CHANGES OBSERVED DURING THE QUICK COOKING AND HEAT TREATMENT PROCESSES

by

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INTRODUCTION

Legume seeds have been used as human food for more than 80 centuries and are still one of the most important sources of protein for a large segment of the world population (Rockland and Jones, 1974). In most of the Central American countries, beans of the Phaseolus vulgaris variety provide 20-50% of the protein in the diets of rural populations (Bressani et al., 1961). Legume seeds which contain relatively high amount of lysine have been reported as a very good complement to cereal protein. For example a combination of ground corn with cowpea legumes can initiate cure in children with Kwashiorkor (Bressani et al., 1962). An excellent nutritional combination of 72% corn and 28% black beans has been reported by Bressani et al. (1962).

Cooking of dry beans is necessary to develop acceptable flavors and textures, to destroy toxic materials and to make the bean protein nutritionally available (Kakade and Evans, 1963). The hard-to-cook phenomenon in seed legumes has been recognized since the 3rd century B.C. (Morris, 1963). This phenomenon increases with seed storage time (Burr et al., 1968). Long soaking periods of 8-12 hr (overnight) are usually required followed by a cooking time which can be several hours (Morris, 1963). These extended cooking times are not only energy intensive but may result in large nutrient losses. Therefore, it would be beneficial to find ways to produce "quick-cooking" seed legumes.

The present study was designed to obtain chemical and morphological information concerning the mechanism of action of two methods which have been proposed to aleviate the hard-to-cook phenomenon in beans: (a) soaking in sodium salt solutions (Rockland and Metzler, 1967; Rockland et al., 1971)

and (b) steam or retort heat treatments (Molina et al., 1976).

The seed legume used in this study was Phaseolus vulgaris, a domestic variety commonly known as black bean. It is believed that once the mechanism of action of these methods has been defined it may be possible to develop more simple and less expensive techniques to control the hard-to-cook phenomenon in beans.

REVIEW OF LITERATURE

The cookability of dry legumes has been associated with a number of environmental factors including climatic conditions after harvest. In addition, the chemical constituents in dry legumes have been studied in an attempt to correlate cooking time with levels and/or changes in lipids, pectic substances, proteins and enzymes.. This literature is discussed below along with the proposed solutions to the hard-to-cook phenomenon.

Environmental Factors

Climatic conditions: Theophrastus (1916) was probably one of the first scientists to recognize that certain climatic conditions affected the amount of time required to cook beans. He related wind conditions to cookability. Gloyer, (1932 a) later reported that hardshell legumes[1] where readily produced in weather conditions which combined high temperatures with low relative humidities. He also indicated that this may be related to variety and stated that "....within the same variety the selection showing the least percentage of hardshell will tend to show the greatest tenderness of the seed coat in the processed product." However, he also found that cold rainy weather just after planting resulted in high percentages of hardshell beans (Gloyer, 1932 b). More recent reports confirm the importance of climate in the development of hard to cook beans (Morris et al., 1950).

Storage conditions after harvest: In 1936, Snyder associated the hard-to-cook phenomenon in dry beans with temperature and moisture conditions

[1] Legumes which do not readily imbibe water.

during storage. She reported that "beans stored under laboratory conditions of temperature and moisture became so hard that they could not be cooked satisfactorily."

A number of authors have reported on relationships between moisture content and the cooking quality of beans (Morris and Wood, 1956; Muneta, 1964; Morris, 1964; Burr et al., 1968). Morris and Wood (1956) found that beans stored under conditions which maintained the moisture content between 12 and 16%, received lower texture rating by taste panelists, i.e. they were not as adequately cooked as beans stored at moisture contents less than 12%. These results were later confirmed by Morris (1964) and Muneta (1964). In fact, Muneta (1964) found a significant positive correlation (+ 0.80) between moisture content and cooking time.

The cookability of dry legumes is also related to storage temperature, and in many cases there is a relationship between storage temperature and moisture content. Morris and Wood (1956) found that if beans containing 10% moisture were stored at 77°F for 2 years their quality was equal to samples stored at 10°F. They found that the loss in quality was related to lipid acid value rather than changes in enzyme activities.

Morris (1964) later defined the temperature moisture relationship by indicating that "....reducing the moisture content six-tenths of one percent is as effective as lowering the temperature 15 degrees." No significant changes occurred in beans stored six months at 40°F (Morris, 1964; Burr et al., 1968).

Chemical Factors

In order to study the cooking phenomenon in dry beans, some components have been determined and an attempt has been made to correlate chemical

constituents with cooking time. For example, Takayama et al. (1965) found no significant correlation between cooking times and the triglyceride, phosphatide, and crude lipid content.

Pectic substances. Pectic substances have also been related to cooking time in seed legumes. Water-soluble pectic constituents have been reported to increase during the cooking of plant tissues (Simpson and Halliday, 1941; Kertesz, 1951; Doesburg, 1965; Hamad and Powers, 1965). The increase in water-soluble pectic substances has been related to the hydrolysis of protopectin to pectin during heating. (Hamad and Powers, 1965; Kon, 1968).

Total pectin content has been inversely related to water imbibition, a factor undoubtedly related to cooking time (Hamad and Powers, 1965). However, the role of the seed coat and the effect of initial moisture content on water uptake was not determined. Earlier investigations have indicated that the seed coat plays an important but undefined role in water absorption by beans (Gloyer, 1932 a; Smith and Mash, 1961) and that lower initial moisture contents in beans results in slower rates of water absorption (Smith and Mash, 1961; Crean and Halisman, 1963). Interactions between pectin content, seed coat structure and moisture content, to date, have not been defined.

Minerals. Studies on mineral constituents of legumes have dealt mainly with nutritional factors (Meiners et al., 1976 a, 1976 b) instead of the cooking phenomenon. However, several authors have shown that calcium present in the cooking water is absorbed into the plant tissues during the cooking process (Noble and Halliday, 1937; Horner, 1939; Marston et al., 1974). This data is important if one recognizes the firming action of calcium on plant tissues (Kertesz, 1951) and the role of calcium in cell

structure (Rains, 1976). In addition if the phytic acid content of legumes is low, insoluble calcium and magnesium pectates may be formed in the cell middle lamella causing poor cookability (Mattson, 1946). Horner (1939) studied the relationship between hard water and its effect on the texture of the vegetable. He found that hard water increased the calcium content of vegetables and decreased the content of other mineral constituents.

Walker and Hymowitz (1972) studied the possible correlations between certain minerals and organic components of common beans. They concluded that "...most correlations between mineral and organic components were negative."

Ginzburg (1958) has suggested that the middle lamella in plant tissues contains a protein component in addition to the known pectic substances and he was able to verify the presence of protein as an important component of the middle lamella (Ginzburg, 1961). This protein component was found to bind divalent ions, e.g. Ca^{++} and Mg^{++} at specific sites. In addition the removal of divalent ions or the addition of proteolytic enzymes was necessary to obtain cell separation. Furthermore, the action of EDTA in separating cells was observed under both acidic and basic conditions.

Proposed Solutions

Several solutions have been proposed to resolve the hard-to-cook phenomenon in dry beans. These solutions include: preparation of precooked dehydrated beans, quick-cooking beans, and heat-treatment of beans.

Precocked dehydrated beans. Precooked dehydrated beans have been suggested since 1942 when Esselen and Davis developed a dehydrated baked bean that could be reconstituted by rehydrating for $1\frac{1}{2}$ hour in cold water,

but this long period of time could not be classied as quick-cooking. Other workers, such as Feldberg et al., 1956; Dorsey et al., 1961, and most recently Steinkraus et al., 1964 have tried to improve the method. Most of those processes involved retort cooking under pressure which requires high uses of energy as well as expensive procedures. Furthermore, those techniques may not be available in countries which consume large quantities of seed legumes. In addition, changes in bean structure and texture make these products unacceptable to consumers.

Quick-cooking beans. Rockland and Metzler in 1967 proposed a method to prepare quick-cooking dry beans. In their method beans were soaked in a solution containing NaCl, $Na_5P_3O_{10}$, Na_2CO_3, and $NaHCO_3$, the seed coats were loosened by vacuum infiltration followed by rinsing and drying. The resulting product cooks in 15 minutes or less. Similar procedures were also used to make frozen and refrigerated quick-cooking beans (Rockland et al., 1971). The process does not greatly affect the structure and appearance of the beans when compared to untreated samples (Rockland and Jones, 1974). However, the removal of the seed coat will change the appearance and flavor of highly colored beans such as black beans and red beans. In addition, losses of vitamins, e.g. thiamine, would occur when treated with the solution which contains sodium bicarbonate. Again this method is expensive and does not readily lend itself to use in developing countries.

Heat treated beans. Heat treatments have been suggested to eliminate the poor cookability of legumes. Morris et al. (1950) dipped beans for 1 min in boiling water before soaking and cooking. Beans treated in such that way were found to be more palatable than untreated samples. The disadvantages of this method were changes in texture, and flavor among others.

Molina et al. (1976) proposed a process to control the development of hard-to-cook phenomenon in black beans. Dry beans were treated with steam or in a retort for different lengths of time. The best results were obtained by using 2 min in the retort or 10 min under steam. Cooking times obtained after 9 months of storage were comparable to untreated samples stored at 4°C for the same time. Because freshly harvested beans take less time to cook than stored beans these findings are valuable. The heat treatment method has advantages over the quick-cooking methods in that it is simple and a technology which would give low production costs. However, even lower-cost technologies would be recommended to encourage increased production and greater availability of beans.

None of the literature explained the mechanism of action involved in their methods. This is what I propose to do in this study.

MATERIALS AND METHODS

Samples

Black beans were purchased from a local market in large enough quantities to use for the entire study. The beans were carefully blended and stored in a plastic container under laboratory conditions.

Heat-treated beans were prepared according to the method of Molina et al., (1976) by placing the beans in one layer into glass jars and submitting them to steam treatments (98°C) for 10 minutes, or placing them in a retort for 2 or 5 minutes. The times chosen were those reported as most desirable in Molina's work. A steamer and a pressure cooker (15 psi) were used.

Analytical Procedures

Soaking treatments. Beans were soaked (50 ml of soaking solution per gram) at room temperature for 24 hr. The following soaking solutions were used: (a) 1% NaCl, (b) 0.75% $NaHCO_3$, (c) 0.25% Na_2CO_3, (d) 0.5% $Na_5P_3O_{10}$, (e) a combination of all of the above chemicals (called "all treatments"), (f) deionized water or (g) tap water. Solution (e) above was used by Rockland and Metzler (1967) in their quick-cooking process.

At the end of the soaking period, the beans were removed from the solutions, placed on absorbent paper to remove surface water, and weighed to determine the amount of water absorbed. Water absorption was expressed as the percentage of water absorbed after soaking per gram of dry beans. The pH of the solutions was determined before and after soaking. The soaking solutions were saved and stored under refrigeration for analysis of ions and pectic substances.

Cooking. The drained soaked beans were placed in a beaker containing deionized water (50 ml/g beans) and heated in an oil bath (American Optical Corporation) at 110°C boiled for 1½ hr. Different periods of soaking and cooking times were tested. A soaking period of 24 hr. and a cooking period of 1½ hrs. was adopted for the present work. The beakers were covered to decrease vaporization and the oil bath shaker was used at a very low speed during the cooking period. After cooking, the cooking water was removed, its volume and pH determined, and it was stored under refrigeration for later analysis. In addition, the cooked beans were analyzed for certain ions and pectic substances were determined.

Ion determinations. The Berry and Johnson (1966) ashing procedure was used with the following modifications. The dried sample of beans was wet with 1.8 N H_2SO_4 in absolute alcohol and the alcohol was burned off, the product was then cooled for about 45 min, and the sample was placed in a cold muffle at 550°C for 5 hours. At the end of that period, the ash was dissolved with 10 ml HCl (1.2 N) on a steam bath and concentrated to dryness. The product was then placed into the muffle at 550°C for 2 hours; this last step was repeated two or three times until a white ash was obtained. The white ash was dissolved and made to 100 ml with 5% HCl. Strontium chloride (500 ppm Sr) was used in the final solutions as a protective agent.

Atomic absorption (Perkin-Elmer Atomic Absorption Spectrophotometer Model 303) was used to determine Ca^{++} and Mg^{++} and flame emission (Perkin-Elmer Spectrophotometer Model 460) was used to determine K^{+} and Na^{+}.

pH, moisture, fat, protein. A Corning Model 10 pH meter, was used and buffer solutions of pH 7 and 10 were used to adjust the apparatus.

The moisture determinations were on raw beans ground with a Wiley Mill using a 60 mesh screen and dried in a vacuum oven at 100°C, 30 p.s.i. for 2 hours.

The fat determinations were done using the Goldfish fat extraction method and pet. ether as the solvent. The ground beans were extracted for 12 hr. The AACC method 46-10, (1963) was used to determine protein (N x 6.25).

Pectic substances. Pectic substances were determined by the colorimetric procedure recommended by Dietz and Rouse (1953) and Kon (1968), with some modifications. The dry beans were ground in a Wiley Mill using a 60 mesh screen and then they were extracted with aqueous ethanol to give a final concentration of 70% ethanol (Kon, 1968). The cooked beans were mixed with the ethanol and ground with sand in a mortar. After extracting with ethanol the mixtures were centrifuged and the supernatant discarded. The procedure of Dietz and Rouse (1953) was then followed.

The pectic substances were extracted from dry beans, without enzymatic digestion, as well as with the utilization of α-amylase or pectinase. The enzymatic methods used were those proposed by Kon (1968) where the enzyme is applied for 12 hr. at 40°C and maintained at a pH of 7.

Microscopy. The scanning electron microscope (SEM) samples were prepared by freezing the soaked or cooked beans in isopentane that was cooled with liquid nitrogen and freeze-drying in an Edwards Freeze-drier for 35-40 hr. at -100°C. The samples were fractured with a razor blade, mounted on stubs, and coated with gold-paladium. Micrographs were taken

on a Etec U - 1[1] SEM. The raw beans were either freeze-dried and mounted on stubs or mounted directly on stubs.

The light microscope samples were prepared by cutting the hydrated beans by hand with a razor and then mounting the sections in refractive oil. Photomicrograph were taken on a Reichert (Austria) light microscope.

Soluble Nitrogen: Soluble nitrogen was determined by method 46-23 of the AACC (1968). Determination of soluble nitrogen in the soaking and cooking waters were performed by direct application of the standard method to a representative volume of that sample (25 ml).

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[1] Etec Company, Hayward, California.

RESULTS AND DISCUSSION

Effects of Sodium Salts

Proximate analysis. The results from the proximate analysis were: 22% protein, 12% moisture, 1.2% fat, 62.5% carbohydrate, and 2.3% ash.

Water absorption and pH. The means and standard deviations for water absorption and pH for beans soaked in various solutions and then cooked in deionized water are presented in Table 1. Significant differences ($P < 0.05$) between treatments were observed for these variables and the L.S.D. values are shown in Table 2.

Beans soaked in $Na_5P_3O_{10}$ had a significantly higher water absorption than beans soaked in $NaHCO_3$, deionized water, tap water, or NaCl solutions. However, water absorption values were similar for beans soaked in $Na_5P_3O_{10}$, Na_2CO_3 and All Treatment (All T.) solutions. The Na_2CO_3 solution had the highest pH (11.25) of the treatments, followed by $Na_5P_3O_{10}$ and All T. Because these three treatments had similar water absorptions, a relationship appeared to exist between W.A. and pH. Statistical analysis showed a correlation between pH of the soaking solutions and water absorption of + .54, $P < 0.04$. Although the correlation was significant the low value suggests that the pH was not the only factor influencing water absorption.

Changes in pH could affect the charges on the protein and pectic components of the middle lamella. The protein at pH's above 9.0 would probably exhibit a net negative charge, and this would enable the protein molecules to bind more water. Albersheim (1959) showed that under alkaline conditions pectic substances breakdown into lower molecular weight products. The degradation of pectic substances could produce separation

TABLE 1. WATER ABSORPTION VALUES FOR BEANS SOAKED IN VARIOUS SOLUTIONS AND THE pH's OF THOSE SOLUTIONS BEFORE AND AFTER SOAKING AND AFTER COOKING.[a]

Treatment	Water Absorption[b] %	pH of Solution		
		Before Soaking	After Soaking	After Cooking
NaCl	97.56 ± 4.03	7.61 ± 0.88	5.97 ± 0.05	6.24 ± 0.19
Na_2CO_3	103.26 ± 4.07	11.25 ± 0.26	10.34 ± 0.08	7.57 ± 0.13
$NaHCO_3$	101.81 ± 2.09	8.58 ± 0.03	8.55 ± 0.25	7.38 ± 0.58
$Na_5P_3O_{10}$	106.37 ± 1.45	9.91 ± 0.19	8.65 ± 0.09	6.45 ± 0.17
All Treatments	102.16 ± 2.69	9.32 ± 0.21	9.22 ± 0.03	8.82 ± 0.26
Tap	99.41 ± 0.39	9.01 ± 0.32	6.34 ± 0.09	6.10 ± 0.17
Deionized H_2O	99.90 ± 1.00	5.45 ± 0.43	5.15 ± 1.04	5.73 ± 0.14

[a] Values are the means plus the standard deviation from the mean.

[b] Expressed as percentage of the dry weight.

TABLE 2. SIGNIFICANT DIFFERENCES ($P < 0.05$) IN WATER ABSORPTION AND pH FOR THE DIFFERENT SOAKING TREATMENTS[a]

Parameter measured							
Water Absorption	$Na_5P_3O_{10}$	Na_2CO_3	All T.	$NaHCO_3$	Deionized	Tap.	NaCl.
pH Before Soaking[b]	Na_2CO_3	$Na_5P_3O_{10}$	All T.	Tap	$NaHCO_3$	NaCl	Deion.
pH After Soaking	Na_2CO_3	All T.	$Na_5P_3O_{10}$	$NaHCO_3$	Tap.	NaCl	Deion.
pH After Cooking	All T.	Na_2CO_3	$NaHCO_3$	$Na_5P_3O_{10}$	NaCl	Tap.	Deion.

[a] Those treatments not underlined are significantly different at .05% level.

[b] These pH's were taken in the soaking and cooking waters.

of the cells and therefore, softening of the plant tissues. In addition, the resulting open spaces could be occupied by more water molecules.

pH changes during soaking. In general, the pH of the soaking solutions decreased during soaking (Table 1). This drop in pH is probably because of the pectic substances. Kertesz (1951) explains the phenomenon as the reaction of alkali with the pectinic acid and later demethylation, although demethylation may not be the only reason why a pectinic acid consumes alkali.

The effect of the initial pH of the soaking solutions on the ionization of proteins, pectic substances and other ionizable molecules present in the legumes could also produce decreases in pH. It has been shown that the consumption of alkali increases with alkali concentration or the duration of the treatment, and the temperature (Kertesz, 1951). However, in the present work beans soaked in $NaHCO_3$, All Treatment and deionized water, gave only small changes in pH. One reason why some treatments caused large changes in pH while others did not, is probably related to the buffering capacity of the different soaking solutions. A simple determination of the buffering capacity was performed and the All Treatment solution showed a relatively high resistence to changes in the pH when a solution of a dilute acid was added.

The small change in pH when beans were soaked in deionized water could possibly be explained by the fact that the initial pH (5.45) would ionize the pectic substances which have a pKa of 2.8-4.2 (Briggs et al., 1961). However, the proteins molecules present in the middle lamella and other molecules with higher pKa values would not be completely dissociated. In the case of the $NaHCO_3$ treatment, we would have in solution Na^+, $CO_3^=$,

HCO_3^-, H_2CO_3 and dissolved CO_2 (Van Wazer and Arvon, 1954). These ions are H^+ acceptors and when the pH (8.58) is high enough to cause ionization of molecules, the H^+acceptors would prevent large changes in the pH of the solution.

pH change during cooking. In general, decreases in pH were observed during cooking and this probably is due to of breakdown and leaching of pectic substances into the cooking water. The results presented in Table 3 verify this observation. It has been shown that heat treatments increase the solubility of pectic substances (Simpson and Halliday, 1941; Kertesz, 1951; Doesbury, 1965; Hamad and Powers, 1965). The breakdown of pectic substances would probably increase the leaching of proteins into the cooking water since interactions between proteins and pectic substances exist in the middle lamella (Ginzburg, 1958; 1961). The two molecules, pectic substances and proteins, exhibit in their structure large proportion of carboxylic acid groups which would become ionized and therefore would drop the pH of the solution.

Pectic substances. The amount of pectic substances extracted from the raw beans depends upon whether or not the extraction was performed on the raw beans or on beans pretreated with α-amylase or pectinase (Table 3). Much higher extraction was observed from the pretreated beans than from the untreated. These results were similar to those found by Kon (1968). He explained the increase in pectic substance extracted with α-amylase to the elimination of starch and postulated that starch may interfere with the carbazole colorimetric determination.

The pectic substances, which are structural component of cell walls, and the middle lamella are carbodydrate derivatives composed of galacturonic

TABLE 3. EXTRACTION OF PECTIC SUBSTANCES FROM BEANS AND S.W.[a] AND C.W. UNDER DIFFERENT TREATMENTS.

	Water Extractable	Hexam Extractable	Sodium Hydroxide Extractable	Total Pectic Substances
RAW	40.47 ± 1.00	10.47 ± 0.13	131.00 ± 8.49	182.45 ± 8.67
RAW + - AM.[b]	239.06 ± 16.16	24.85 ± 0.00	40.90 ± 14.46	304.81 ± 1.70
RAW + PECT.[c]	324.97 ± 18.70	16.24 ± 1.43	78.66 ± 2.69	419.87 ± 17.44
NaCl				
Cooked Beans	6.88 ± 0.06	9.41 ± 1.56	39.03 ± 0.81	279.42 ± 8.01
S.W.	61.60 ± 7.78			
C.W.	162.51 ± 2.55			
Na_2CO_3				
Cooked Beans	3.45 ± 0.40	4.77 ± 0.14	32.13 ± 2.68	230.38 ± 5.32
S.W.	61.67 ± 15.42			
C.W.	128.38 ± 6.87			
$NaHCO_3$				
Cooked Beans	5.29 ± 0.16	4.99 ± 0.25	25.81 ± 1.83	250.02 ± 0.54
S.W.	49.30 ± 1.55			
C.W.	164.65 ± 0.91			
Tap Water				
Cooked Beans	6.75 ± 0.16	5.95 ± 0.04	34.43 ± 0.82	213.59 ± 23.94
S.W.	33.85 ± 8.28			
	132.62 ± 14.72			
$Na_5P_3O_{10}$				
Cooked Beans	9.54 ± 3.58	11.05 ± 2.06	33.85 ± 1.68	301.88 ± 10.84
S.W.	116.68 ± 48.66			
C.W.	130.77 ± 41.78			
All Treatments				
Cooked Beans	7.90 ± 0.79	1.58 ± 0.16	11.15 ± 0.40	295.02 ± 6.42
S.W.	89.91 ± 2.57			
	184.48 ± 7.65			

Deionized				
Cooked Beans	6.63 ± 0.25	5.09 ± 1.07	33.34 ± 2.21	267.28 ± 18.16
S.W.	54.76 ± 2.43			
C.W.	167.65 ± 12.45			

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[a] Soaking Water and Cooking Water. Values are the means plus the standard deviation from the mean.

Values are expressed as mg per gram of dry basis.

[b] α-AM means α-amylase.

[c] Pect. means pectinase.

acid residues joined in linear units by α-1-4 linkages like those which occur in amylose (Albersheim, 1976). The cell wall contains protein molecules and some non-structural components such as xyloglucans and arabinogalactans (Albersheim, 1976). It is also possible that glucose polymers exist in those cell structures and interact with pectic substances. If this were true then the α-amylase used in the pectic substances determination described above could degrade these glucose polymers as well as the starch. The result would then be increased extractability of pectic substances. Some authors feel that similarities exist between pectic substances and starch, including the enzyme susceptibilities (Kertesz, 1951).

The amounts of pectic substances extracted with pectinase were much higher than with α-amylase. Again, this result is in agreement with Kon (1968). How pectinase degrades pectic substances is not clear, but undoubtedly results in the production of smaller units which are easier to extract.

The effects of cooking on the extractability of pectic substances are also presented in Table 3. The heat treatment resulted in an increase in the extractability of high methoxyl compounds (water extractable). The protopectin portion (NaOH extractable) was much higher in the raw rather than heat treated beans. The literature states that protopectin is transformed into soluble pectic substances during heat treatments. (Simpson and Halliday, 1941; Kertesz, 1951; Doesbury, 1965; Hamad and Powers, 1965).

The amount of low methoxyl components (Hexametaphosphate extr.) extracted from All Treatment was significantly less ($P < .05$) than the other heat treatments and the raw beans. The high pH (9.32) of this solution could result in the degradation of pectic substances to produce smaller molecules.

In addition, the high Na^+ content could produce ion exchanges. The chelating ability of $Na_5P_3O_{10}$ could result in the removal of the calcium and magnesium which binds those molecules together. This could produce an easier extraction of these molecules. However, the data (Table 4) shows than no more calcium was lost into the soaking water when beans were soaked in All T. than when they were soaked in $Na_5P_3O_{10}$ alone. Significantly higher amounts ($P < .05$) of magnesium, however, were lost into the soaking water in the former case. Therefore, those pectic substances could have been leached into the soaking and cooking waters.

Minerals. Losses of minerals during the cooking of plant tissues has been reported in the literature (Horner, 1936; Marston et al., 1974). The data presented in Table 4 is in agreement with those results. However during the present work greater losses of minerals occurred during soaking than during cooking. Such data has not been previously reported. The losses of these ions during soaking would result in a nutritional disadvantage for those persons who make the practice of changing the soaking water before cooking the beans.

A gain of sodium can be observed in those treatments with Na^+ in the soaking solution, and the losses of this ion were significant ($P < 0.05$) during soaking in tap and deionized water, two treatments with little or no Na^+.

Large amounts of K^+ were lost into the soaking water in all of the treatments. Since beans gained Na^+ during all of the sodium salt treatments and K^+ was lost, it is possible that some interchanges of monovalent ions occurred during soaking.

TABLE 4. MINERALS IN BEANS AND IN SOAKING AND COOKING WATER AFTER SOAKING IN VARIOUS SOLUTIONS[a]

Treatment	Na^+(mg/g)	K^+(mg/g)	Ca^{++}(mg/g)	Mg^{++}(mg/g)
Raw Beans	1.53 ± .34	13.50 ± 3.41	0.32 ± 0.64	1.95 ± .11
NaCl				
Cooked Beans	3.87 ± 0.31	2.13 ± 0.15	0.18 ± 0.01	0.63 ± 0.08
S.W.[b]	89.73 ± 5.90	7.83 ± 0.38	0.21 ± 0.02	0.54 ± 0.02
C.W.	7.50 ± 0.53	0.23 ± 0.06	0.02 ± 0.00	0.53 ± 0.17
Na_2CO_3				
Cooked Beans	3.13 ± 0.46	2.45 ± 0.06	0.18 ± 0.02	1.01 ± 0.03
S.W.	28.63 ± 1.53	6.68 ± 0.42	0.07 ± 0.01	0.28 ± 0.01
C.W.	3.73 ± 0.83	0.21 ± 0.03	0.03 ± 0.00	0.47 ± 0.21
$NaHCO_3$				
Cooked Beans	3.87 ± 0.46	2.43 ± 0.21	0.16 ± 0.03	0.80 ± 0.02
S.W.	41.77 ± 2.14	7.07 ± 0.64	0.12 ± 0.03	0.44 ± 0.02
C.W.	4.53 ± 0.95	0.20 ± 0.00	0.03 ± 0.00	0.36 ± 0.03
Tap				
Cooked Beans	0.47 ± 0.15	4.40 ± 0.26	0.25 ± 0.01	1.05 ± 0.04
S.W.	1.17 ± 0.06	2.17 ± 0.12	0.25 ± 0.07	0.37 ± 0.04
C.W.	0.23 ± 0.06	4.00 ± 0.89	0.03 ± 0.00	0.37 ± 0.10
$Na_5P_3O_{10}$				
Cooked Beans	2.97 ± 0.25	2.73 ± 0.46	0.13 ± 0.03	0.49 ± 0.03
S.W.	38.17 ± 1.94	7.47 ± 1.07	0.15 ± 0.01	0.72 ± 0.07
C.W.	4.20 ± 0.30	0.33 ± 0.15	0.02 ± 0.00	0.47 ± 0.04
All Treatments				
Cooked Beans	7.67 ± 0.57	1.90 ± 0.00	0.14 ± 0.00	0.65 ± 0.08
S.W.	184.00 ± 5.85	14.20 ± 1.57	0.14 ± 0.00	0.93 ± 0.03
C.W.	15.93 ± 0.90	0.30 ± 0.17	0.02 ± 0.01	0.30 ± 0.04
Deionized H_2O				
Cooked Beans	0.30 ± 0.00	3.00 ± 0.00	0.21 ± 0.01	0.92 ± 0.00
S.W.	1.45 ± 0.49	2.90 ± 0.00	0.07 ± 0.00	0.27 ± 0.04
C.W.	0.25 ± 0.07	3.95 ± 0.21	0.02 ± 0.00	0.48 ± 0.08

[a] Values are the means plus the standard deviation from the means and are expressed on the dry basis.

[b] S.W. and C.W. means soaking water and cooking water, respectively.

Calcium was lost from beans in all of the solutions but smaller amounts were lost when beans were soaked in Tap water, and this was probably due to the high concentration of Ca^{++} present in tap water (.20 - .23 mg/ml). Other authors have also shown that there was less loss of Ca^{++} when plant tissues were treated in tap water (Horner, 1936; Noble and Halliday, 1937; Horner, 1939; Marston et al., 1974).

Magnesium was also lost from the beans during the different soaking treatments. However, lower amounts of Mg^{++} were lost from the beans when soaked in Na_2CO_3, tap and deionized water. At this time, the complex interactions between pH and ion concentration in the different solutions cannot be explained.

Several relationship appeared to exist between ions with respect to gains and losses during soaking. Within treatments, high amounts of Na^+ in soaking solutions resulted in large losses of Mg^{++} during soaking. For example, the L.S.D.'s showed that when beans were soaked in the All T. solution (highest content of Na^+) they lost more Mg^{++} than when beans were soaked in any other solution. It is possible that Mg^{++} is replaced by Na^+ in the intercellular cement. Ginzburg (1961) has suggested that divalent cations can be replaced by monovalent cations in the intercellular cement. However, the concentration of Na^+ is not the only factor that influences the loss of Mg^{++} from the beans. For example, only small amounts of Mg^{++} were lost from beans treated with Na_2CO_3 or deionized water. The former solution has a relatively high amount of Na^+ compared to the deionized water, but the amount of Mg^{++} lost in both solutions were essentially the same. The pH probably also affected the interchange between Na^+ and Mg^{++}. ($pH_{Na_2CO_3}$ = 11.25; $pH_{deionized}$ = 5.45).

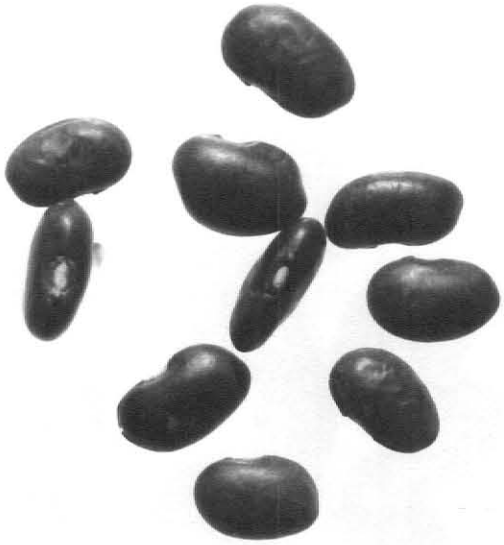
A relationship also appears to exist between the retention of K^+ and Ca^{++} in the cooked beans. A linear correlation of 0.60 ($P < .02$) was found. This suggests that if K^+ is retained in the cooked beans Ca^{++} is also retained. Ginzburg (1961) proved that K^+ cannot be replaced by divalent ions. Our data seems to support that conclusion.

One of the mechanisms involved in the quick-cooking process developed by Rockland et al. (1967) undoubtedly involves ions since interchanges between Na^+ and Ca^{++} were observed in beans treated with the All T. solution. This solution probably has the ideal concentration of sodium and hydrogen ions which would produce the changes required to soften the tissues of seed legumes.

Ultrastructure of raw beans. The gross structure of raw beans is presented in Fig. 1 and the SEM structure in Fig. 2. The seed coat structure is shown in Figs. 2a and b. The seed coat consist of 4 layers: the pigment layer, the palisade layer, the subepidermal layer (hourglass cells), and the parenchyma cells. The cotyledon portion is shown in Figs. 2 c-f. Cells in the cotyledon contain starch granules embedded in a protein matrix. The cells are very compact and it is difficult to distinguish the cells and cell walls (Fig. 2c). A film of protein around the starch granules is honeycomb shaped as can be seen in Figs. 2d and e. In addition protein bodies (p) can be seen embedded in the protein matrix. These protein bodies were verified by polarizing microscopy. The junction between three cells can be seen in Fig. 2f.

Ultrastructure of soaked and cooked beans. The structure of beans soaked and cooked in deionized water is presented in Fig. 3. When beans were soaked the constituents became hydrated. Individual cells are easier to recognize than in the raw beans (Figs. 3a & b). However, little separation

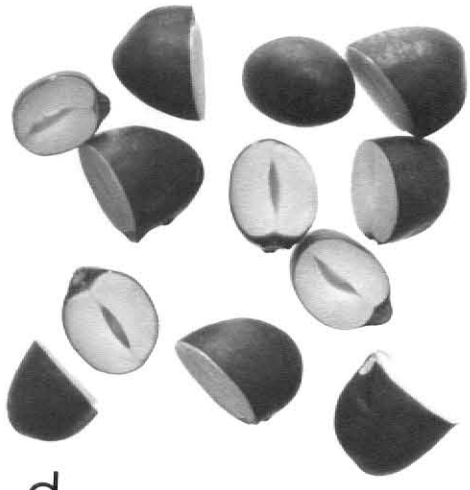
Figure 1. Black beans (Phaseolus vulgaris): dry beans (a, b) and beans soaked 24 hr. (c, d).



a

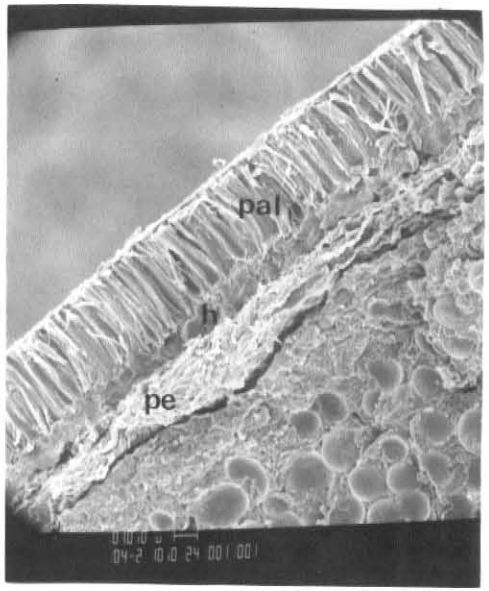
b

c

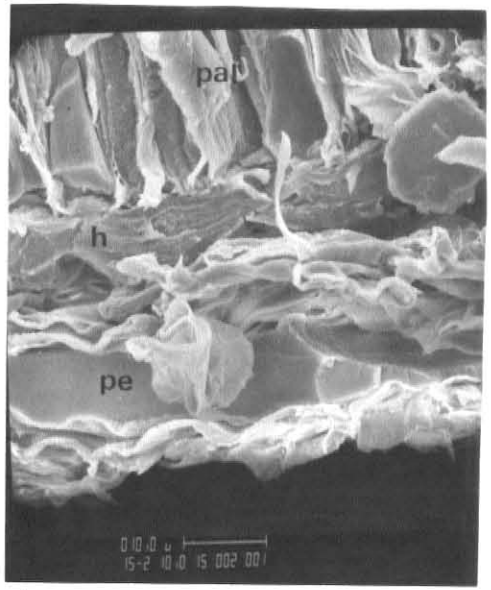


d

Figure 2: Scanning electron micrographs (SEM) of cross sections of raw black beans: (a) seed coat showing pigment layer (pi), palisade layer (pal), hourglass cells (h), parenchyma cells (pe), and cotyledon (cot); (b) higher magnificatien of seed coat; (c) middle of cotyledon. Arrows show boundaries of one cell, starch granule (s); (d) higher magnification of the center of the cotyledon, protein bodies (p), starch granule (s); (e) micrograph showing the honeycomb protein material on the starch granule; and (f) higher magnification of protein matrix.



a



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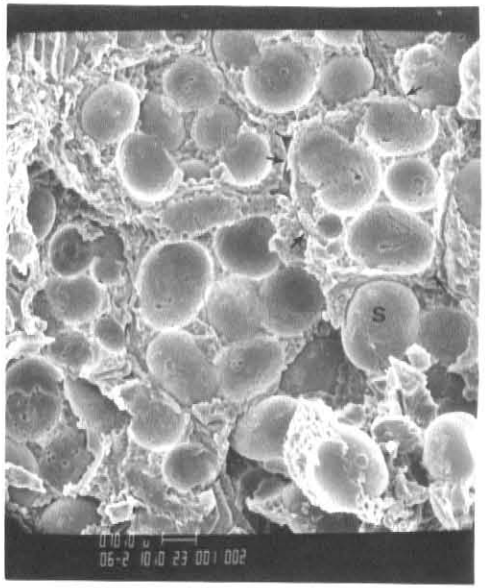
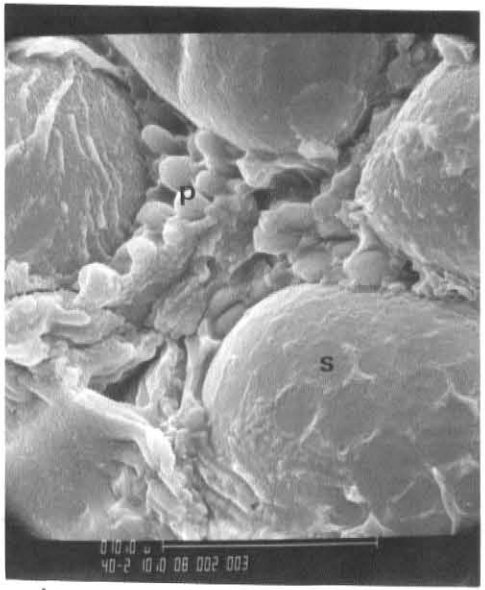
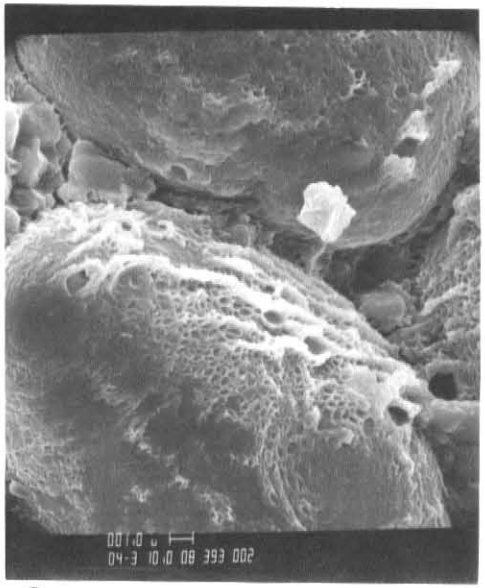


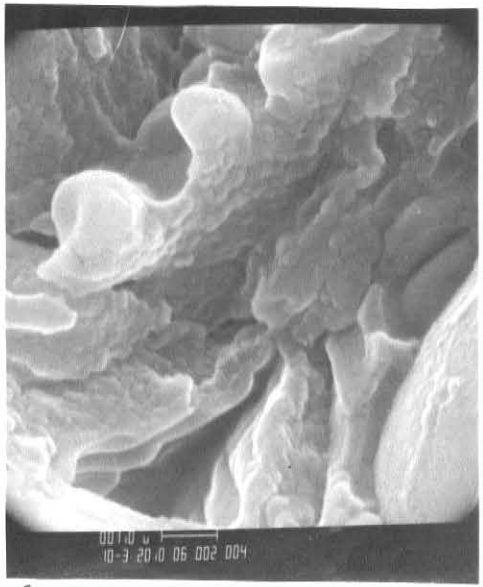
c



d

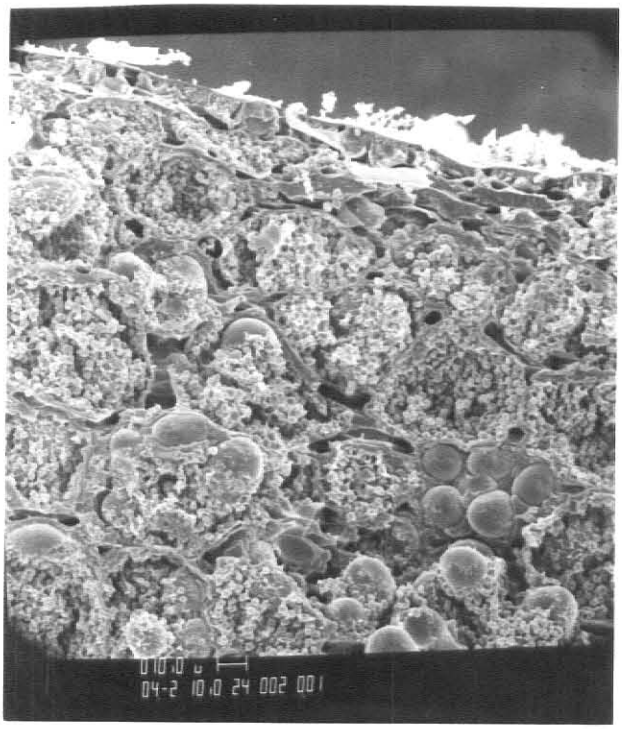


e

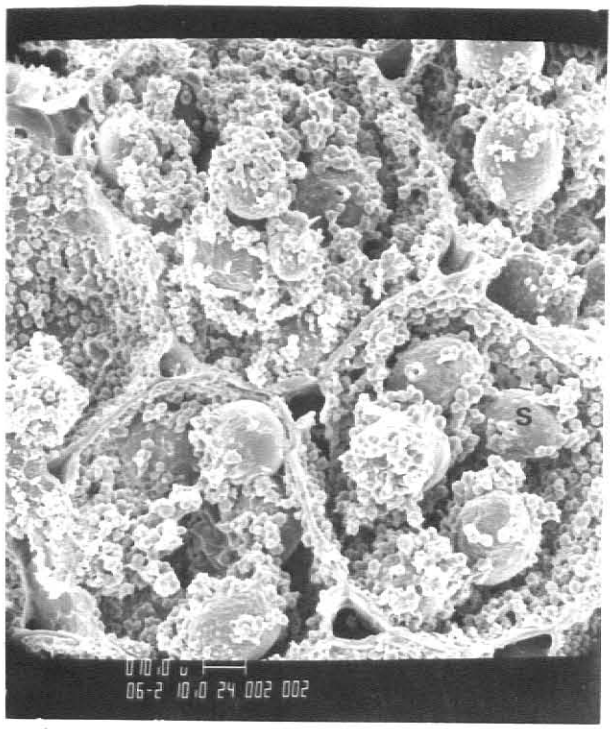


f

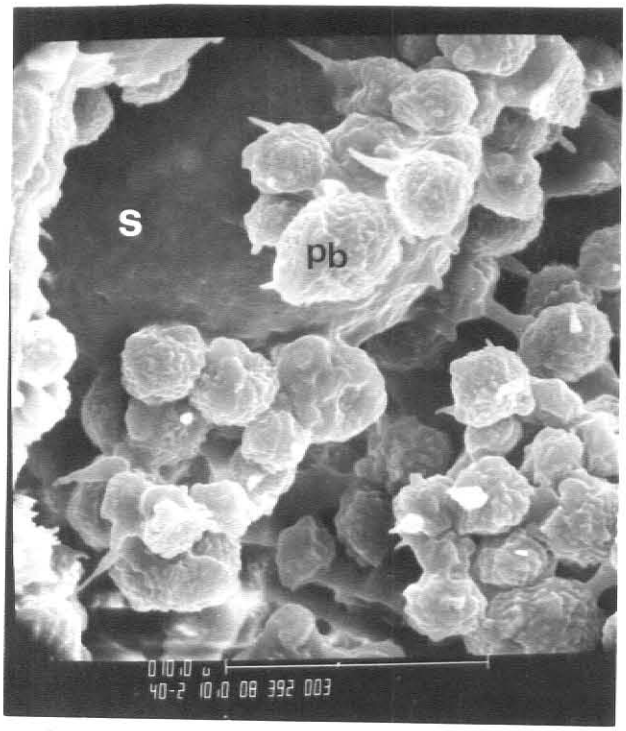
Figure 3: SEM's of beans that were soaked (a, b, c) and cooked (d) in deionized water. S indicates starch granules and pb indicates protein bodies.



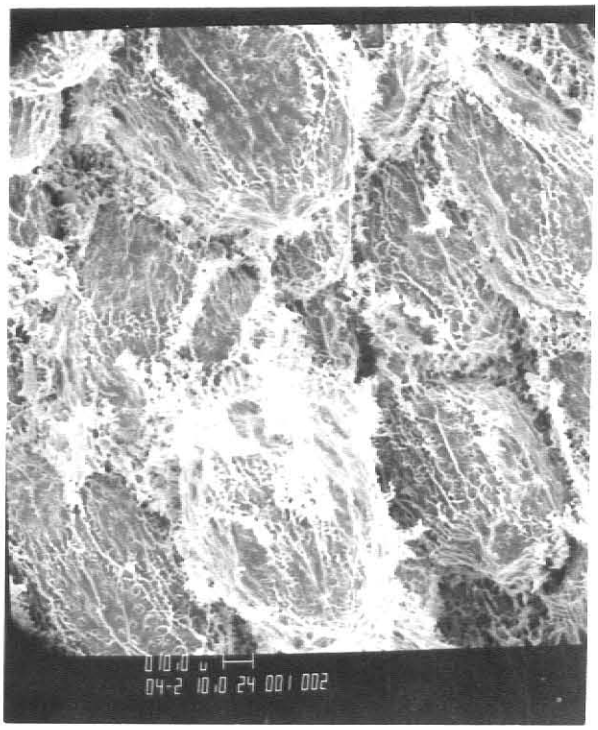
a



b



c



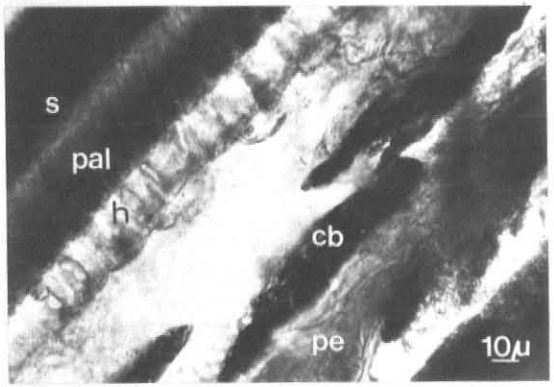
d

exists between the cells. Protein bodies can be easily seen in Fig. 3c. Some of the protein bodies appear to be pulling away from the starch granules, and bridges between these bodies can be observed.

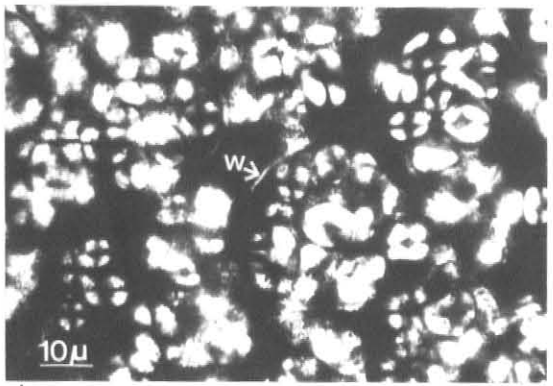
Cooking the beans for $1\frac{1}{2}$ hrs. at 110°C caused a drastic change in the structure. The protein bodies are no longer present as spherical shapes but appear to have formed films around the entire cell mass (Fig. 3d). It is quite certain that some of the material which surround each cell is protein since the light micrographs (Fig. 4) indicate that this cooking period did not completely gelatinize the starch granules to produce an exudate to cover the cell content. In addition, there is cell wall material covering these cell masses. The separation between cells is not very appreciable and the interrelationship between cells remained similar to that in the raw.

The effects of soaking in a 1% NaCl solution and cooking in deionized water can be seen in Fig. 5. There are fewer protein bodies than in the deionized water soaked beans, and they do not appear as hydrated (Figs. 5a and b). However, no significant differences ($P < 0.05$) were observed between these treatments for W.A. Therefore, those differences observed in structure could be a result of differences in pH and Na^+ concentration of the soaking solutions. The NaCl solution has a higher pH which would produce greater ionization of the protein and this would cause attraction of Na^+ to the ionized negative groups in the protein and result in changes in the structure that were observed. The data on mineral analysis (Table 4) support this theory since the amount of sodium increased in cooked beans which were first soaked in NaCl. Furthermore, salt solutions could increase the solubility of the proteins and this is probably the

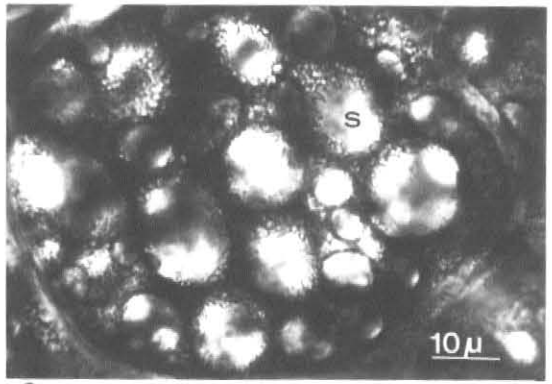
Figure 4. Representative light photomicrographs of black beans: (a) brightfield image of the seed coat of beans cooked in deionized water after soaking in deionized water; S, pal, cb, and pe indicate bean surface, palisade layer, colored bodies and parenchyma cells, respectively; (b) polarizing microscope image of the cotyledon from deionized soaked beans showing starch granules with maltese crosses enclosed by a cell wall, w indicates cell wall; (c) polarizing microscope image of All T. soaked beans showing birefringent starch granules covered with protein bodies; (d) polarizing microscope image of beans cooked in deionized water after being soaked in All T. solution; arrows point to one starch granule which still has its maltese cross; (e) brightfield image of bean cotyledon showing fibrous material.



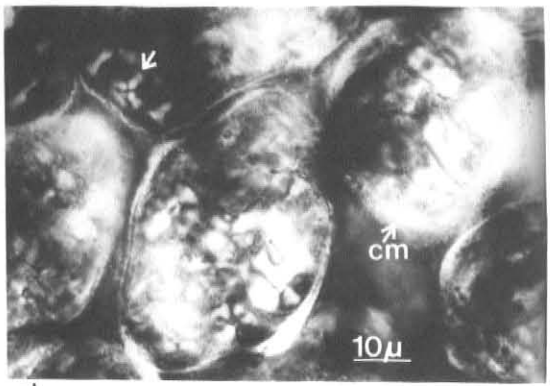
a



b



c



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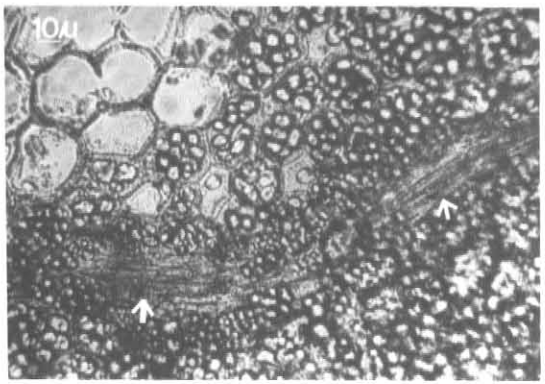
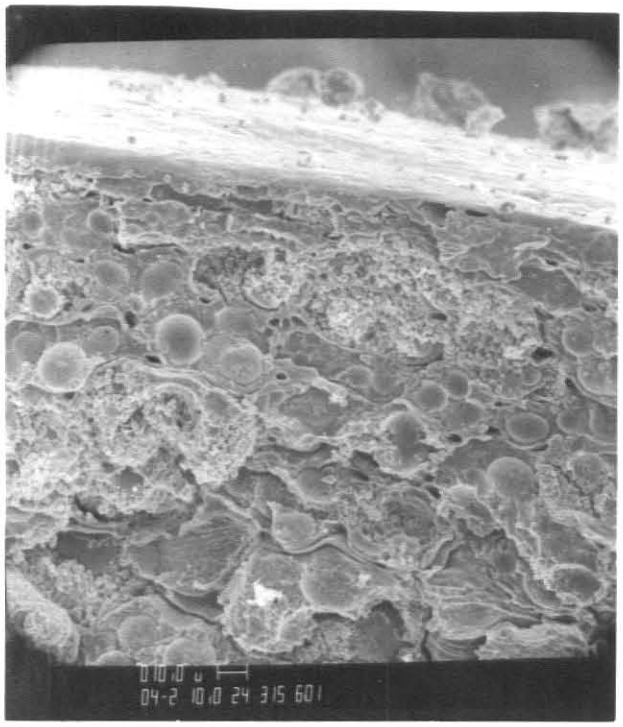


e

Figure 5. SEM's of beans that were soaked (a, b) in NaCl and cooked (c, d) in deionized water. S indicates starch granules, pb indicates protein bodies and cw indicates cell wall.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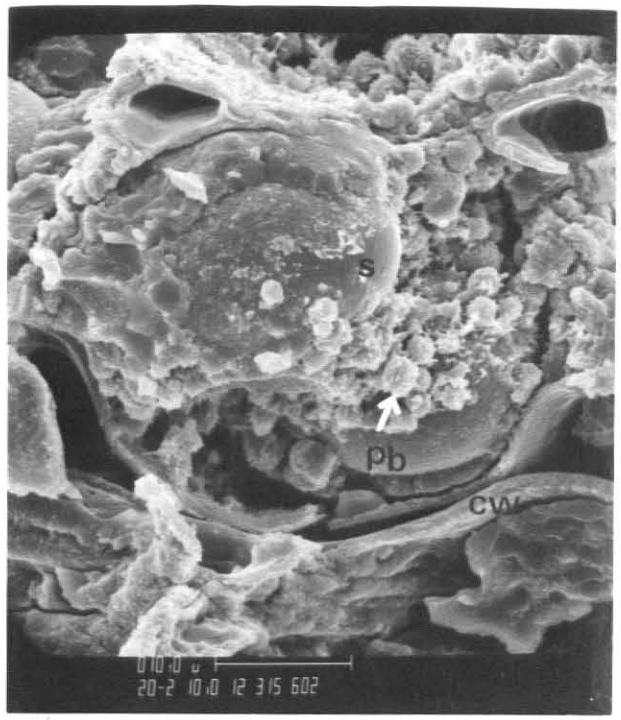
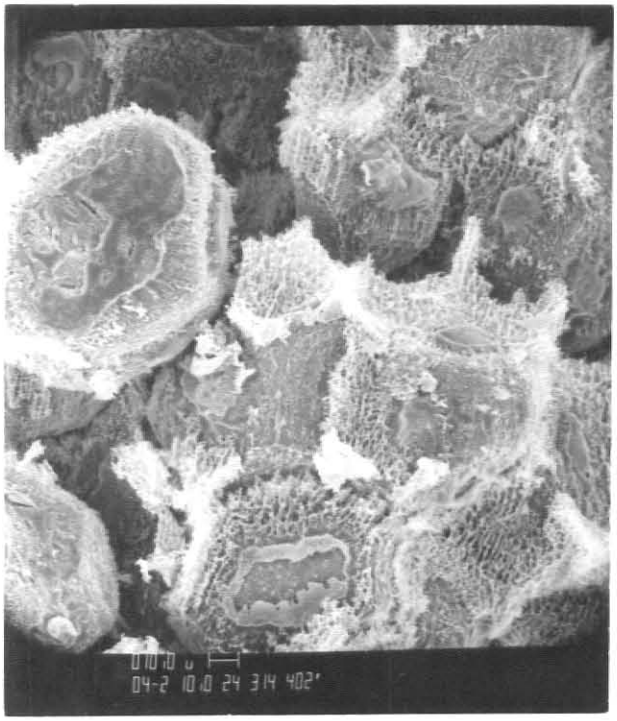
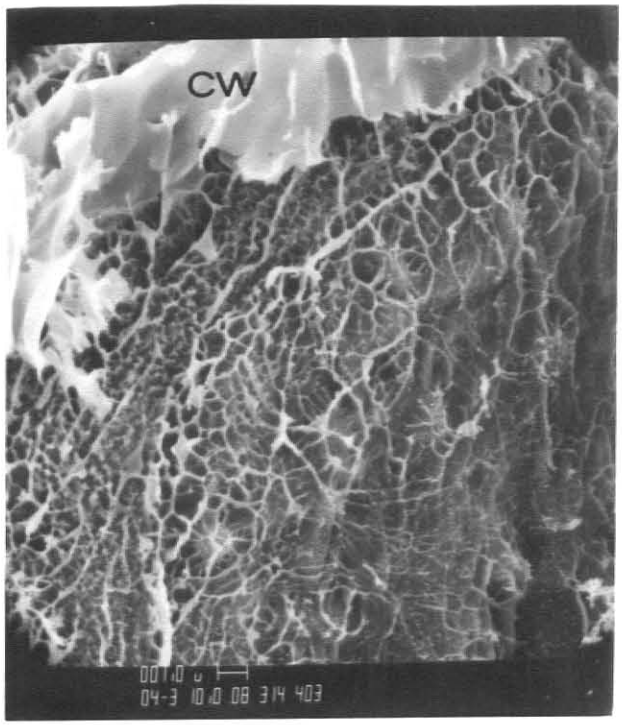
a


S
Pb
CW
20-2 10.0 12 315 602

b


04-2 10.0 24 314 402

c


CW
04-3 10.0 08 314 403

d

reason why we have fewer protein bodies present in the NaCl treated beans.

Cooking the NaCl soaked beans in deionized water produced a greater separation between cells (Fig. 5c) compared to the beans that were soaked and cooked in deionized water. Fig. 5d is used as an example to illustrate the branching of the cell wall material during cooking, and this was true for all the cooked beans. To verify that these structures were cell wall material, the polarizing microscope was used (see Fig. 4).

The SEM's from beans soaked in a 0.25% solution of Na_2CO_3 are presented in Fig. 6a & b. The separation between cells can be easily observed. This separation appears greater than that observed with deionized and NaCl soaking solution. Small fibers between two cell walls (Fig. 6a) appear to be holding the cells together. These bridges must be derived from the cell wall and they probably interact with the components of the middle lamella forming a stable matrix in untreated beans. The large separation of cells could be a result of a higher value of water absorption for Na_2CO_3 treated beans than for deionized and NaCl treated beans ($P < 0.05$). The Na_2CO_3 treatment has a very high pH (11.25) which would increase the water absorption because of the ionization of protein and pectic substances that are present in the middle lamella.

Protein bodies can be easily seen in the Na_2CO_3 treated beans (Figs. 6a & b). At higher magnification (Fig. 6b), the protein bodies look very disrupted and are embedded in a honeycomb protein matrix. Again, the high pH of this treatment could have affected the protein structure, coupled with the movement of ions from the protein structure during soaking (Table 4). After these soaked beans are cooked in deionized water a large separation

Figure 6. SEM's of beans soaked in 0.25% solution of Na_2CO_3 (a & b) and cooked in deionized water (c). pb = indicates protein bodies.

a
b
s
pb
c

and disorganization of the cell masses can be observed. The cell wall material is disrupted and is randomly distributed between the remaining cell masses. The alkaline pH of this treatment in combination with the heat effects probably changed the interaction between protein and pectic substances which would caused the leaching of the components from the middle lamella.

Beans that were soaked in a 0.75% solution of $NaHCO_3$ are shown in Figs. 7a and b. The cells remained very close together after the soaking period. Large amounts of protein bodies are observed and at a higher magnification the honeycomb shape of the protein material can be easily observed in Fig. 7b. The protein bodies exhibit a very rough appearance and protrutions appear on their surface. Beans during soaking in $NaHCO_3$ solution absorbed Na^+ and lost K^+ (Table 4) and this together with the alkaline pH could produce interruptions in the protein structure to form these protrutions which could allow more water to enter and interact with the protein bodies.

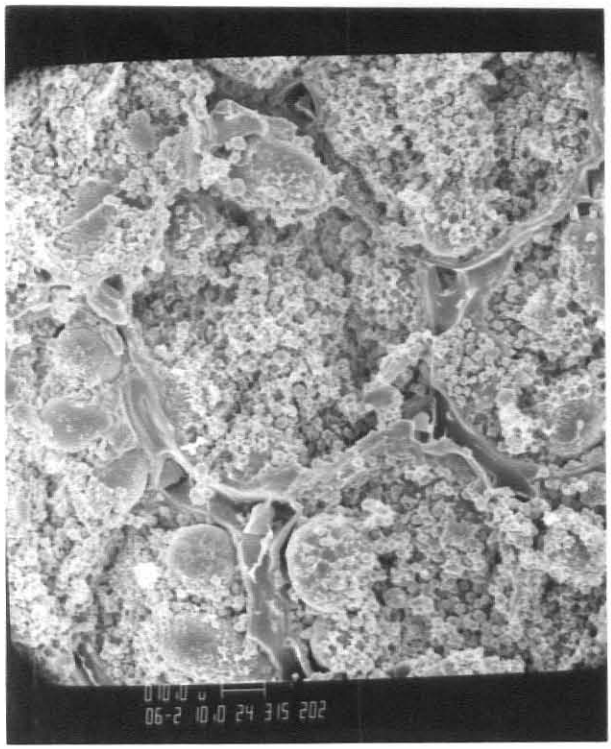
The cell masses observed after cooking beans soaked in $NaHCO_3$ solution (Fig. 7c) show little separation. A branching film appears to be spread on the surface of the cell masses. This film could be the result of the leaching of cell wall material and the denaturation of protein during the cooking period.

The SEM's of beans soaked in tap water (Figs. 8a & b) exhibited protein bodies but the honeycomb protein matrix cannot be seen as it appeared in those treatments with high concentrations of Na^+ (Figs. 6 & 7). Based on the composition of tap water, the changes in the protein bodies could be explained as a result of the Ca^{++} concentration in tap water. The cooked

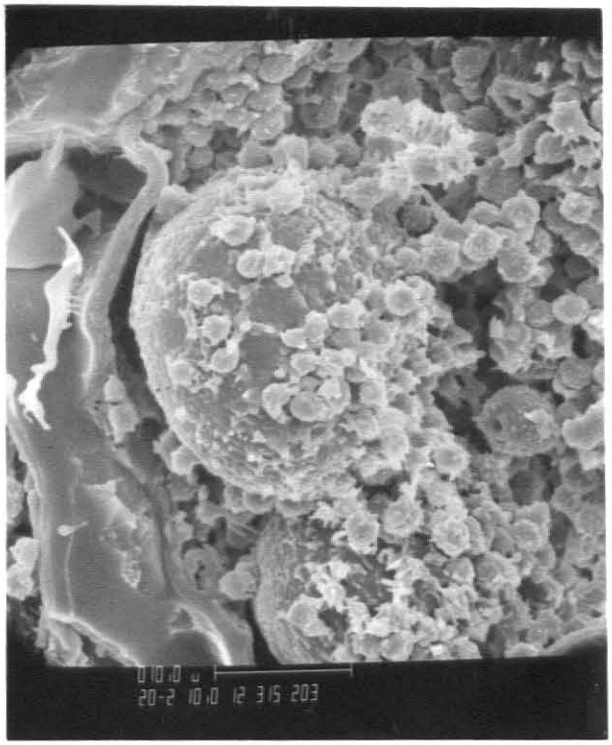
Figure 7. SEM's of beans soaked in a 0.75% solution of $NaHCO_3$ (a & b) and cooked in deionized water (c). S indicates starch granules.

a
b
c
S

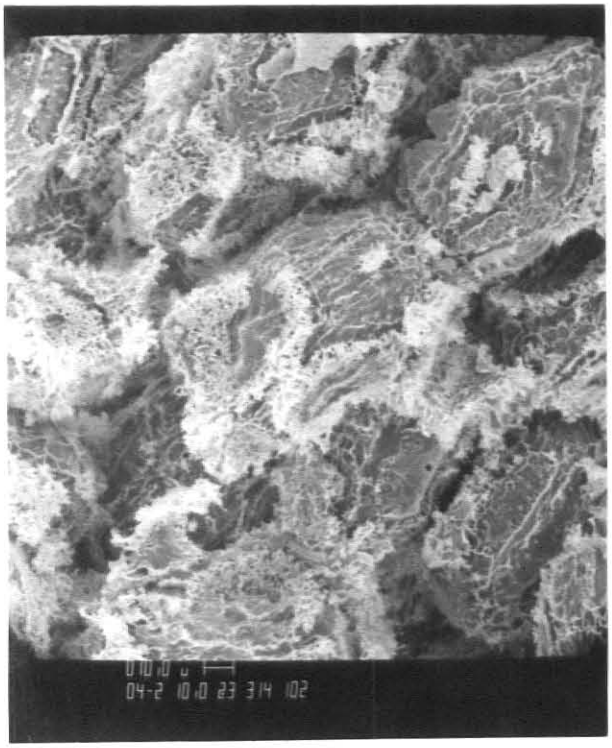
Figure 8. SEM's of beans soaked in tap water (a & b) and cooked in deionized water (c & d).

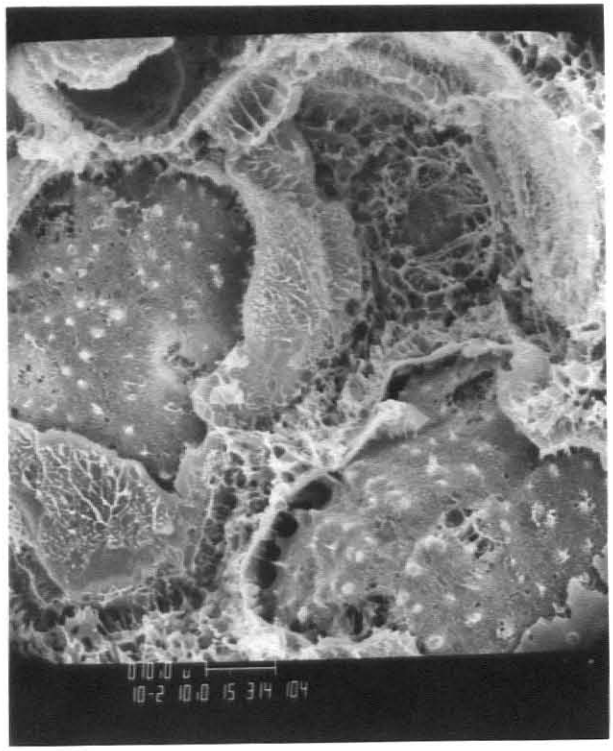

06-2 10.0 24 315 202

a


20-2 10.0 12 315 203

b


04-2 10.0 23 314 102


10-2 10.0 15 314 104

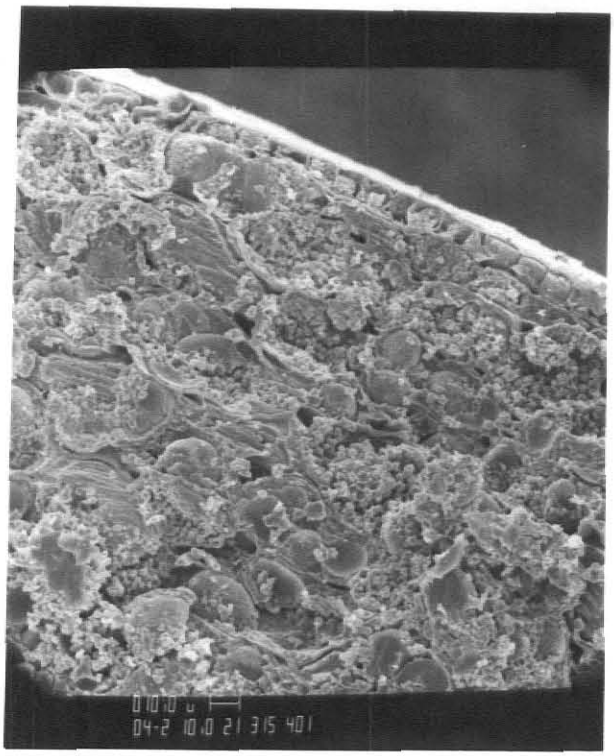
beans treated with tap water during the soaking period lost less Ca^{++} ($P < 0.05$) than the other treatments which contain Na^{+} in solution. Probably the material from the middle lamella is easier to leach out when the Na^{+} is present in the solution than when Ca^{++} exists in the solution. This theory agrees with the results from pectic substances (Table 3) where the beans treated with tap water resulted in less amounts of total pectic substances extracted.

The SEM's from beans cooked in deionized water after soaking in tap water is shown in Fig. 8c & d. The cell masses with attached cell wall material are separated from each others. Also, small bright spots are observed on the surface of the cell masses after cooking (Fig. 8d). These spots are probably what remains of the protein bodies after denaturation during the cooking period.

The protein bodies observed when beans were soaked in a 0.5% solution of $Na_5P_3O_{10}$ (Figs. 9a & c) appear to have bridges between them and they are not as disrupted as those in Na_2CO_3 soaked beans. In addition, the honeycomb protein matrix is not observed here (Fig. 9c). High concentrations of Na^{+} as well as the alkaline pH are present in this treatment, and both are capable of altering the structure of the proteins.

Structures of beans cooked in deionized water after being soaked in the $Na_5P_3O_{10}$ treatment are shown in Figs. 9b and d. The palisade cells have cracks in their surface after cooking (Fig. 9b), and this was observed in all of the treatments. Appreciable separation between cells after the cooking period was observed (Fig. 9d). This separation was undoubtedly due to the prior changes during soaking in $Na_5P_3O_{10}$. The $Na_5P_3O_{10}$ probably was able to assist in the removal of the substances from the middle lamella

Figure 9. SEM's of beans soaked in a 0.5% solution of $Na_5P_3O_{10}$ (a & c) and cooked in deionized water (b & d). Palisade cells (pal), hourglass cells (h), parenchyma cells (pe) are identified in Fig. 9 b.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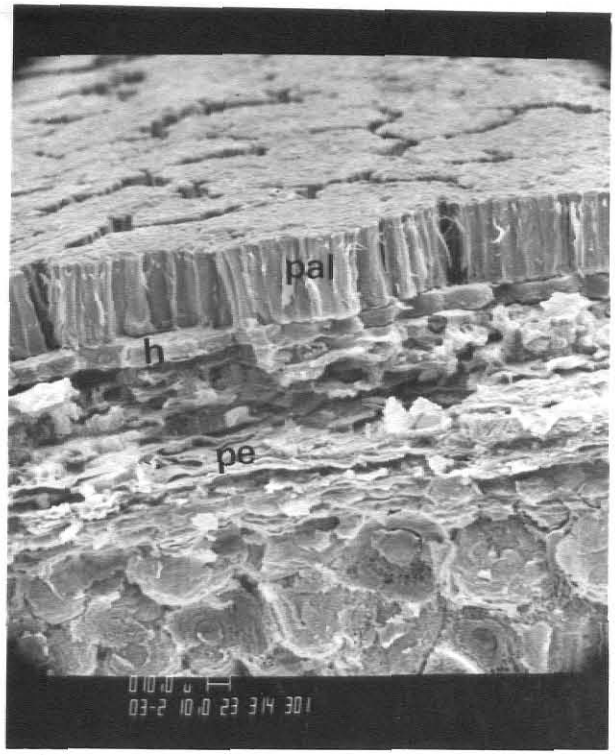
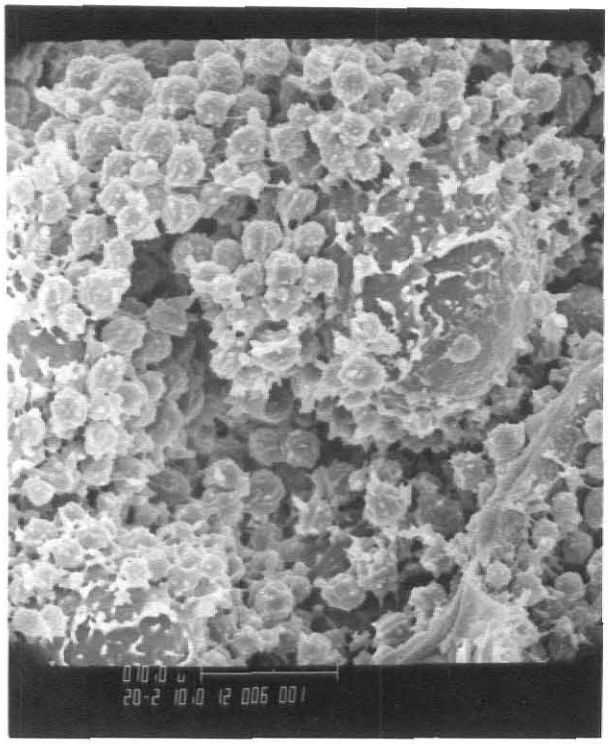
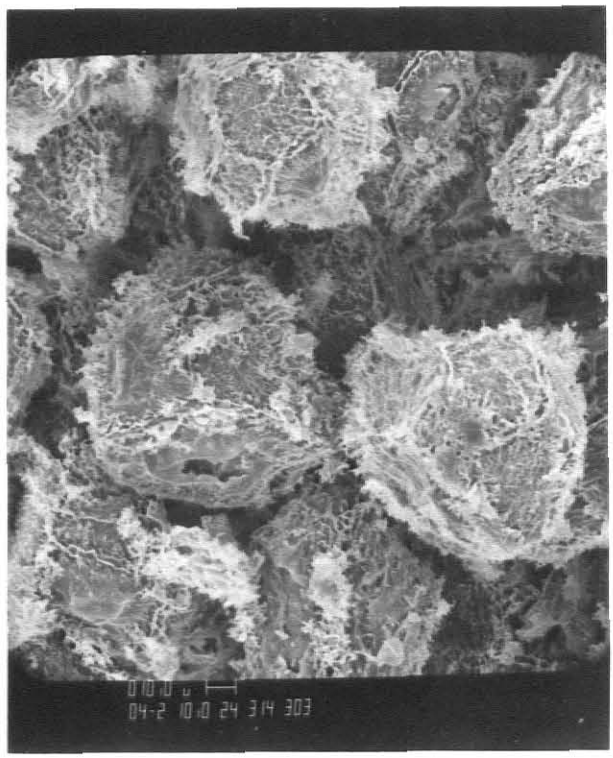
a


pal
h
pe
03-2 10.0 23 314 301

b


20-2 10.0 12 006 001

c


04-2 10.0 24 314 303

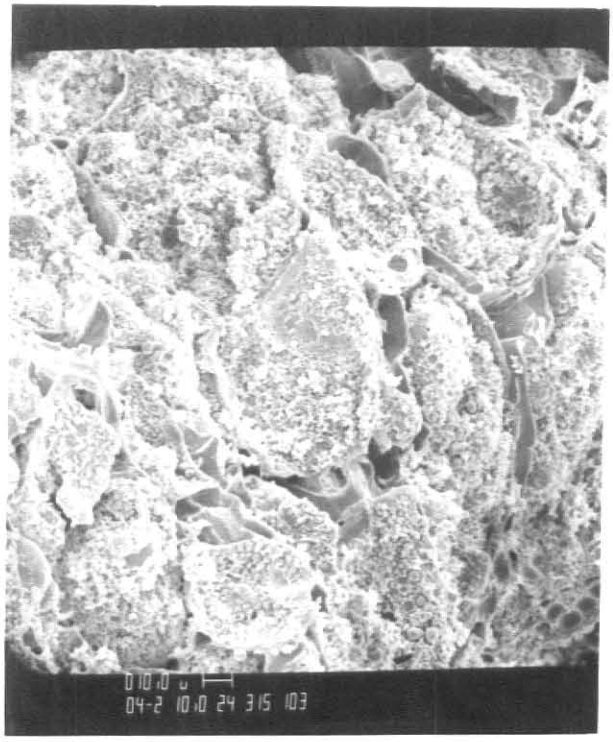
d

because of its chelating ability. The fact that large amounts of Ca^{++} and Mg^{++} were lost from the beans during soaking corroborates these speculations (Table 4). In addition, this is in agreement with the determination of pectic substances (Table 3) where one of the highest values obtained was from the beans treated with $Na_5P_3O_{10}$, and the amount of pectic substances present in the water extract was the highest amount of all of the other treatments.

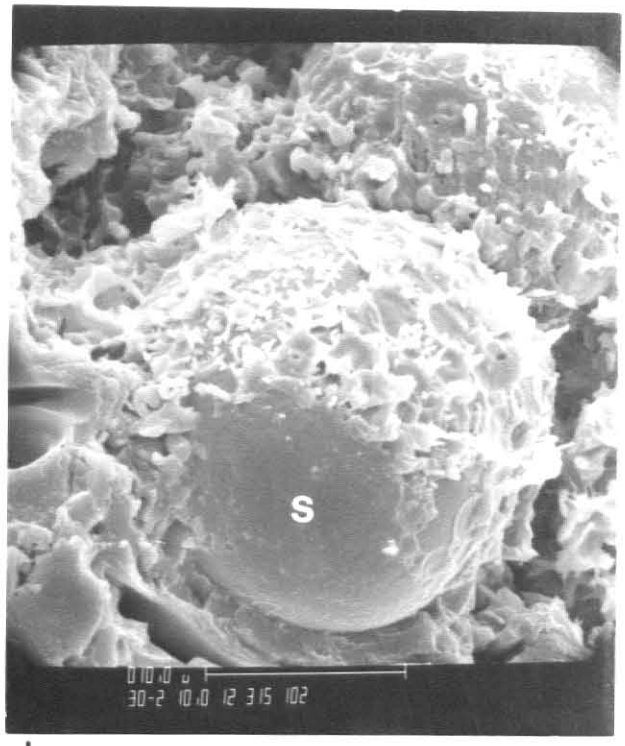
The SEM's of beans soaked in the All Treatment solution are presented in Figs. 10a & b. Protein bodies appear smaller than the ones observed in the other treatments. At higher magnifications, the typical protein bodies that were previously observed do not appear here. The starch surface does not show a honeycomb structure with this treatment. The concentration of Na^+ could produce increased solubility of the protein which would result in the leaching of the protein from the beans.

When the All T. beans were cooked in deionized water, a very large separation between cells as well as disorganization among them appeared. An exhaustive removal of the substances in the intercellular cement could be responsible for this large separation of the cell masses after cooking. This result is in agreement with the values obtained from the pectic substances determination (Table 3). The portions of cell wall material that remain appear as bright structures (Fig. 10c). A fibrous material was also observed transversing the cotyledon (Fig. 10d). This fibrous material could be lignified protein which has been mentioned in the literature as responsible for the hardness of bean legumes (Molina et al. , 1976). Light microscope studies also showed these fibrous structures (Fig. 4e).

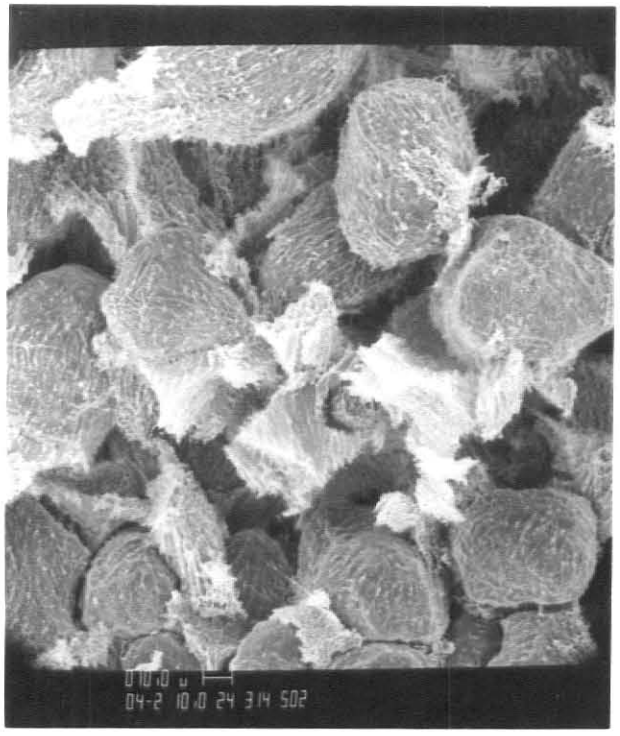
Figure 10. SEM's of beans soaked in a All Treatment solution (a & c) and cooked in deionized water (c & d). S = starch granule. f = fibrous material.


04-2 10.0 24 315 103

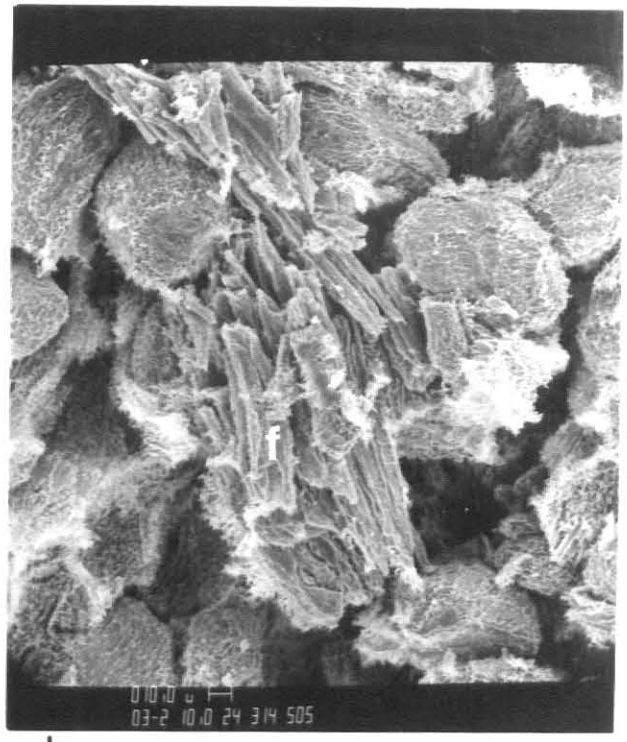
a


S
30-2 10.0 12 315 102

b


04-2 10.0 24 314 502

c


f
03-2 10.0 24 314 505

d

Effects of Heat Treatments

Moisture content. Beans that were steam or retort treated lost moisture during the heat process. Raw beans contained 12.00% moisture while steam treated beans contained 10.72%, retort 2 min. contained 10.15% and retort 5 min. contained 10.19%. These data are not in agreement with those of Molina et al. (1976). They found that retort treatments did not produce any changes in moisture content, but that steam treatments caused an increase in the moisture content of the beans. The differences in results between the two studies may be due to slight differences in processing treatments. The dehydration (loss of moisture) that was observed in this study undoubtedly affected the components or interaction between components of the beans.

Water absorption and pH. The results of water absorption and pH for the different heat treated samples using the Molina et al. (1976) method are presented in Table 5. No significant differences existed between the three treatments.

Increases in the pH's of the soaking waters were observed when heat treated samples were soaked in deionized water. It is possible that during the heat treatments the bean proteins were altered so that more groups were exposed which could attract protons from the soaking water and therefore, result in an increase in the pH.

Minerals. The data from the mineral analysis are shown in Table 6. Potassium was lost from the beans during the processing steps. Most of this loss occurred during soaking. The steam 10 min. treatment resulted in significantly less leaching ($P<0.05$) of K^+ from the beans compared to the retort treatments. During the heat treatment with steam, the beans

TABLE 5. WATER ABSORPTION VALUES FOR HEAT TREATED BEANS AND pH's OF DEIONIZED WATER BEFORE AND AFTER SOAKING AND AFTER COOKING

Treatment	Water Absorption[b]	pH of Deionized Water		
		Before Soaking	After Soaking	After Cooking
Untreated beans	99.90 ± 1.00	5.45 ± 0.43	5.15 ± 0.4	5.73 ± 0.4
Steam 10'	104.04 ± 5.71[a]	5.10 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Retort 2'	95.32 ± 3.63	5.10 ± 0.00	5.98 ± 0.04	6.00 ± 0.00
Retort 5'	98.39 ± 2.28	5.10 ± 0.00	5.90 ± 0.00	5.95 ± 0.00

[a] Values are the mean plus the standard deviation from the mean.

[b] Expressed as percentage of the dry weight.

TABLE 6. MINERALS IN BEANS AND IN SOAKING AND COOKING WATERS[a] AS AFFECTED BY HEAT-TREATMENTS.

Treatment	K^{+}(mg/g)	Ca^{++}(mg/g)	Mg^{++}(mg/g)
Raw beans	13.50 ± 3.40	0.32 ± 0.06	1.85 ± 0.04
Steam 10'			
Cooked beans	1.05 ± 0.05	0.27 ± 0.04	0.74 ± 0.06
S. W.[b]	9.00 ± 0.00	0.08 ± 0.01	0.65 ± 0.09
C. W.	3.15 ± 0.19	0.01 ± 0.00	0.14 ± 0.04
Retort 2'			
Cooked beans	1.01 ± 0.19	0.31 ± 0.04	0.59 ± 0.01
S. W.	10.95 ± 0.35	0.08 ± 0.00	1.04 ± 0.01
C. W.	3.15 ± 0.20	0.01 ± 0.00	0.04 ± 0.01
Retort 5'			
Cooked beans	0.89 ± 0.07	0.30 ± 0.10	0.65 ± 0.06
S. W.	10.50 ± 0.14	0.09 ± 0.01	0.94 ± 0.10
C. W.	1.88 ± 0.49	0.01 ± 0.01	0.04 ± 0.03

[a] Values are the means plus the standard deviation from the means and are expressed on the dry basis.

[b] S. W. and C. W. mean soaking and cooking water, respectively.

were subjected to 98°C which is a milder treatment than with the retort where the beans are submitted to 15 p.s.i. and 121°C. Therefore, the retort treatment would produce much larger changes than steam treatment. The macro-molecular complexes present in the intercellular structure would suffer changes and become unstabilized. Therefore, the ions present in the complex would be released easier than the beans treated with steam. Potassium is one of the ions which plays an important role in the stabilization of the intercellular structure (Ginzburg, 1961).

Calcium principally remained in the cooked beans. No significant differences ($P < 0.05$) appeared between the 3 heat treatments. Unlike K^+, only small amounts of Ca^{++} went to the solution during soaking and still less was observed to migrate during cooking. Any one of these heat treatments are strong enough to affect the binding of Ca^{++} in the middle lamella. Apparently the Ca^{++} ion strongly interacts with the large molecular components of the intercellular cement.

The magnesium in heat treated beans was observed to leach from the beans especially during the soaking period. The losses of Mg^{++} were also higher in the retort treated samples than in the steam treated one. The same reasoning that was applied to K^+ could also be applied here. Monovalent and divalent ions have been reported to be bound at different sites on the protein in the middle lamella (Ginzburg, 1961), it is possible that K^+ and Mg^{++} show less affinity for binding to the protein under these conditions than does Ca^{++}.

Soluble nitrogen. In general, the amount of soluble nitrogen extracted from raw heat treated beans was comparable to the amount extracted from the raw untreated beans (Table 7). However, when the steam and retort treated

beans were soaked in deionized water higher amounts of protein (soluble nitrogen) were lost into the soaking water as compared with the soaked untreated beans ($P < 0.05$). The high value of soluble nitrogen obtained in the soaking water of heat treated samples probably represents the breakdown of the protein or the breakdown of the interactions between proteins and other molecules in the middle lamella to result in increased solubilization of proteins.

Smaller amount of nitrogen were lost from the heat treated beans during cooking than during soaking. This is contrary to what was found for the cooking water from the beans with no heat treatment. Apparently most of the changes in (degradation of) the protein in the heat treated beans occurs during the heat treatments while the opposite is observed for the untreated beans. Undoubtedly, some of the proteins that were denaturated in the heat treated samples were enzymes which could produce changes in the beans during storage to result in the development of the hardshell phenomenon.

Ultrastructure of heat treated beans. The SEM's of untreated and heat treated beans are presented in Fig. 11. The center of the cotyledon shows the starch granules embedded in the protein matrix. After the beans were heat treated no apparent differences were observed in the protein matrix of the steam 10 min. retort 2 min. and in raw untreated. However, the protein matrix of the retort 5 min. treated beans appears more dehydrated showing open spaces in the matrix. Probably these differences are due to the decrease in moisture found after the beans were heat treated.

The SEM's of steam treated beans after soaking (a & c) and after cooking (b & d) are presented in Figure 12. Irregularly shaped cells as

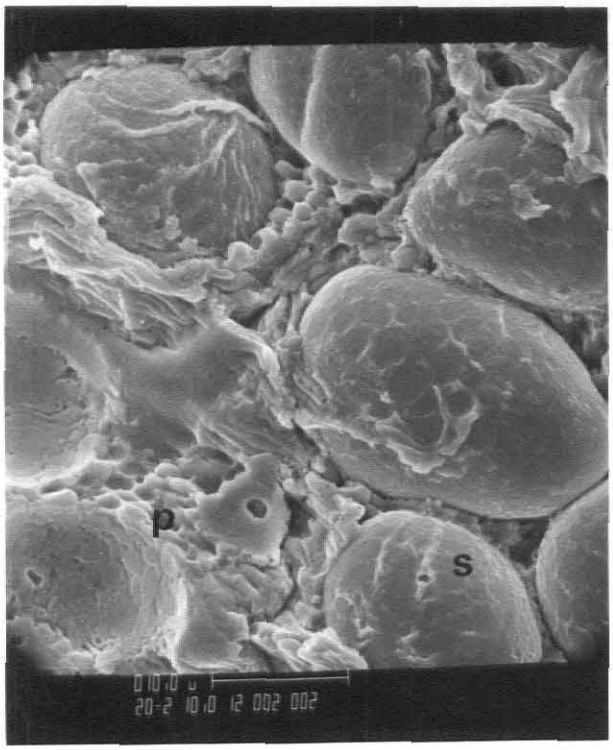
TABLE 7. PERCENTAGE OF WATER SOLUBLE NITROGEN IN HEAT TREATED BEANS AND IN SOAKING WATER AND COOKING WATER[a]

Treatment	% Water-Soluble Nitrogen
Steam 10'	
Raw	2.19 ± 0.09
S. W.[b]	0.50 ± 0.13
C. W.	0.38 ± 0.15
Retort 2'	
Raw	2.33 ± 0.01
S. W.	0.55 ± 0.00
C. W.	0.30 ± 0.00
Retort 5'	
Raw	2.02 ± 0.05
S. W.	0.60 ± 0.02
C. W.	0.37 ± 0.00
Raw Beans	2.16 ± 0.18
S. W.	0.16 ± 0.02
C. W.	0.56 ± 0.03

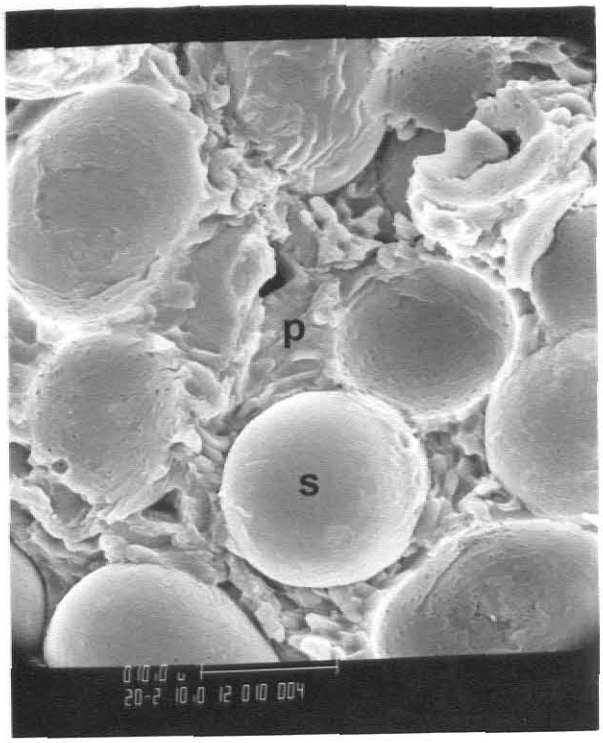
[a] Values are the mean plus the standard deviation from the mean.

[b] S. W. and C. W. mean soaking and cooking water respectively.

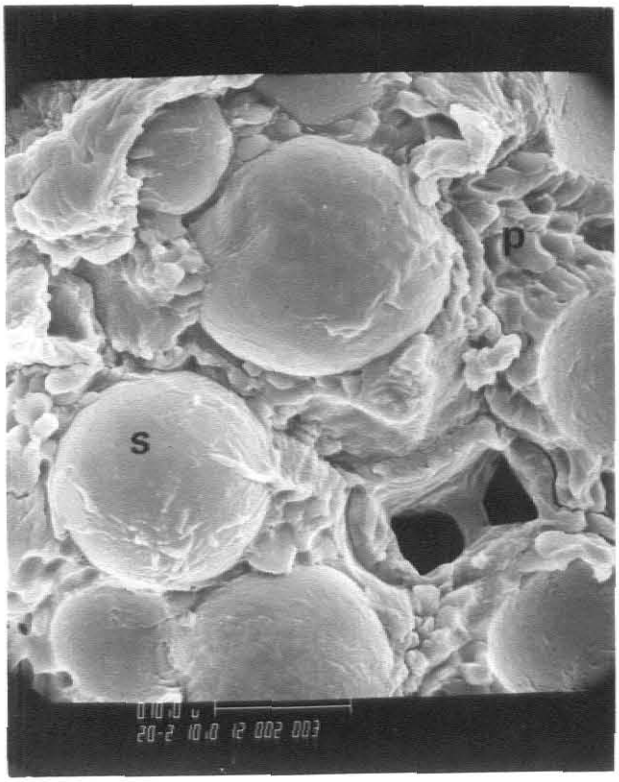
Figure 11. SEM's of raw beans (a), steam 10' heat treated beans (b), retort 2' heat treated beans (c), and retort 5' heat treated beans (d).


p
s
20-2 10.0 12 002 002

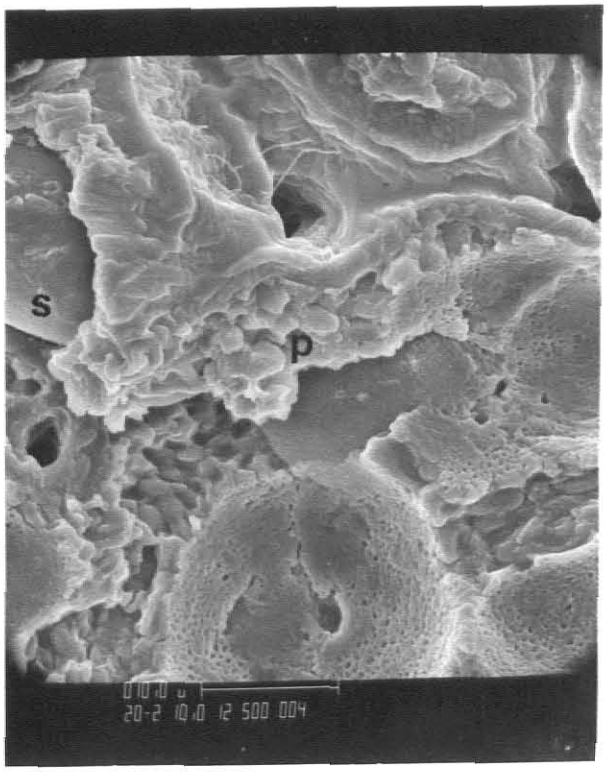
a


p
s
20-2 10.0 12 010 004

b


p
s
20-2 10.0 12 002 003

c


s
p
20-2 10.0 12 500 004

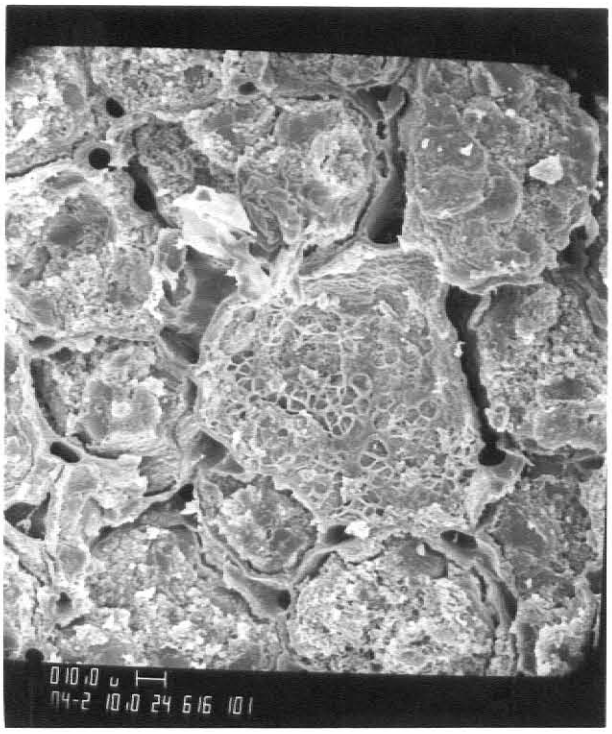
d

well as separation between cells is observed after the soaking period (Figure 12a). The protein bodies (Figs. 12a & c) that are present are unlike those observed in the first experiment (see pages 27, 30, 32). They appear disrupted and coral-like in shape (Fig. 12c) compared to the untreated soaked beans which have spherical protein bodies (Fig. 3c). In addition, some of the protein bodies appear as films covering the cells (Fig. 12a). Undoubtedly, the heat treatment produces some changes in the protein components of the beans since the heat treatments significantly ($P < 0.05$) increased the amount of soluble protein leached into the soaking water (Table 7).

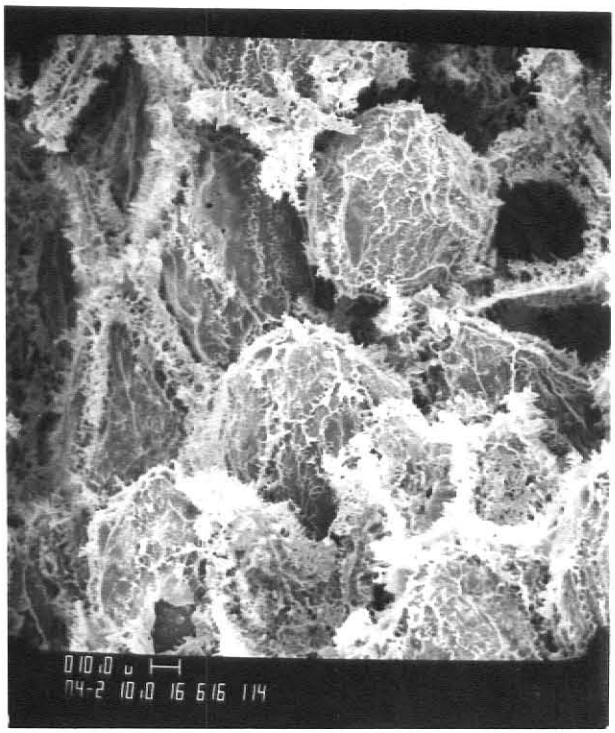
The cell masses of the steam treated soaked beans remained close to each other after cooking (Fig. 12b), and they appeared to be covered with a bright material branching over the cell masses which has been identify as cell wall material (see Fig. 4). At higher magnification the interior or the cell mass showed the starch granules organized within the cell (Fig. 12d). It can also be observed in Fig. 12 d that there is a layer covering the starch granules in the cell mass. This layer is probably a combination of denatured protein and degraded cell wall material.

Micrographs of retort 2 min. heat treated beans after soaking and cooking in deionized water are presented in Fig. 13. A double wall can be observed in the soaked sample (Fig. 13a) suggesting that the cell contents are pulling away from the cell wall. This could be the result of plasmolysis which occurs due to the combined effects of the heat treatment and the minerals in the soaking water. The data in Table 6 shows that during soaking large amounts of K^+ and Mg^{++} were lost into the soaking water. This could result in the movement of water from the beans (which contain low concentrations of these ions) to the soaking solution (with high concentration

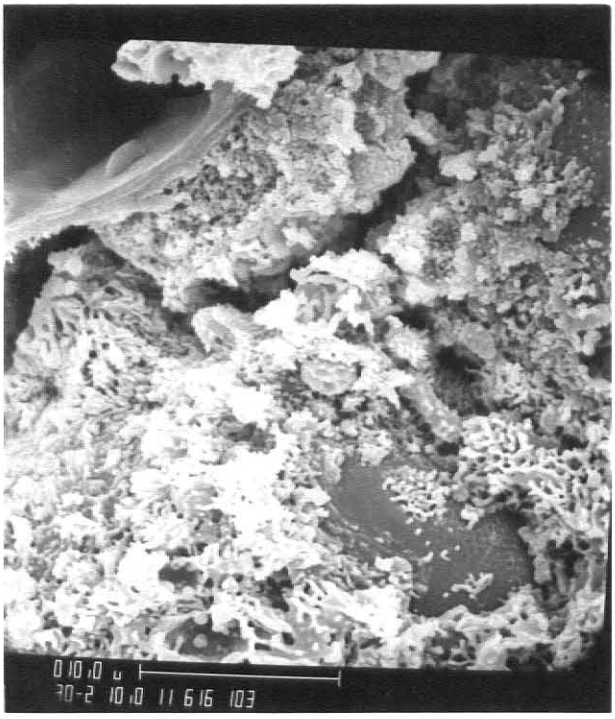
Figure 12. SEM's of heat treated beans: steam 10' after soaking in deionized water (a, c) and after cooking in deionized water (b, d).


010,0 u
04-2 10,0 24 6 16 101

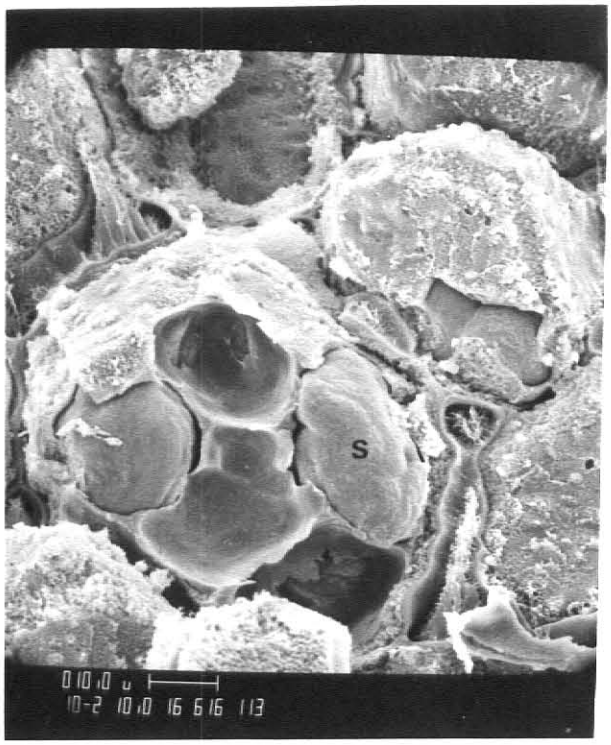
a


010,0 u
04-2 10,0 16 6 16 114

b

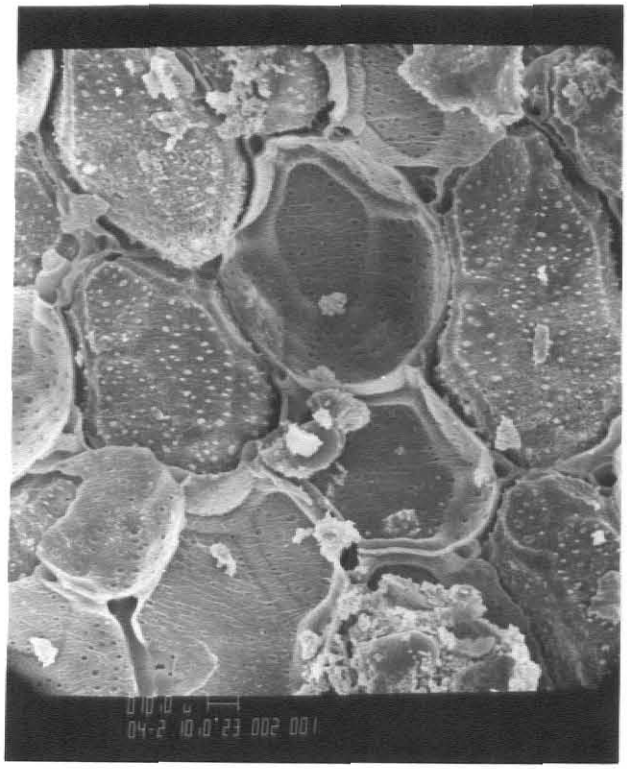

010,0 u
30-2 10,0 11 6 16 103

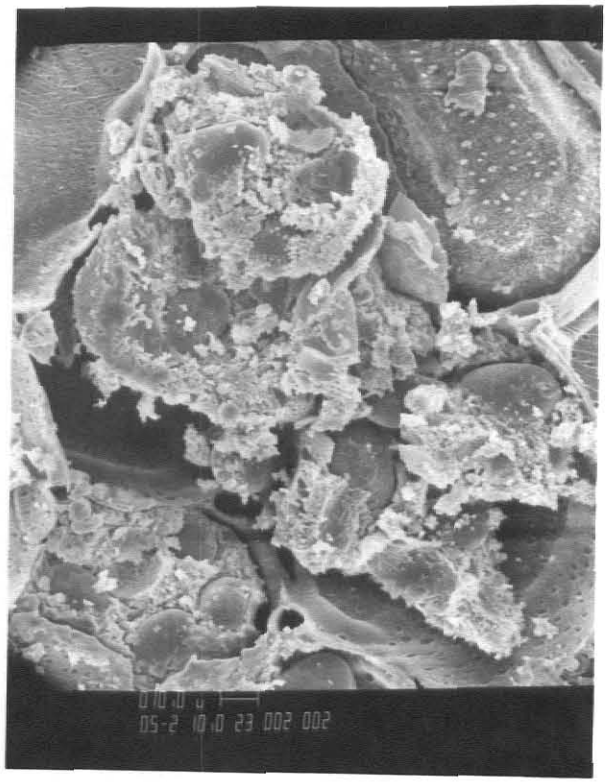
c


S
010,0 u
10-2 10,0 16 6 16 113

d

Figure 13. SEM's from heat treated beans: retort 2' after soaking in deionized water (a, b) and after cooking in deionized water (d). Light micrograph of retort 2' after cooking in deionized water (c).


04-2 10.0 23 002 001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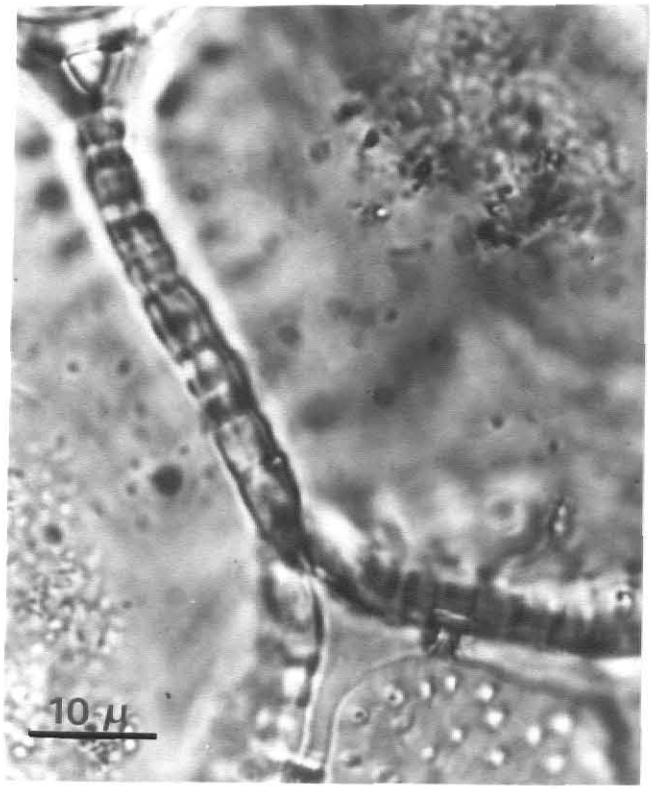
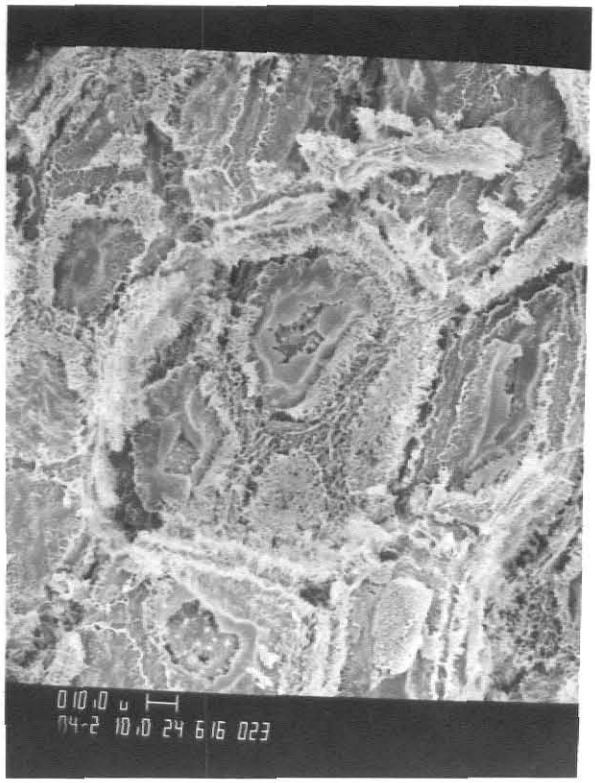

10 µ


04-2 10.0 24 6 16 023

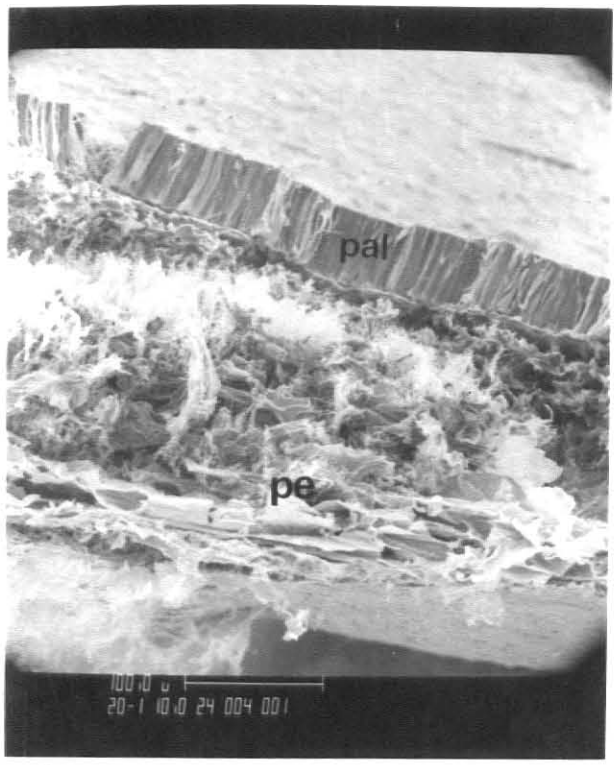
of K^+ and Mg^{++}) by the mechanism of osmosis.

Small bright spots can be observed on the surface of the cells (Figs. 13 a & b). These spots could be the structure remaining from the protein bodies which were altered during the heat treatment, leaving only the centermost part of the protein bodies. Yould and Huand (1976) found that protein bodies isolated from castor beans had a crystalloid center. It is possible that the spots that appear in Figs. 13 a & b are the crystal portion of the protein bodies present in black beans. These structures exhibited slight birefringence (Fig. 13d) suggesting at least that some ordered structure exists.

The retort 2 min. sample that was cooked in deionized water is shown in Fig. 13 c. Unlike the steam 10 min. treatment (Fig. 12b) the inter-relationship between the cells remained intact. High concentrations of cell wall material are located at the edges of the cells, and the cell wall material appears to be covering the cell masses. In addition, some remnants of the protein bodies can still be seen. The overall structure of the cooked product is similar to that present when beans were soaked in NaCl, deionized water, tap water and $NaHCO_3$ in experiment 1 (Figs. 5, 3, 8, 7, respectively).

The SEM's of retort 5' treated beans soaked and cooked in deionized water are presented in Fig. 14. The palisade layer of the seed coat appears very enlarged after soaking (Fig. 14a). This effect was easily observed with the naked eye after the beans were soaked. The heat treatment may have altered the palisade layer of the seed coat so that increased amounts of water were allowed to move through this layer and accumulate outside of the cotyledon. This effect was not observed in experiment 1, and indicates that the heat treatment affected the seed coat. Therefore, this could be

Figure 14. SEM's from retort 5' heat treated beans after soaking (a, b, c) and after cooking (d) in deionized water. pal, pe and s indicates palisade layer, parenchyma cells and starch granules, respectively.


pal
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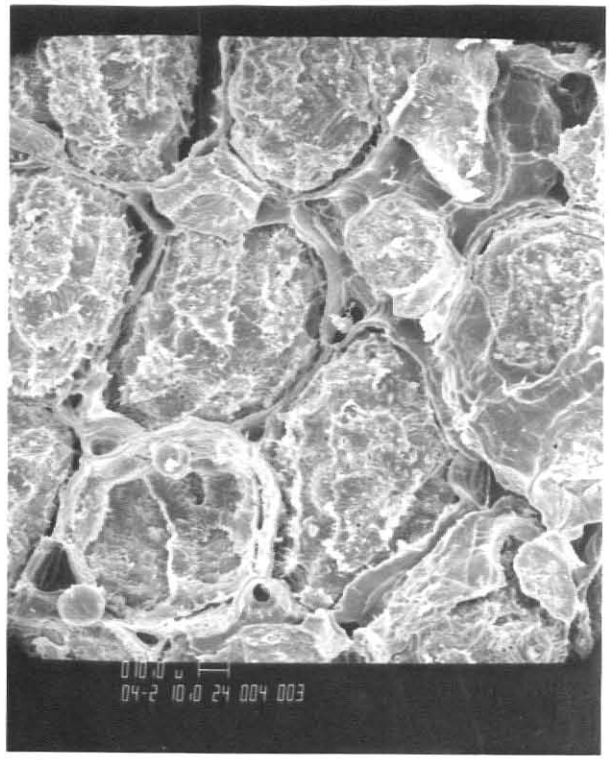
20-1 10.0 24 004 001

a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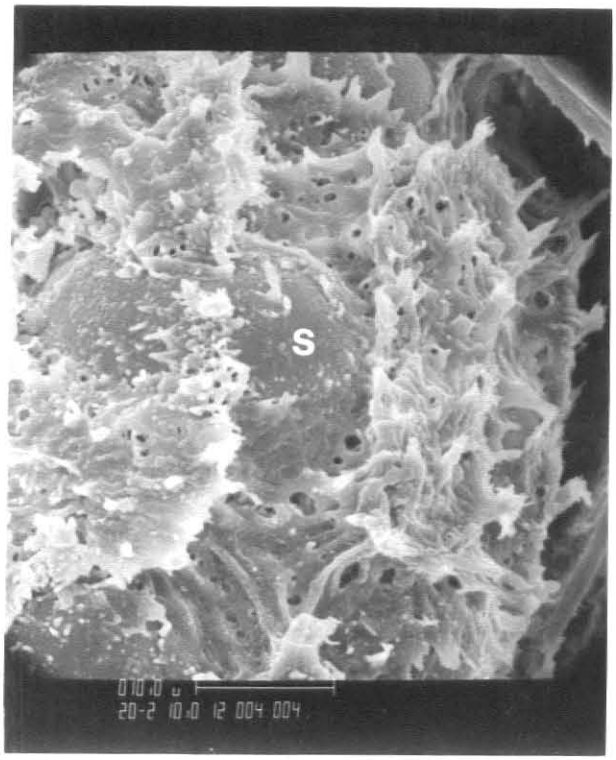
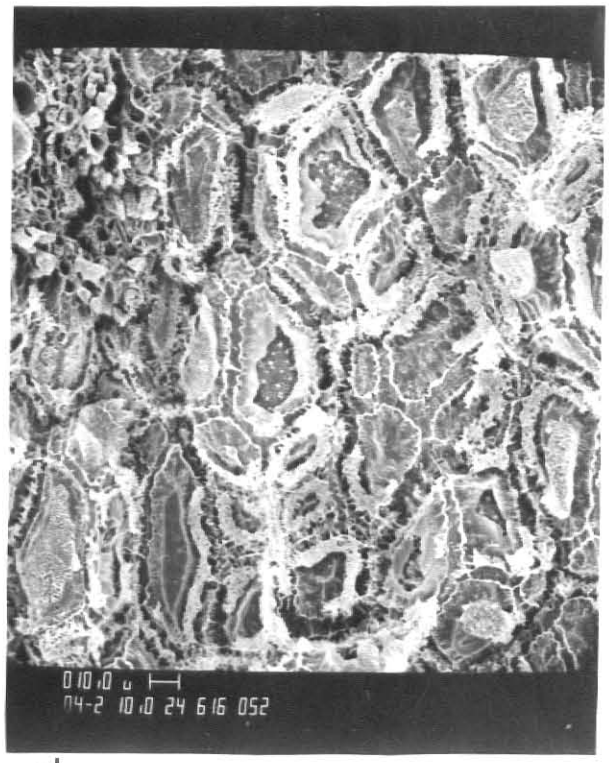
04-2 10.0 24 004 003

b


s
010.0 u
20-2 10.0 12 004 004

c


010.0 u
04-2 10.0 24 616 052

d

an indication of how these heat treatments prevent the development of the hardshell phenomenon in beans.

The retort 5 min. treatment had a greater effect than the steam 10 min. and retort 2 min. on the protein bodies, (Figs. 14b & c) even though the amount of protein that was solubilized did not differ for the two retort treatments (Table 7). It is possible that the slightly longer retort treatment had a greater effect on the structure without greatly affecting the chemistry of the beans. The other structural effects observed in the retort 2 min. soaked treatment and the osmotic changes that were discussed above could also be applied here. Finally, the cooked beans in (Fig. 14 d) show the same structural characteristics as retort 2 min. cooked (Fig. 13 c).

SUMMARY AND CONCLUSIONS

Black beans (Phaseolus vulgaris) were used as a model to study the effects of sodium salt solutions and short-time heat treatments on the chemistry and structure of these seed legumes. In the first experiment, black beans were soaked in the following solutions: 1% NaCl, 0.75% $NaHCO_3$, 0.25% Na_2CO_3, 0.5% $Na_5P_3O_{10}$ or a solution called "All Treatments" (All T.) which is a combination of all of the above salts. Beans were also soaked in deionized and tap water to use as control comparisons. After soaking, the beans were drained and cooked in deionized water. Water absorption (W.A.), pH, pectic substances, and mineral determinations were made. In addition, light and scanning electron microscopical studies were conducted.

Water absorption values determined after 24 hr. of soaking were lower for beans soaked in NaCl, tap water and deionized water than for beans soaked in $Na_5P_3O_{10}$ ($P < 0.05$). However beans soaked in Na_2CO_3, All T. and $NaHCO_3$ solutions exhibited similar values for W.A. as did $Na_5P_3O_{10}$. The pH of the soaking solutions and W.A. showed a correlation coefficient of + 0.54, $P < 0.04$. Although the correlation was significant, the low value suggested that the pH was not the only factor influencing water absorption.

The pH of the soaking solutions in general decreased during the soaking period. This decrease in pH was probably caused by the ionization of proteins, pectic substances and other ionizable molecules present in the seed legumes.

Decreases in the pH were also observed during cooking. This effect was probably due to the breakdown and leaching of the intercellular material into the cooking water. The pectic substances contain large

proportions of carboxylic acid groups and therefore, a drop in pH would be expected.

The amount of pectic substances extracted from raw beans was low compared to beans treated with the different soaking solutions or the raw beans treated with pectinase or α-amylase. The high amounts of Na^+ in the solutions and the chelating ability of $Na_5P_3O_{10}$ could result in the removal of divalent ions. This would increase the extractability of the pectic substances.

The mineral analysis showed that greater losses of minerals occurred during soaking than during cooking, and that large losses of K^+ occurred in every treatment. Sodium was found to be absorbed by the beans when beans were soaked in solutions containing Na^+. In addition, when high amounts of Na^+ were present in the soaking solution, large losses of Mg^{++} were observed. The pH also appeared to affect the interchange of Na^+ and Mg^{++} but the exact relationship is complex and unknown at this time.

Some relationships existed between the chemical and microscopical data. The separation of cells in the cooked beans appeared to be related to W.A., i.e. the higher the water absorption the greater the cell separation. Cell separation was also related to the leaching of pectic substances from the beans into the soaking and cooking waters. Those treatments which resulted in the leaching of large amounts of pectic substances from the beans ($Na_5P_3O_{10}$, All T.) showed large cell separation. In addition, the structure of the protein bodies changed with the treatments; the higher the pH the more disrupted the protein bodies were.

In the second experiment when the beans were steam and retort heated, no significant differences were observed in the W.A. values for the 3 heat

treatments. However, the pH increased after soaking the heat treated beans in deionized water. This effect is probably due to the protonation of the ionized groups present on the macromolecules of the heat treated beans.

Losses of minerals during soaking of the heat treated beans were appreciable. This effect was especially observed with K^+ and Mg^{++}.

The heat treatments, especially retort 5', caused dehydration of the protein matrix and this could be seen using the SEM. The ultrastructural studies also showed changes in the appearances of the protein bodies. Denaturation of the protein occurred and left what appeared to be the crystalline centermost portion of the protein bodies deposited on the cells. Chemical studies showed that the amount of soluble nitrogen leached into the water during soaking was increased by the heat treatments.

Structural changes in the seed coat after soaking were also observed. Water had accumulated between the seed coat and the cotyledon resulting in great expansion of the seed coat. These structural changes could be related to the hardshell phenomenon observed during the storage of seed legumes.

In conclusion, these studies showed that the mechanisms of action of the quick cooking process proposed by Rockland et al. (1967) involve a combination of alkaline pH and Na^+ salt concentrations which resulted in the lost of minerals and pectic substances from the beans to produce easily cookable beans. On the other hand, the short heat treatment proposed by Molina et al. (1976) to improve the storage properties of black beans caused increased solubility of protein during soaking and changes in the seed coat characteristics. These changes could contribute to the prevention of hardshell.

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CHANGES OBSERVED DURING THE QUICK COOKING AND HEAT TREATMENT PROCESSES

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ABSTRACT

Chemical and structural studies on the quick cooking process for beans were conducted. In addition, the mechanisms of action of a short-time heat treatment process were studied.

The results showed that the mechanisms of action of the quick cooking process proposed by Rockland et al. (1967) involved a combination of alkaline pH and Na^+ salt concentration which resulted in the loss of minerals and pectic substance from the beans to produce more readily cookable beans.

The short-heat treatment proposed by Molina et al. (1967) to improve the storage properties of black beans caused increased solubility of protein during soaking and changes in the seed coat characteristics. These changes could contribute to the prevention of hardshell.