correlated with NaHMP levels. The ANOVA showed significant (P=0.01) differences in total nitrogen (%) in soak water; but no difference in the soaked beans among the NaHMP levels. As the NaHMP levels increased, the total nitrogen (%) in soak water increased on the fresh weight basis but decreased on the dry weight basis. This suggested that soluble solids, in addition to total nitrogen compounds, leached out during soaking, supporting the research of Varriano-Marston and De Omana (1979). These researchers reported "Pectic substances were leached out from beans during the soaking period. The greatest losses occurred when beans were soaked in either the

Table 9. Mean values of NaHMP level effects on the quality characteristics of soaked and processed beans

Measure	NaHMP (%) 0	0.25	0.50	0.75	1.0	1.5	CV%
Soaked beans							
Water uptake (g)	231.43	234.26	239.02	247.64	244.08	248.35	3.35
Shear resistance (lbs/50g)	922.50	903.75	801.00	750.00	729.00	715.50	3.11
Soak water N(%)							
Fresh wt. basis (x100)	3.20	5.90	6.50	7.90	7.40	6.80	11.04
Dry wt. basis	4.13	3.51	3.04	2.61	2.24	1.80	2.96
Soaked beans N(%)							
Fresh wt. basis	1.67	1.75	1.68	1.60	1.59	1.77	6.64
Dry wt. basis	4.11	4.09	4.12	4.01	4.05	4.23	0.12
Processed beans							
Drained wt. (g)	343.50	316.67	306.60	329.44	312.40	315.24	3.45
Shear resistance (lbs/100g)	51.03	50.23	49.61	48.56	48.42	48.67	7.63
Hunter L	45.65	46.57	47.30	48.59	48.55	48.14	2.48
Hunter $+a_L$	2.54	2.74	2.62	2.64	1.11	0.52	2.44
Hunter $+b_L$	11.22	11.64	12.12	12.88	12.52	11.94	3.33
ΔE	51.01	50.23	49.61	48.56	48.42	48.67	2.22
Brine N(%)							
Fresh wt. basis (x10)	1.93	2.25	1.74	1.95	1.78	1.64	5.12
Dry wt. basis	3.36	3.22	2.78	2.72	2.40	2.22	2.96

Table 9 (cont'd.).

Measure	NaHMP (%)						CV%
	0	0.25	0.50	0.75	1.0	1.5	
Beans N(%)							
Fresh wt. basis	1.35	1.09	1.08	0.96	1.03	1.10	11.62
Dry wt. basis	4.24	4.36	4.23	3.73	3.93	4.15	1.33

Table 10. Statistical summary of NaHMP level effects

Measure	Source of variation	D.F.	M.S.
Soaked beans			
Water uptake	Treatment	5	397.40^{**}
	Error	42	65.22
Shear resistance	Treatment	5	16149.60^{**}
	Error	6	624.19
Soak water N(%)			
Fresh wt. basis	Treatment	5	$5.49\times10^{-4**}$
	Error	6	4.18×10^{-5}
Dry wt. basis	Treatment	5	1.45^{**}
	Error	6	7.31×10^{-3}
Soaked beans N(%)			
Fresh wt. basis	Treatment	5	1.27×10^{-2}
	Error	6	1.24×10^{-2}
Dry wt. basis	Treatment	5	9.24×10^{-3}
	Error	6	5.55×10^{-3}
Processed beans			
Drained wt.	Treatment	5	272.68
	Error	6	122.72
Shear resistance	Treatment	5	53.33
	Error	6	14.22
Hunter L	Treatment	5	2.81
	Error	6	1.39
Hunter a	Treatment	5	1.92^{**}
	Error	6	4.94×10^{-2}
Hunter b	Treatment	5	0.71^{*}
	Error	6	1.61×10^{-1}
ΔE	Treatment	5	2.22
	Error	6	1.20
Brine N(%)			
Fresh wt. basis	Treatment	5	$9.20\times10^{-4**}$
	Error	6	9.30×10^{-5}
Dry wt. basis	Treatment	5	$3.98\times10^{-1**}$
	Error	6	6.80×10^{-3}
Beans N(%)			
Fresh wt. basis	Treatment	5	6.16×10^{-2}
	Error	6	1.64×10^{-2}
Dry wt. basis	Treatment	5	5.89×10^{-3}
	Error	6	3.00×10^{-3}

**denotes significant F, P=0.01
*denotes significant F, P=0.05

Table 11. Selected quality characteristics of processed bean regression on NaHMP levels

Dependent measure	Regression	r_{NaHMP}	SEr
Soaked beans			
Water uptake	Y=234.98+8.03X	0.8437*	0.2684
Shear resistance	Y=905.71-153.13X	-0.9203**	0.1956
Soak water N(%)			
Fresh wt. basis	Y=0.05+0.02X	0.6920	0.3609
Dry wt. basis	Y=3.92-1.55X	-0.9816**	0.0955
Processed beans			
Hunter a_L	Y=3.06-1.57X	-0.8626*	0.2529
Hunter b_L	Y=11.66+0.58X	0.5249	0.4256
Brine N(%)			
Fresh wt. basis	Y=0.21-0.03X	-0.6730	0.3698
Dry wt. basis	Y=3.32-0.81X	-0.9718**	0.1179

**denotes significant r, P=0.01

*denotes significant r, P=0.05

$Na_5P_3O_{10}$ solution or the combined salts solutions. Considerably less pectic substance was solubilized when beans were soaked in tap water."

Processed beans -- The drained weight and shear resistance of processed beans were not significantly (P=0.05) affected by soak water and process brine NaHMP levels. This could possibly be explained by the fact that the retorting masked the effects of NaHMP on these two dependent measures. As the concentration of NaHMP increased, the Hunter $+a_L$ decreased dramatically and Hunter $+b_L$ tended to increase, which implied that in the higher levels of NaHMP, beans became less red and more yellow. The regression equations and correlation coefficients are also reported in Table 11. The Hunter L and Δ E were not significant (P=0.05) affected by soak water and process brine NaHMP levels.

The total nitrogen analyses in processed brine and beans were similar to those in final soak water and soaked beans. The data reported on dry weight basis more clearly demonstrate these effects.

Combination of calcium ion and NaHMP

The mean values of combination of calcium ion and NaHMP effects are reported in Table 12. The summary of statistical analyses of these data is reported in Table 13.

Table 12. Mean values of combination of Ca^{+2} and NaHMP effects on the quality characteristics of soaked and processed beans

Measure	Ca^{+2}(ppm) + NaHMP (%)						CV%
	0+0	50+0	100+0	0+1	50+1	100+1	
Soaked beans							
Water uptake (g)	229.69	227.32	226.05	244.03	246.54	244.21	1.58
Shear resistance (lbs/50g)	892.50	918.75	947.50	1729.00	748.50	772.50	3.00
Soak water N(%)							
Fresh wt. basis (x100)	3.19	3.21	3.16	7.45	7.11	6.84	6.34
Dry wt. basis	4.13	3.61	3.65	2.24	2.31	2.29	0.35
Soaked beans N(%)							
Fresh wt. basis	1.67	1.65	1.71	1.59	1.70	1.74	4.22
Dry wt. basis	4.11	3.80	3.82	4.05	4.24	4.25	3.75
Processed beans							
Drained wt.	430.26	387.66	342.22	312.40	320.92	318.08	2.50
Shear resistance (lbs/100g)	65.82	82.13	106.63	73.13	73.13	70.50	12.30
Hunter L	46.10	46.59	47.15	48.56	49.33	50.80	1.58
Hunter $+a_L$	2.54	2.73	3.48	1.01	2.62	2.75	10.39
Hunter $+b_L$	11.20	11.81	12.64	12.57	13.19	13.77	3.09
ΔE	51.01	50.73	49.71	48.33	47.43	46.68	1.26
Brine N(%)							
Fresh wt. basis (x10)	1.95	1.73	1.74	1.79	1.78	1.87	5.06
Dry wt. basis	3.36	3.11	3.25	2.40	2.54	2.52	3.53
Beans N(%)							
Fresh wt. basis	1.08	0.89	1.05	1.06	1.08	1.08	3.89
Dry wt. basis	4.14	3.85	4.06	4.02	4.13	4.08	2.00

Table 13. Statistical summary of combination of Ca^{+2} and NaHMP effects

Source of variation	D.F.	M.S. of dependent measures on soaked beans					
		Water uptake	Shear resistance	Soak water N(%) F.W.B.[1]	Soak water N(%) D.W.B.[2]	Soaked bean N(%) F.W.B.	Soaked bean N(%) D.W.B.
Ca	2	4.15_{**}	2512.31_{**}	1.06×10^{-5}	3.40×10^{-3}**	3.00×10^{-4}	1.15×10^{-2}
NaHMP	1	891.65^{**}	82419.18^{**}	4.66×10^{-6}	6.87^{**}	0	1.68×10^{-1}
CaxNaHMP	2	6.5	91.70	6.99×10^{-6}	2.08×10^{-1}	7.00×10^{-3}	6.60×10^{-2}
Error	6	13.99	712.32	1.07×10^{-5}	1.07×10^{-2}	5.00×10^{-3}	2.30×10^{-2}

Source of variation	D.F.	M.S. of dependent measures on processed beans				
		Drained wt.	Shear resistance	Hunter L	Hunter $+a_L$	Hunter $+b_L$
Ca	2	1712.59^{**}	367.09^{**}	2.76	1.87^{**}	1.82^{*}
NaHMP	1	14524.12^{**}	476.53^{**}	26.05^{**}	1.87^{**}	4.89^{**}
CaxNaHMP	2	2201.91^{**}	481.42^{**}	3.89×10^{-1}	5.04×10^{-1}	2.02×10^{-2}
Error	6	77.30	93.35	5.72×10^{-1}	6.87×10^{-2}	1.50×10^{-1}

Source of variation	D.F.	M.S. of dependent measures on processed beans				
		ΔE	Brine N(%) F.W.B.	Brine N(%) D.W.B.	Bean N(%) F.W.B.	Bean N(%) D.W.B.
Ca	2	2.20^{*}	1.30×10^{-4}	2.52×10^{-5}	8.40×10^{-3}**	6.20×10^{-2}
NaHMP	1	27.03^{**}	2.90×10^{-6}	1.71^{**}	1.28×10^{-2}*	1.00×10^{-2}
CaxNaHMP	2	9.35×10^{-2}	2.20×10^{-4}	4.30×10^{-2}	1.24×10^{-2}*	8.00×10^{-2}**
Error	6	3.81×10^{-1}	8.39×10^{-5}	1.02×10^{-2}	1.63×10^{-3}	6.60×10^{-3}

[1]F.W.B. = Fresh wt. basis, [2]D.W.B. = Dry wt basis

**denotes significant F , P=0.01

*denotes significant F , P=0.05

Soaked beans -- NaHMP in soak water softened the beans and accelerated water uptake. The ANOVA did not indicate the calcium ion significantly (P=0.05) affected the water uptake or shear resistance. No interaction effects between the two additives on these measures were observed. The total nitrogen (%) both on dry weight and fresh weight bases was significantly different in final soak water, but not significantly different in soaked beans. No effects on total bean or soak water nitrogen were due to calcium ions. As the levels of NaHMP increased, total nitrogen (%) in final soak water increased on the fresh weight basis, but decreased on the dry weight basis.

Processed beans -- The calcium ion and NaHMP significantly (P=0.01) decreased the drained weight of processed beans. This was consistent with the results from separate calcium ion and NaHMP studies. The interaction effect was significant (P=0.01). Both calcium ion and NaHMP significantly (P=0.01) affected shear resistance of processed beans. Calcium ion increased, NaHMP decreased, and the interaction of these two additives remained as no calcium ion effect on the shear resistance of processed beans. Hunter $+a_L$ values were not affected by this combination treatment. No significant (P=0.01) interaction effects between these two additives on any color measurements were presented. However, NaHMP increased Hunter L values. Both calcium ion and NaHMP increased Hunter $+b_L$ values

and decreased ΔE. The effects due to NaHMP were more than those due to calcium ion. The total nitrogen (%) in the final process brine was not significantly ($P=0.01$) affected by calcium ion or NaHMP on the fresh weight basis, but was significantly ($P=0.01$) affected by NaHMP on the dry weight basis. The interaction effect on total nitrogen (%) in processed beans was significant ($P=0.01$) on the dry weight basis. However, on the fresh weight basis, no significant differences were observed.

Effects of Selected Enzyme Treatments

Soaked beans -- The mean values of selected enzymes on water uptake and shear resistance of beans soaked with and without seedcoats for 5 hours at different temperatures are presented in Tables 14 and 15, respectively. Seedcoats were removed to facilitate enzyme penetration into the beans. The summary of multiple factor ANOVA is presented in Table 16. The histograms of main factor effects on water uptake and shear resistance of soaked beans are reported in Figures 1 and 2.

The ANOVA indicated there were no significant differences ($P=0.05$) in water uptake among enzyme types and between groups (control and treatment) in either beans with seedcoats intact or removed. The non-difference in accelerating water uptake due to enzyme types implied that no changes in water uptake were due

Table 14. Mean values[1] of water uptake (g) of beans soaked with and without seedcoats in selected enzyme solutions for 5 hours at different temperatures

	Whole Beans (Seedcoat Intact)		Whole Beans (Seedcoat Removed)	
Temperature	Control	Treatment	Control	Treatment
		α-Amylase at pH 6.9		
20°C	56.65	55.40	67.80	65.90
40	56.90	57.45	65.25	66.03
60	59.80	59.94	61.80	65.00
80	60.95	60.75	64.50	65.20
		Glucoamylase at pH 4.5		
20	56.80	61.05	66.30	65.50
40	57.10	54.10	64.35	64.10
60	58.05	60.75	61.15	62.05
80	60.00	62.80	66.20	61.30
		Pectinase at pH 3.5		
20	56.65	59.70	66.80	65.20
40	57.45	57.70	64.40	63.60
60	56.35	60.80	58.05	57.00
80	60.60	64.15	64.35	63.65
		Cellulase at pH 6.5		
20	57.35	60.15	67.20	63.15
40	57.25	54.60	63.50	67.25
60	56.15	61.25	57.75	62.10
80	59.85	64.20	62.45	61.60
		Protease at pH 3.5		
20	57.80	60.25	68.90	61.95
40	56.85	52.75	64.15	64.05
60	55.75	59.50	59.60	59.35
80	61.80	62.70	64.35	67.65

[1] n=2 replications; CV=4.34 %

Table 15. Mean values[1] of shear resistance (lbs/50g) of beans soaked with and without seedcoats in selected enzyme solutions for 5 hours at different temperatures

	Whole Beans (Seedcoat Intact)		Whole Beans (Seedcoat Removed)	
Temperature	Control	Treatment	Control	Treatment
		α-Amylase at pH 6.9		
20°C	1113.75	1102.5	967.00	907.50
40	1087.50	1095.00	900.00	892.50
60	986.25	1050.00	900.00	952.50
80	472.50	495.00	277.50	318.75
		Glucoamylase at pH 4.5		
20	113.75	1095.00	903.75	892.50
40	1087.50	1091.25	911.25	885.00
60	1020.00	1046.25	930.00	930.00
80	637.50	652.50	401.25	415.50
		Pectinase at pH 3.5		
20	1102.50	1117.50	915.00	907.50
40	1081.50	1083.75	897.75	900.00
60	1001.25	1035.00	971.25	982.50
80	577.50	585.00	487.50	480.00
		Cellulase at pH 6.5		
20	1185.00	1106.25	945.00	915.00
40	1107.75	1085.25	907.50	862.50
60	1005.00	1057.50	918.75	952.50
80	652.50	652.50	427.50	446.25
		Protease at pH 3.5		
20	1128.75	1117.50	903.75	930.00
40	1080.00	1081.50	897.75	888.75
60	1001.25	1023.75	922.50	922.50
80	600.00	615.00	397.50	357.00

[1] n=2 replications, CV=4.48%

Table 16. Statistical summary of soak studies with selected enzymes

Source of variation	D.F.	M.S. Water uptake	M.S. Shear resistance
Main effects			
Enzyme type	4	3.43	8157.33**
Temperature	3	91.15**	2510688.34**
Seedcoat	1	1012.04**	1107392.01**
Group	1	13.23	61.26
Interactions			
ExT	12	8.24	8566.17**
ExSC	4	10.98	4497.94*
TxSC	3	130.38**	33925.07**
ExG	4	3.57	344.65
TxG	3	17.94	3668.19
SCxG	1	33.49*	1476.22
ExTxSC	12	3.48	1368.66
ExTxG	12	3.59	790.81
ExSCxG	4	5.67	292.41
TxSCxG	3	25.10*	553.54
ExTxSCxG	12	6.03	415.69
Error	80	7.07	1527.69

**denotes significant F, P=0.01

*denotes significant F, P=0.05

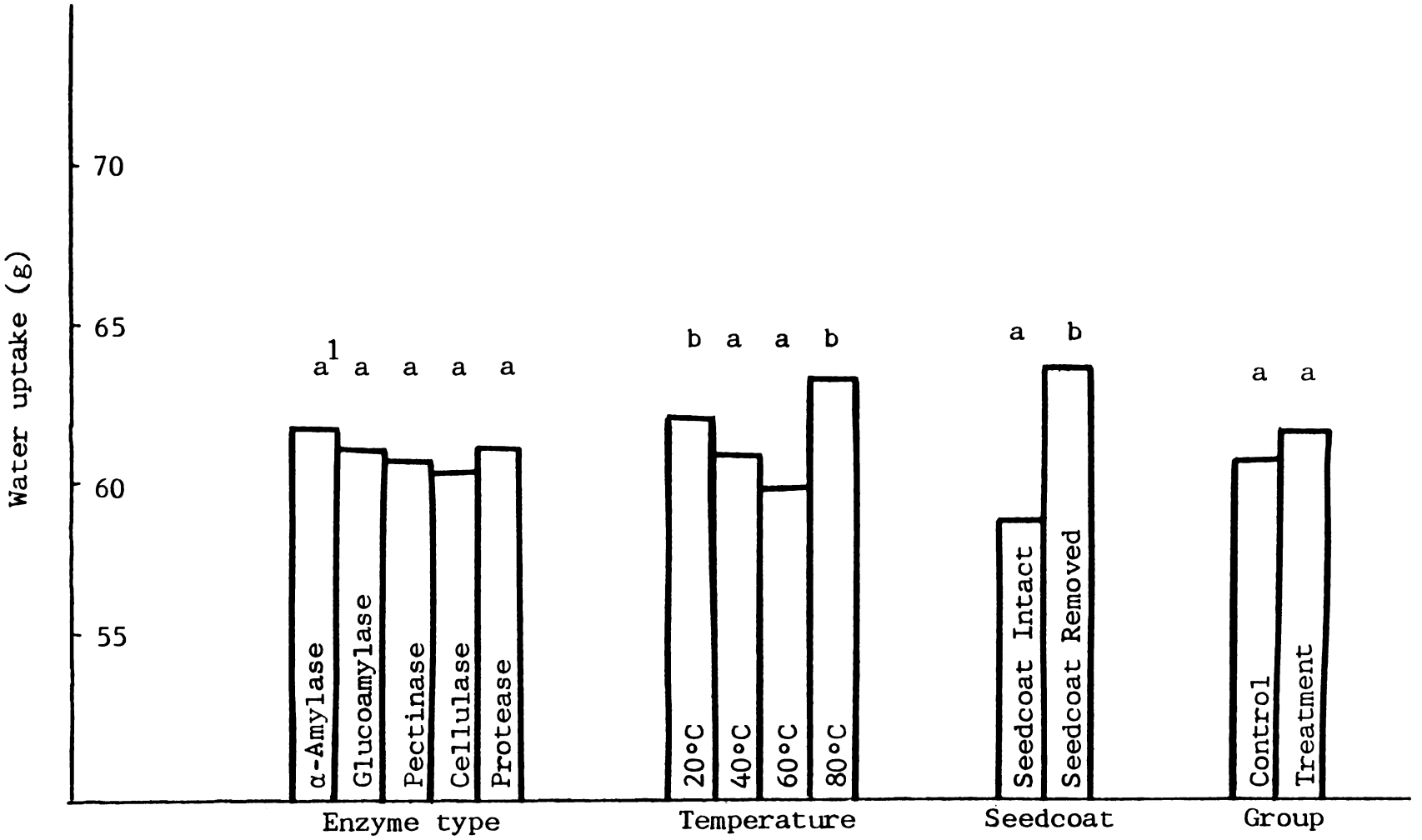

Figure 1. Enzyme soak studies -- The main factor effects on water uptake of soaked beans

[1]Like letters denote nonsignificant differences

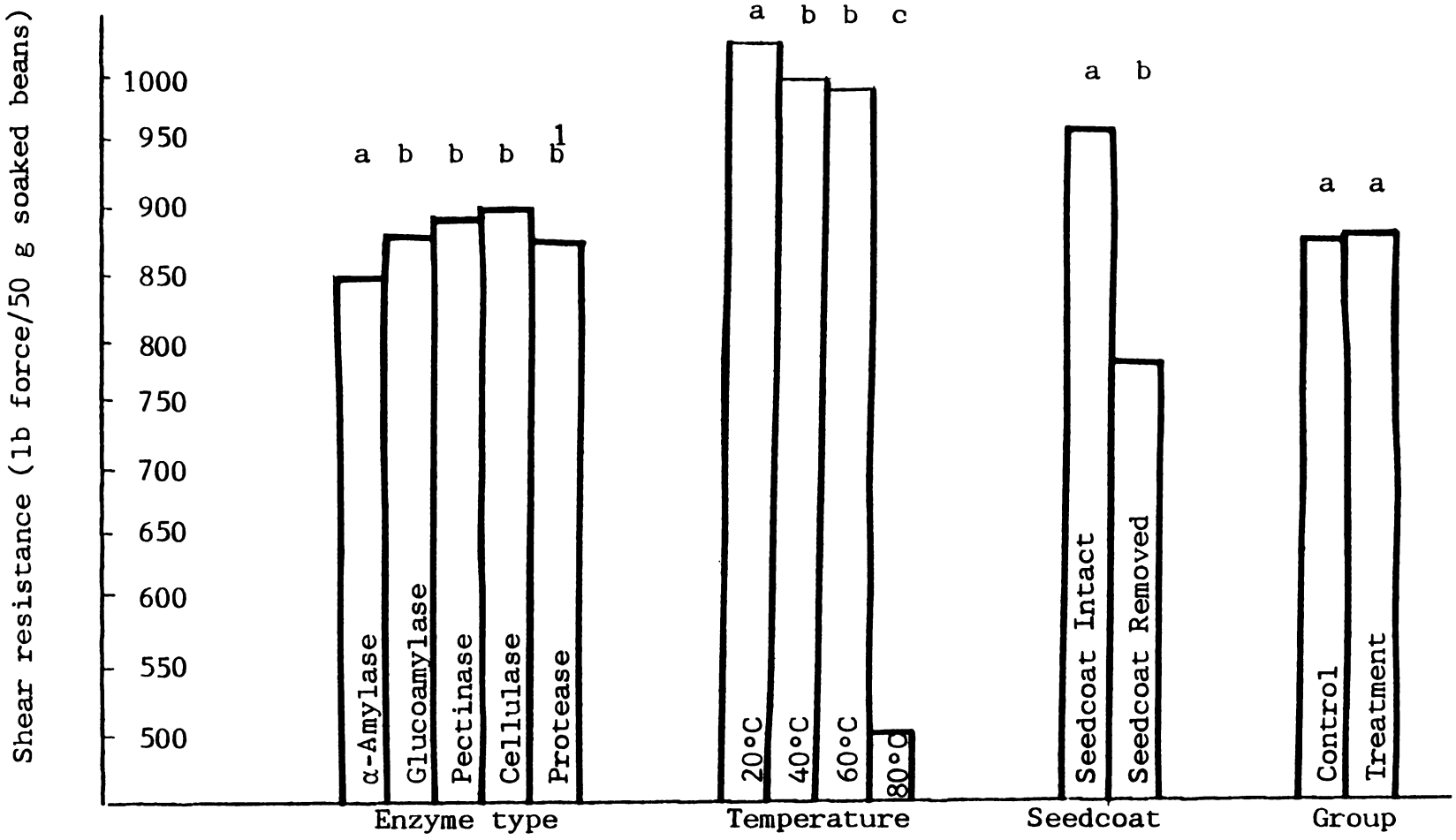

Figure 2. Enzyme soak studies -- The main factor effects on shear resistance of soaked beans

[1]Like letters denote nonsignificant differences

to different pHs, since beans were soaked in each enzyme solution at its stable pH. The temperature and seedcoat contributed significantly (P=0.01) to water uptake. The interaction effects of temperature and seedcoat; seedcoat and group; temperature, seedcoat and group were significant (P=0.05). Tukey's mean separations of the main factor effects are also included in Figure 1.

The ANOVA showed that the effects on shear resistance of soaked beans due to seedcoat, enzyme type and temperature were significant (P=0.01). But there was no significant (P=0.05) difference between treated and control samples on this measure over all the main factors. The main factor effects and Tukey's mean separations are presented in Figure 2. The soaked beans with seedcoats intact possessed higher shear resistance than those with seedcoat removed. The shear resistance of beans soaked in α-amylase solution at pH 6.9 was significantly different (P=0.01) from other enzyme treatments. Higher temperature soaking resulted in softer beans. However, the relationship between temperature and shear resistance of soaked beans was not linear. Tukey's mean separations showed no differences in shear resistance of beans soaked at 40°C and 60°C. The beans soaked at 80°C were much softer than those soaked at lower temperatures.

Gelatinization -- The experimental results reported above indicated that the selected enzymes were inactive

on water uptake and shear resistance in either beans with seedcoats intact or removed. It was questioned whether the enzymes were able to contact their substrates in the cotyledons. Therefore, the whole ground bean flour was used to demonstrate the activities of selected enzymes on the viscosity of bean flour during gelatinization. The results are presented in Table 17 and Figure 3. The summary of statistical analyses of these data is reported in Table 18. Only α-amylase and pectinase significantly ($P=0.01$) reduced the viscosity of bean flour. Glucoamylase, cellulase and protease remained inactive. It is known that the glucoamylase is inactive in this experiment (Reed, 1975). The inactivities of cellulase and protease may be due to their low concentrations and short reaction time, or due to these substrates'limited effects on viscosity. Table 18 contains the effect of pH on the viscosity of bean flour during gelatinization. The viscosity increased as pH decreased. The phenomenon may be explained by the denaturation of some proteins and suppression of hydration of the proteins and starch in the bean flour by hydrochloric acid. The plot of the viscosity data indicated a linear relationship between pH and viscosity (Figure 4). Increased degrees of freedom would be needed to establish the significance of a linear relationship.

Table 17. Means values[1] of viscosity of bean flour gelatinized in selected enzyme solutions

Enzyme type	pH	Viscosity (Brabender unit) Control	Treatment	% CV
α-Amylase	6.9	757.5	477.5	5.64
Glucoamylase	4.5	905.0	880.0	3.92
Pectinase	3.5	1030.0	955.0	1.59
Cellulase	6.5	858.5	827.5	8.66
Protease	3.5	1030.0	1025.0	1.09

[1] n=2 replications

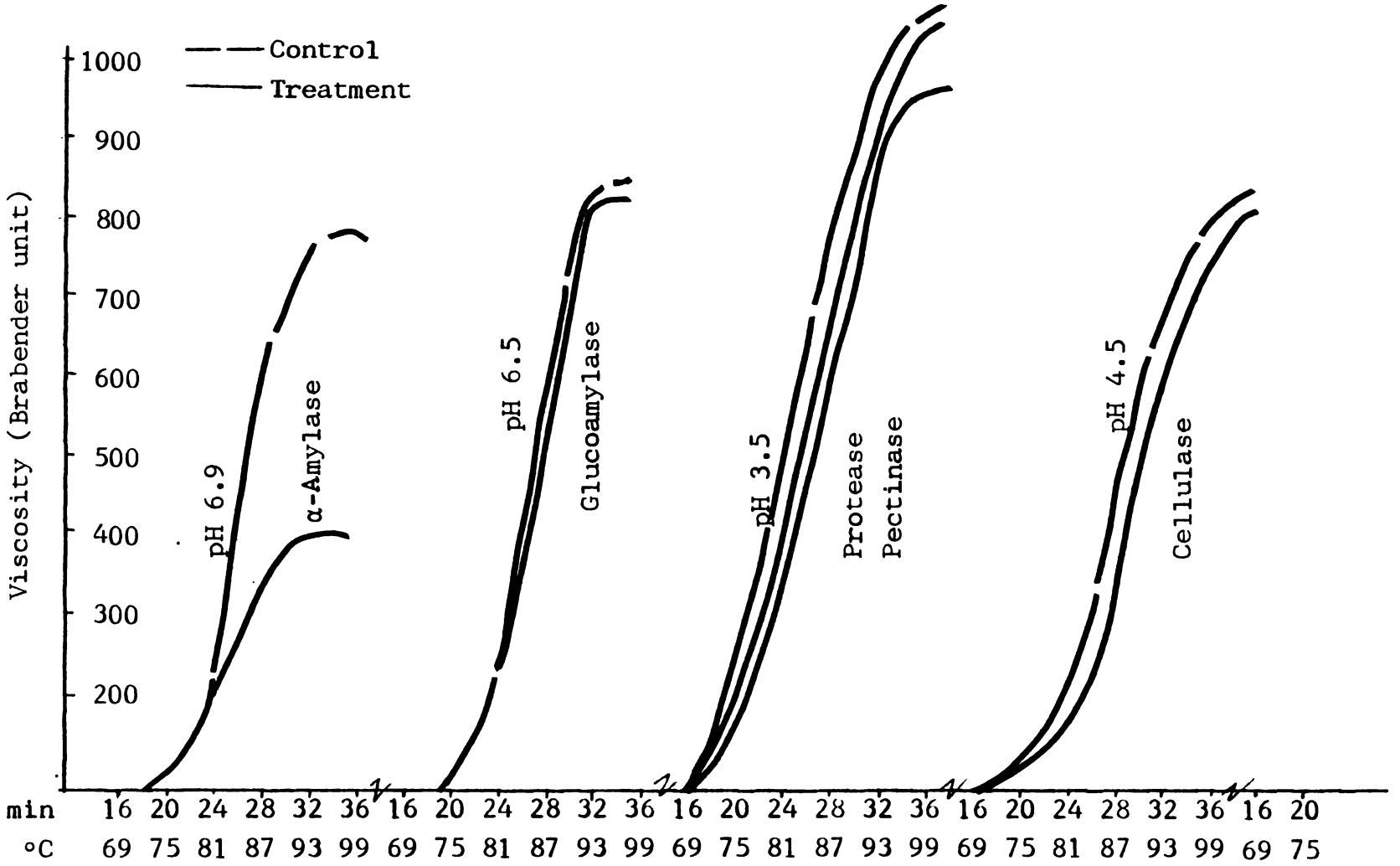

Figure 3. Effects of selected enzymes on the viscosity of whole ground bean flour

Table 18. Statistical summary of selected enzyme gelatinization studies

Measure	Source of variation	D.F.	M.S.
α-Amylase	Treatment	1	78230.5**
	Error	2	1212.5
Glucoamylase	Treatment	1	625.0
	Error	2	1225.0
Pectinase	Treatment	1	11250.0**
	Error	2	250.0
Cellulase	Treatment	1	2881.2
	Error	2	5335.5
Protease	Treatment	1	25.0
	Error	2	125.0
pH	Treatment	3	21216.7**
	Error	4	675.0

**denotes significant F, P=0.01

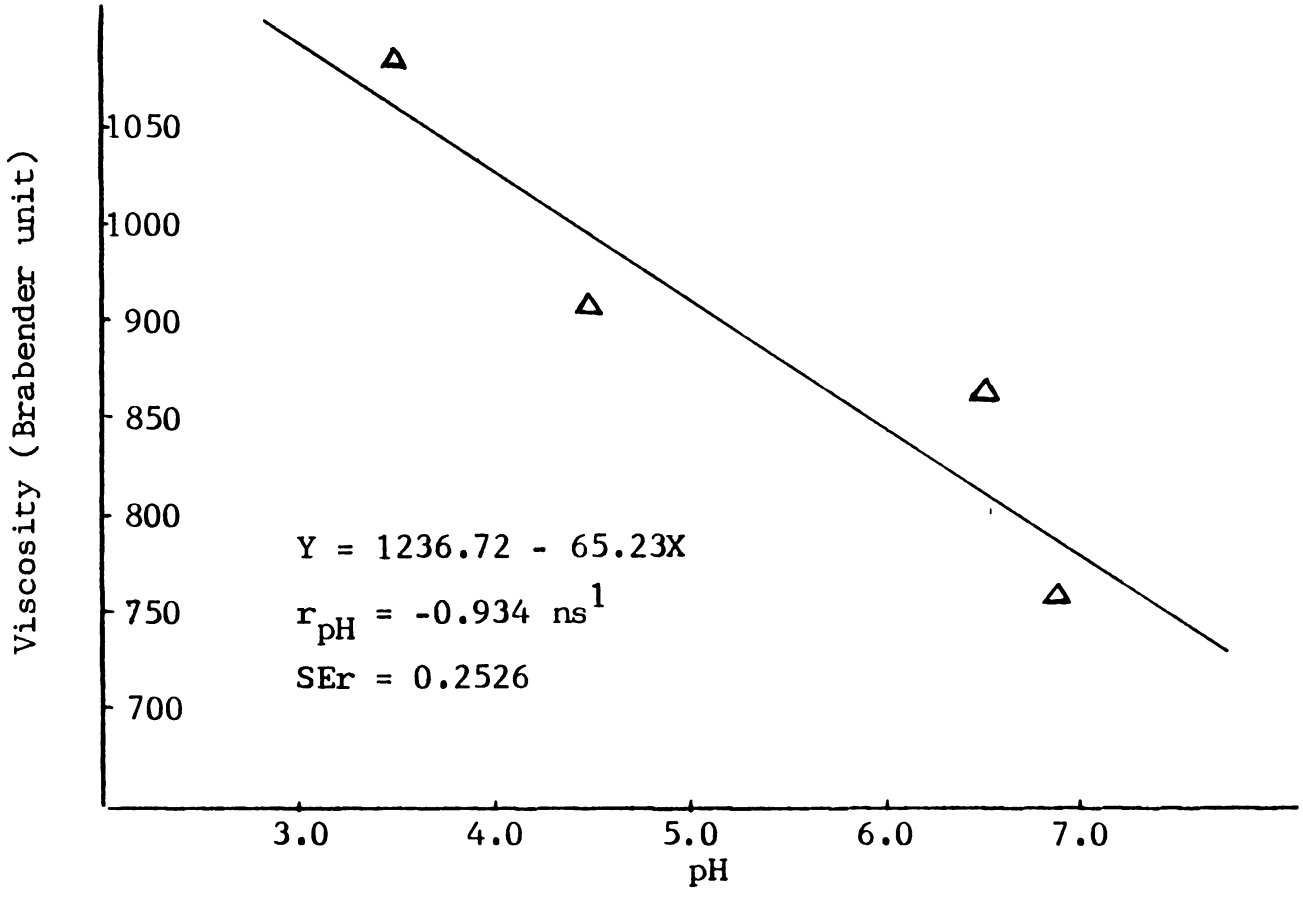

Figure 4. Bean flour viscosity regression on pH

[1]Non significant

SUMMARY

Two critical factors of processed navy bean quality and subsequent consumer acceptance are degree of bean hydration and firmness. Addition of divalent ions, Ca^{+2} and Mg^{+2}, to processed navy beans not only increased their firmness but had a pronounced effect on the drained weight. The calcium content of processed beans maintained a negative relationship with processed bean drained weight and a direct relationship with processed bean firmness throughout soaking and processing. These results were in agreement with the theory that pectin and calcium ion retarded water uptake and increased firmness through the formation of tough pectin-metal ion complexes (Matz, 1962). The addition of calcium ions into soak water and process brine did not significantly (P=0.01) affect the color of processed beans.

The effects on drained weight and shear resistance of processed beans by magnesium ions were similar to those caused by calcium ions. However, bean color Hunter L, $+a_L$ and especially Hunter $+b_L$ were affected by magnesium ions.

Increasing levels of NaHMP increased water uptake, softened soaked beans and affected color Hunter $+a_L$ and $+b_L$

of processed beans. No significant changes in drained weight or shear resistance of processed beans were observed. As the NaHMP level increased the total nitrogen (%) in the final soak water increased on the fresh weight basis, but decreased on the dry weight basis. A similar phenomenon was observed in the processed products. These results suggested that soluble solids, in addition to nitrogen compounds, leached out during soaking and processing of beans in different levels of NaHMP additive.

The study of combining Ca^{+2} and NaHMP as an additive indicated that NaHMP softened the soaked beans and accelerated water uptake. The calcium ion retarded water uptake and increased shear resistance of soaked beans. No interaction effects were observed. Both calcium ion and NaHMP, as well as their interaction, decreased drained weight of processed beans. The calcium ion increased, but NaHMP decreased and the combination treatment remained as no calcium ion effect on the shear resistance of processed beans. Increasing Hunter $+b_L$ and decreasing ΔE values by the combination treatment were observed. The two additives did not yield interaction effects on any color measurements. Total nitrogen (%) in the final soak water increased on a fresh weight basis, but decreased on a dry weight basis due to the addition of NaHMP to soak water. The final process brine total nitrogen (%) decreased on a dry weight basis. The

significant interaction effects of Ca^{+2} and NaHMP on the processed beans total nitrogen (%) were observed, on a dry weight basis.

The selected enzyme soaking studies indicated that the enzyme types did not show effects on water uptake in whole beans, as well as not showing differences between treated and control samples. The enzymes also remained inactive in beans with seedcoats removed under the identical soaking conditions. The removal of seedcoats increased the rate of water uptake. No difference in shear resistance was observed between control and treated samples over all the other main factors. Shear resistance of beans soaked in α-amylase solution at pH 6.9 was different from those soaked in other enzymes. This difference was likely due to the function of pH, since each enzyme was incubated at its stable pH. The large difference between soaked beans with and without seedcoats in shear resistance peak height indicated that seedcoats contributed a major component to the shear resistance. The beans soaked at 80°C, either with or without seedcoats, were much softer than those soaked at lower temperatures.

To evaluate whether enzymes were in contact with their substrates in cotyledons in the soaking studies with or without seedcoat, whole ground bean flour was used to test the activities of enzymes in reducing the viscosity of gelatinized bean flour. Only α-amylase and pectinase decreased the viscosity, while glucoamylase,

cellulase and protease remained inactive possibly due to low concentration and short reaction time, or due to these substrates' limited effects on viscosity. The hydrogen ion increased the viscosity of gelatinized bean flour. This phenomenon may be explained by the denaturation of some proteins and suppression of hydration of the proteins and starch in the bean flour with decreasing pH.

FURTHER RESEARCH

1. Use a radioactive isotope to trace the distribution of divalent ions from soaking and processing to confirm the relative firming action of soak and process brine.
2. Analyze the pectic substances in the final soak water and process brine for NaHMP treatment to confirm that NaHMP softening is partly due to solubilizing pectic substances.
3. Conduct morphological and chemical studies on relative effects of magnesium and calcium ions in intercellular firming of processed beans.
4. Study effects of cellulase and protease on the viscosity of whole bean flour by increasing the concentration and reaction time of these enzyme treatments.

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