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GENETIC, PHYSICO-CHEMICAL AND STRUCTURAL PARAMETERS AFFECTING TEXTURE OF DRY EDIBLE BEANS

By

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ABSTRACT

GENETIC, PHYSICO-CHEMICAL AND STRUCTURAL PARAMETERS AFFECTING TEXTURE OF DRY EDIBLE BEANS

By

Agbo N'Zi Georges

The purpose of the present study was to develop fundamental information concerning physical and chemical characteristics of dry bean (*Phaseolus vulgaris* L.) seeds and relate them to cooking quality. Two dry bean isolines San-Fernando (a black seeded cultivar of tropical origin) and Nep-2 (a white seeded mutant from San-Fernando) presumably differing only in seed coat color were compared for proximate analysis, soaking and cooking, and flour characteristics. Sanilac, a navy bean cultivar used widely in dry bean studies was employed as a control.

Proximate composition of Nep-2 and San-Fernando showed no significant differences for moisture, protein, fat, starch and ash contents. Cumulative proximate composition over the three crop years (1978, 1979 and 1980) showed significant differences in fat contents only and Nep-2 had higher fat values than San-Fernando.

In the cotyledon, α-oligosaccharide contents were similar in all three genotypes with stachyose being in the greatest quantity. The oligosaccharides were not detected in the seed coat of any of the three cultivars used.

The rate of water absorption was lower in San-Fernando than Nep-2 and Sanilac. After beans were thermally processed, sample of Sanilac and Nep-2 had higher washed drained weights and lower texture than for San-Fernando. No significant differences were detected among the strains for moisture content after soaking and processing. The viscosity of bean flour indicated high initial pasting temperatures with continuously rising pasting curves for all strains. The removal of the bean seed coat increased the viscosity potential of Sanilac and San-Fernando flours. However, Nep-2 flour showed a decreased curve pattern.

Variability among genotypes was observed under Scanning Electron Microscope for seed structural characteristics. Nep-2 had partially occluded micropyle, slightly elevated hypocotyl area, and a "plastic-like" seed coat with holes while San-Fernando had closed micropyle, elevated hypocotyl area, rigid seed coat without holes, firmly packed parenchyma cells organization in the dry seeds, and partially liberated starch granules in the soaked and processed seeds.

To

my Mother

For her patience and moral support

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INTRODUCTION

The World Food Problem

The major problems facing the world today are two-fold: one of population; the other of not enough food. Modern medicine and improved sanitation have brought about a spectacular increase in the average life span while the birth rate in many countries remains practically unchanged. The result is the much-discussed population explosion. In developed countries, it is recognized that the difference between birth rate and death rate is small due to birth control and high medical practices while in developing and lesser-developed countries that difference is larger due to the fact that birth rate is highly by lack of birth control, and also the death rate reduced by improvement of sanitation and better medication availability.

Efforts to check the birth rate and to increase the food supply are severely handicapped by lack of education, adherence to customs, and lethargy from malnutrition and undernutrition.

The problem of population and food is not new; it has confronted man for nearly all the million years that human beings have been on the earth. The present problems differ only in magnitude and intensity from problems that have been faced many times before. Today, one-half billion people out of four billion (12.5% of the world population) do not receive close to an adequate quantity of food; moreover, possibly one-half the world is marginally fed. This non-availability of food to that portion of the world population, mainly Latin America, Africa and Asia, could be associated to many factors such as drought, infertility of the soils, inadequate farming lands (Sahel, Africa subsaharian regions), inadequate food production due to the fact that the farmer produces food only to feed his family and not for commercial purposes; lack of mechanical means for large production; lack of preservation, transformation and transportation means of the food; political and economical limitations; low salary leading to inability to buy enough food and food of adequate aesthetic quality; and lack of nutritional information and improvement of quality and yield of the food available. All those factors contribute to the low calorie intake of the one-half billion of the world population.

Coupled with the general food shortage is the shortage of specific nutrients. There are six main categories of nutrients that are needed to supply energy, promote growth and repair body tissues and to regulate body processes. These are water, carbohydrates, protein, lipid, vitamins and minerals. Those nutrients are quite present in a wide variety of food but may be limited in some others. By lack of nutritional information and inadequate distribution, one may be exposed to inadequacy of one of those nutrients leading to different sickness. One such nutrient that has received considerable attention is protein.

"Protein Problem"

The deficiency in animal protein in the diet deficit regions of the world has been generally associated to poor physical development of adults and even more markedly to the protein deficiency diseases of children. Kwashiorkor and marasmus account for a very high child mortality between weaning and 6 years of age and often leave the children that survive with stunted physiques and retarded mentality.

Different authorities have set different goals for animal protein in the diet. The U.S. Department of Agriculture would provide 10 grams of animal protein per person per day, and 10 grams of pulse protein, the latter from increased cultivation and use of leguminous crops and from increased human consumption of the press cake from oil seeds. Cook (1962) set a goal of 16 grams of animal protein per person per day for the under-nourished countries. Pawley (1963), looking to the year 2000, set a goal of 20 grams of animal protein per person per day with the rest of the requirement from other food sources.

In view of the paramount importance of a complete protein in the diet, particularly for child development, it is not quite easy to accomplish the goals. Four causes of this "protein problem" have been reported by the Protein-Calorie Advisory Group (PAG) of the United Nations (PAG Statement No. 20, 1973) as:

1) Low wages and income and under-employment or unemployment in rural or urban centers, all of which limit the purchase of the relatively costly foods that contain protein of good quality.

2) Difficulties associated with the production of protein-rich food of animal or plant origin because of ecological and agricultural limitations with the result that they are usually expensive and relatively short supply.

3) The lack of effective food processing, distribution and marketing system resulting in large loss of food crops.

4) The lack of knowledge of food values and food preparation for children and specific prejudices against giving some protein foods to young children, especially when they have infectious disease.

Possible Solutions to the "Protein Problem"

Several approaches have been proposed to solve the protein gap but one should recognize that the situation is very complex. Altschul (1969) mentioned that the conventional approach to increasing food supply is to increase grain production, and that to increasing protein quality of a diet is to increase animal production. He proposed two ways beyond conventional means:

1) Raise the protein impact of the major contributor to protein in the diet; 2) develop new nutritious foods to supplement those now available from conventional sources. His views are directed toward synthetic amino acids, vegetable protein mixtures, improved cereal products, protein beverages, texture foods and others. He suggested that the major increase by far in protein supply for the near future will come by increase in total supply of cereal grains and the next will come by increase in conventional protein sources - legumes and animal protein.

The United Nations Advisory Committee on the Application of Science and Technology to development (1968) suggested seven policy directions.

1. Promotion of increased quantity and quality of conventional plant and animal protein sources suitable for direct human consumption.

2. Improvement in the efficiency and scope of both marine and fresh-water fisheries operations.

3. Prevention of unnecessary losses of proteinaceous foods in field storage, transport and home.

4. Increase in the direct food use of oilseeds and oilseed-protein concentrates by human population.

5. Promotion of production and use of fish-protein concentrate.

6. Increase in the production and use of synthetic amino acids to improve the quality of protein in cereals and other vegetable sources, and the development of the use of other synthetic nutrients.

7. Promotion of the development of single-cell protein for both animal feeding and direct utilization by man.

Kahn (1981) reported that the world food needs for 1985 are estimated to be 44% greater than that required in 1970, and about 70% of that 1985 estimate will be needed to feed the Third World. His approach to solve the problem is a multisectoral coordination. First, slowing of population growth should be a paramount objective of any developing country's program to alleviate hunger. Second, the choice of strategy that should be undertaken for eradicating poverty associated with low protein intake should be left to each government. But it must be recognized by planners, economists and policy makers that increasing national income alone will not solve the poverty-hunger problem. Last, he stated that food production yield per acre could be improved in many areas of the world, even though factors such as climate, water, soil, energy, etc., influence productivity.

Of all the suggestions that have been made for solving the protein problem, the one that has received the most attention is the improved production and utilization of conventional animal and plant protein sources. Animal products are ideally suited for meeting the protein needs of people through out the world but they are costly, scarce or not utilized fully because of ignorance, taboos or poverty.

Needs of Vegetable Protein

Vegetable protein which are the basic plant protein sources, typically supply over 80% of dietary protein in developing countries. Cereals and legumes give the largest contributions, and they are utilized in various forms in different regions. Protein content among cereals ranges from 6 to 14%; rice is low and wheat is high among the predominant cereals eaten by humans. Within each variety there is a range in protein content; in wheat alone the range can be 8 to 14%. The quality of the cereal protein is not as good as that from animals, primarily because the amino acid pattern is deficient in one or more amino acids, principally lysine; others are tryptophan, threonine and methionine.

Because of that, attention has been paid to legume protein. Legumes grown in different parts of the world enjoy widespread use, have a high degree of acceptability, relatively low cost and possess good nutritional properties. They are known to be rich sources of lysine but deficient in sulfur amino acids. This places them as natural complements to cereal based diets.

In general, the aim to the solution of the protein problem is the production of protein rich foods within the developing countries or regions. Several indigenous crops which are still unknown to the western world need to be investigated in order to evaluate their nutritional status and incorporate that into local agricultural systems. Grain legumes which fall in that category have become important crops.

Yield Problem

Carperter (1981) reported that world production of grain legumes relative to that of cereal grains is falling, mainly because of considerably greater yields obtained with cereal. Yet, governments and international agencies continue to encourage production of these legumes because they "fix" nitrogen, and because of a belief that they make a special contribution to the diet of people unable to afford high levels of milk and other animal products.

In the U.S., it has been shown that average bean yield has a downward trend in the past 20 years suggesting that a barrier to higher yields has been reached in beans. In Michigan, through Michigan Improved Bean and Seed Program, Michigan State University has been made responsible for the development, evaluation and release of new varieties. Hosfield and Uebersax (1980) stated that there is a number of reasons for yield barriers in crops, but the least common denominator is the variety because variety possesses a certain genetic potential or program prescribed by its genetic complements to produce under a given set of circumstances. They explained that when circumstances are optimum, the variety will produce at a maximum, and if circumstances change and prevailing conditions limit the genetic potential of a variety, the variety then must be replaced with one that can maximize its genetic potential in terms of productivity. This replacement of variety could be important for the seed quality. Frazier (1978) indicated that some of the highest bean yields being achieved in Michigan wide trials are for beans with tropical germplasm in their pedigrees. Smucker et al. (1978) precised that preliminary results from several field experiments suggest that black beans derived from tropical accessions have root systems more tolerant to partial soil compaction and water stress.

Bean Production and Utilization

Figure 1 shows the family and subfamily of beans. The papilionaceae sub-family grown in tropical and subtropical regions provide the most edible beans.

The subject of this dissertation is *Phaseolus vulgaris* L., the common dry bean which is the grain legume consumed in greatest quantity in the world as a whole, and it exemplifies all the problems associated with these materials. Because of the high nutritional value of beans, various workers have conducted compositional analyses: investigations generally include protein, fat, moisture, fiber, ash, mineral, vitamin, carbohydrate and texture.

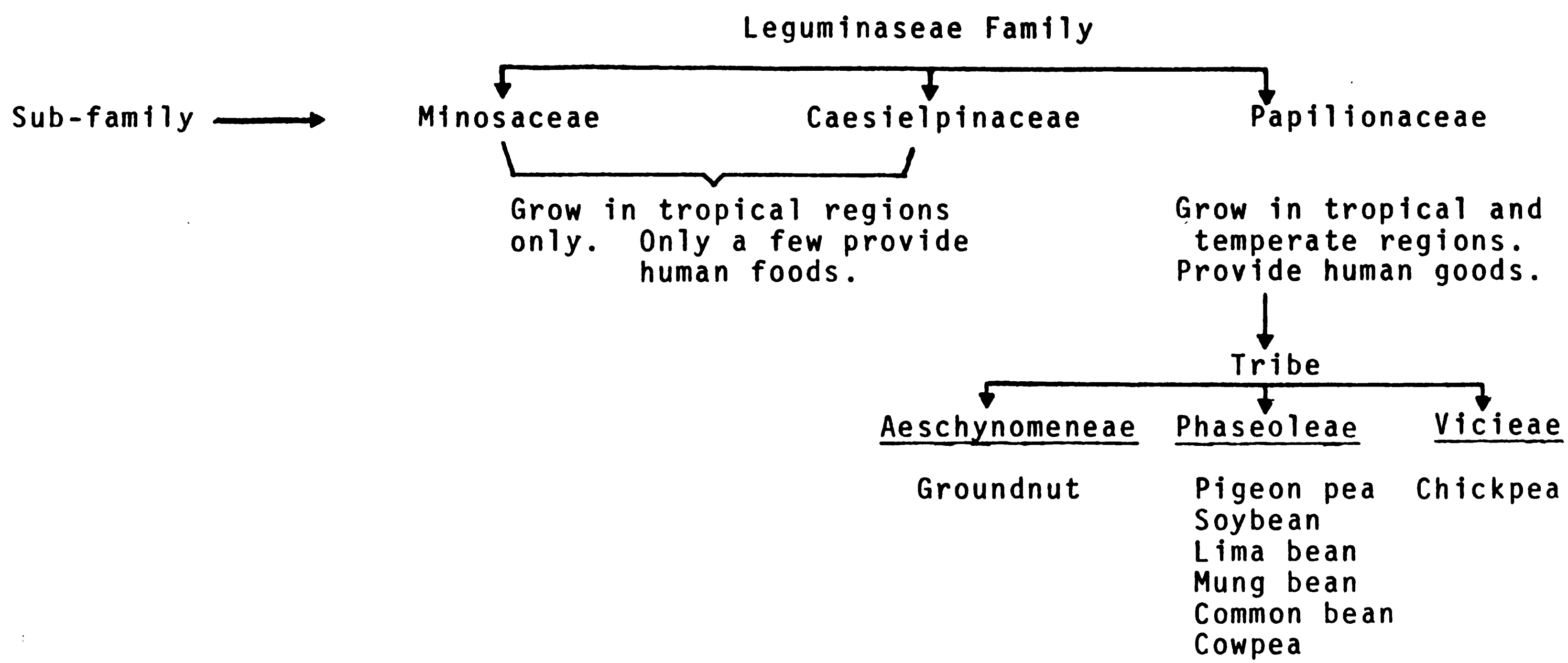

Figure 1. Phylogenetic relationship of several dry edible legumes.

Dry beans often require long cooking periods to become eating soft. Hydration of the beans prior to cooking is an important factor in the legume industries. Conventional methods of preparing and cooking dry beans involve soaking overnight in water at room temperature (16-24 hrs) followed by cooking in boiling water for 45 min to 3 hrs. Time of cooking depends on bean variety and storage history. Many investigations have been carried out to solve that problem and it has been suggested that information on chemical composition of specific tissues and localization of chemical constituents in those tissues is a prerequisite for an explanation of physical and chemical changes in bean tissues during mechanical, thermal, chemical and enzymatical treatment (Powrie et al., 1960). Texture is an important quality characteristic of cooked beans. One should also bear in mind that texture of adequate acceptability by consumer is of quite importance in bean production and now to plant breeders. Although there have been numerous attempts to measure objectively the texture of cooked beans, there is very little information on the texture of raw and soaked beans.

Beans have also been associated with excessive intestinal gas production known as flatus. Studies have been directed toward elucidating the cause of flatulence in beans. Several indigestible sugars namely stachyose and raffinose have been implicated in the flatus associated with eating cooked dry beans. To study this problem, research has been directed toward:

1. characterizing the specific component.
2. developing physical and chemical means of extracting these components.
3. developing practical cooking or processing techniques which may be used to reduce the problem.
4. attempting to identify genetic variants possessing low levels of oligosaccharides which may be used to biologically remove the flatulence factors of beans.

Although yield is the most important solution criteria in bean breeding, the breeder is cognizant of consumer preferences when introducing new cultivars. Consumers have acquired specific preferences for various combinations of visual characteristics of seeds and, beans are generally soaked and must be cooked to make them tender and palatable and to render seed protein nutritionally available before eating (Rockland and Jones, 1974).

The use of modern technology has provided the beans by which food quality evaluations may be conducted with a high degree of objectivity and precision on small amounts of seed (Hulses <u>et</u> <u>al</u>., 1977). However, little information is available as to what characteristics lead to the variability for physical and chemical characters noted in dry edible beans (Hosfield and Uebersax, 1980).

Objectives

Strains that differ from each other genetically at one locus only are referred to as isolines. Since "Nep-2" was the result of an induced mutation in "San-Fernando" that presumably only affected the color locus in common bean (Moh, 1971), the differences in physico-chemical properties were surprising and prompted this investigation because of the possible impact on cooking quality. Earlier processing evaluations of these strains showed that "San-Fernando" and "Nep-2" differed in the rate of water absorption during soaking and "San-Fernando" required almost 2 times the force as "Nep-2" to shear a sample of beans to the point of deformation using a Kramer Shear Press Instrument.

The objective of this research was to investigate physico-chemical differences noted between the above isolines. The dry bean cultivar, Sanilac, was used as a point of reference (standard) for all evaluations. For this purpose experiments were designed to ascertain the:

1. Proximate composition of the two isolines and Sanilac (a commercial standard *Phaseolus vulgaris* L. bean).

2. Possible flatulence sugars for potentiality of reduction of the levels through breeding.

3. Microstructure of the three cultivars of beans by scanning electron microscope (SEM) and relate differences to texture.

4. Hydration rate of the three varieties and relate that to their microstructures.

5. Physical properties (viscosity) of the flour from the beans and relate that to pasting characteristics.

6. Processing quality and texture of the three cultivars.

7. Effect of soaking and cooking on the microstructure of the beans and relate that to texture.

LITERATURE REVIEW

Nutritional Contribution of Food Legume Seeds to World Hunger Problem

Legume seeds are grown and used for food in nearly all the temperate and tropical areas of the world. Rockland and Nishi (1979) mentioned that 12 varieties have commercial importance and legumes require less energy per unit protein production than cereal grains and particularly animal protein. Tobin and Carpenter (1978) in their critical review of the literature concerning protein quality evaluations and other properties of the common beans, *Phaseolus vulgaris* L., reported PER (Protein Efficiency Ratio) values for varieties and cultivars ranged from 1.0 to 2.0. Rockland and Nishi (1979) reported values of 1.2 - 1.4 for authentic strains of cooked standard and 1.2 - 1.6 for analogous quick-cooking products prepared from various *P. vulgaris* L. cultivars. Rockland (1978) reported that a maximum PER for quick-cooking beans was observed after cooking for 5 min in boiling water. After cooking for 15-30 min, the PER values were slightly lower. Standard-cooked beans, cooked for 5 min, had about the same PER as the quick-cooking beans. There, the time and energy factors are involved in the efficiency of the bean protein. Tobin and Carpenter (1978) suggested that extended cooking, especially at higher pressure and temperature, tends to lower nutritional quality. Elias et al. (1979) and Rockland and Radke (1981) obtained lower PER values for beans and cook water combined than for drained beans. They suggested that seed-coat tannins in colored beans may prevent complete utilization of legume proteins.

Beans, generally, are fairly good sources of riboflavin and vitamin E and others such as thiamin, niacin, vitamin B_6 and folacin (Bunnel et al., 1965; Harris et al., 1950; and Patwardhan, 1962). Guerrant et al. (1946), Lamb et al. (1946), Lantz (1938) and Shroeder (1971) reported riboflavin retention of 72 - 105% in processed bean. Bunnel et al. (1965) and Harris et al. (1950) reported vitamin E losses of 70 - 90% in canned beans. Normal bean processing methods may not prevent the formation of hydroperoxides which allow the tocopherol side-chain to be oxidized and further degraded to peroxides which in turn further decompose to cleavage to aldehydes and ketones. Riboflavin was higher in steam blanched beans but vitamin E was not affected since vitamin E is not water soluble (Connie et al., 1979) using navy beans, Pinks, Pinto and snap beans. As storage time increased, the vitamin retention decreased. Nordstrom and Sistrunk (1977) reported vitamin E loss in canned bean during storage. Bunnel et al. (1965) and others (Ames, 1972; Harris et al., 1950) reported riboflavin losses of 90% in canned bean during storage.

Beans are also source of minerals such as iron, calcium, phosphorus, magnesium, sodium, potassium, manganese and copper. Augustin *et al*. (1981) reported retention of those minerals ranging from 38.5% to 103.2% with sodium and calcium being the lowest and highest, respectively.

All the above factors are of great interest to plant breeders in the development of a new variety.

Cooking and Processing

Legume seeds often require long cooking periods to become soft. Cooking is necessary not only to tenderize the seed coat and cotyledon and develop acceptable flavor and texture, but also to make the bean protein nutritionally available. Factors affecting cooking characteristics have been associated with seed coat (Synder, 1936; Gloyer, 1932) and cotyledon (Mattson, 1946). Adams (1975) by relating soaking time and cookability mentioned that the hilum and micropylar areas usually admit water readily, but seed coats differ strikingly in this regard. What he did not specify was the structural characteristics of the micropyle and seed coat of the beans he used. He stated that both the age of the seed and the genotype of the maternal parent are important regulatory factors. Powrie *et al*. (1960) stated that information of chemical composition of specific tissues and localization, of chemical constituents in those tissues is a prerequisite for an explanation of physical and chemical changes in bean tissues during mechanical, thermal, chemical and enzymatic treatments.

The mechanical factors are mainly seed coat breakage and splitting of cotyledons resulting from handling in harvesting and cleaning. Because of temperature, rainfall and other weather conditions during growth, dry beans are subject to cracks, hard seed coats and other problems that can affect processing procedures (Connie et al., 1979). Splits were affected by bean type, initial moisture content and storage time. Adams and Bedford (1975) stated that, as a general rule, the larger and more irregular shaped seeds are the more sensitive to mechanical abuse than are the smaller, more nearly rounded seeds.

Bressani and Elias (1974) reported a minimum of two hours for cooking soaked dry beans (*Phaseolus vulgaris* L.) at atmospheric pressure. There have been numerous attempts (Esselen and Davis, 1942; Feldberg et al., 1956; Dorsey et al., 1961) to find a way of lowering the cooking time for legumes. An important development in the utilization of whole legumes has been the preparation of quick-cooking legume products.

Steinkraus et al. (1964) reported on a new process for preparation of quick-cooking dehydrated beans by hydrating the dry beans through soaking in water for 15 min, followed by a precooking in steam and coating by dipping in a 20%

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sucrose solution at 160^{o}F, then dehydrating. Rockland and Metzler (1967) reported a process for quick-cooking large dry lima beans using an intermittent vacuum treatment for 30 to 60 min in a solution of inorganic salts (sodium chloride, tripolyphosphate, bicarbonate and carbonate), soaking for 6 hours in the same salt solution, rinsing and drying. They indicated that their process facilitated infusion of the salt solution through the hilum and fissures in the hydrophobic outer layer of the seed coat. Wetted by the solution, the inner membrane hydrates rapidly, plasticizing the seed coat and causing it to expand to its maximum dimensions within a few minutes. As a result, cotyledons imbibe the solution rapidly. This causes about 80% reduction in the cooking time. Another method of processing legume seeds involves dehulling of the beans prior to cooking. Practices of dehulling differ from legume to legume and with the same legume in different parts of the world. Although the processing of legumes for home consumption with or without dehulling differs to a considerable extent in different countries and regions, the similarities outweigh the differences (Aykroyd and Doughty, 1964). Some other methods are fermentation, roasting, parching, agglomeration and germination, but the important one is the hard-to-cook phenomena.

Moisture Factor

For processing, the generally desired moisture content in dried beans is in the range of 14 - 18%. USDA (1975) permits moisture content up to 18% without being designated as "high moisture beans". Morris and Wood (1956) reported that beans with a moisture content above 13% deteriorate significantly in texture and flavor after 6 months at 25°C. Beans stored at less than 10% moisture maintained good quality and stored well even after 24 months. Too low moisture content can also create other processing problems such as not imbibing water normally; bean seed coats becoming brittle, thus being subjected to cracking. But Connie et al. (1979) reported that low original moisture level before soaking resulted in higher hydration ratios in all beans used (Pinks, Red kidney, common navy, hite, Pinto, snap beans) except Pinks and Avenger. Bean samples containing 16% initial moisture were firmer in texture after thermal processing. Burr and Morris (1968), Kon (1968), Morris and Wood (1956), Muneta (1964) and Ruiloba (1973) reported that the use of a low storage temperature (4°C) or the practice of storing beans with a low moisture content (8-10%) at a relatively low humidity environment has been shown to minimize the development of a hard-shell condition in legume seeds, including black beans. Adams and Bedford (1975) stated that when various types of beans at about 12.5% moisture level are dropped 10 meters onto a slanting steel surface, they usually will incur sufficient damage to permit detection of genetic differences. Morris (1964) studied the effect of moisture content and temperature during storage on the cooking time of Pinto, Sanilac and lima beans. He found that cooking time increased with storage time, especially at moisture contents above 10%. At 13% moisture content the cooking time after 12 months was three times the initial cooking time. At 10% the cooking time after 12 months was only slightly longer than the initial time. Cooking time also increased with storage temperature, especially at high moisture contents. Barriga-Solorio (1961) reported that at 9.7% moisture 27.8% of Sanilac seed were damaged but at 15.5% moisture damage was reduced to only 5.3%. Muneta (1964) found a correlation of +0.80 between moisture content of stored dry beans of four cultivars and cooking time. He thought moisture content per se was not the primary factor in determining cooking time and postulated that _factor_ or _factors_ connected with high moisture content resulted in a longer time to breakdown (TTB) phenomena. From all the above views, the speculation of Muneta (1964) gives space for identification of other factors either chemically or structurally in the understanding of the hard to cook beans phenomenon.

Hydration

It is well known that when a sample of beans is soaked in water at room temperature, some of the beans do not a

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imbibe water, presumably because the seed coats are impermeable (Burr, 1973). This condition is referred to as hard-shell or seed-hardness.

Hydration of the beans prior to cooking is an important factor in the legume industries. Conventional method of preparing and cooking dry beans involves soaking overnight in water at room temperature (16-24 hours) followed by cooking in boiling water for 45 min to 3 hrs (Oguntunde, 1980). Time of cooking depends on bean variety and storage history. Inadequate soaking lengthens cooking time and affects the cooked bean texture. Longer cooking times lead to the use of more energy than necessary in the processing of beans.

Adams (1975) mentioned that hydrating seed to 53 to 57% will insure uniform expansion in the can during the thermal process and insure product tenderness. He specified that a long, soft-water soak will leave the bean more tender or even mushy. Hard water tends to toughen the skins and firm the texture of the cotyledon. He suggested that water containing 25 - 50 ppm calcium is considered optimum. He added that if too-hard water cannot be softened chemically or by ionic exchange resins, the firm effect can be counteracted by addition of 0.1 to 0.2% of sodium polyphosphate to the soak.

Gloyer (1932), Morris _et al_. (1950), and Steinkraus _et al_. (1964) reported a favorable effect of a heat treatment on the water absorption of beans which minimized the hard-shell development in the grain. However, other authors (Burr and Morris, 1968) reported that beans that rehydrate as quickly as normal beans usually need a prolonged cooking time, thus indicating no correlation between water absorption capacity and cooking time. These findings reveal that, at least in some bean varieties, a higher water absorption capacity (lower hard shell) is not necessarily correlated with a shorter cooking time. Molina _et al_. (1976) indicated that heat treatment did not affect the physical appearance of black bean grains (_Phaseolus vulgaris_ L.) but significantly ($P \leq 0.05$) decreased the development of the hard-to-cook phenomenon in foods. Powrie _et al_. (1960) reported that the seed coats from soaked navy beans possessed an average moisture level of 76.6%. This high capacity of the seed coat for water uptake suggested the possibility of water migration through the seed coat for the hydration of other bean tissues during the soaking period. Snyder (1936) reported that for some bean seeds, the entrance of water at ordinary temperature was largely through the micropyle and germinal area. Hamly (1932) suggested that for some seeds, the micropyle is probably no more than a hole for exchance of gases.

The importance of the hilum in relation to the ripening of the seed and the permeability of the testa in some Papilionaceae was reported by Hyde (1954). He found that the hilum in some seeds performs a function essential for the hardshell condition. But it is not still very clear what anatomical structures in the legume seed are responsible for water absorption or the development of the hardshell condition. According to Powrie _et al_. (1960) most of the water, both bound and free, presumably residues in the proteinaceous and cellulosic portions of the cells. In general, one should recognize that the seed coat, hilum and micropyle together may form an integrated specialized water absorption/removal system.

To understand the slow hydration rate and the hard-to-cook phenomenon, various investigations have been carried out. Most of the research and reports have been concentrated on the relation of the external structures like hilum, seed coat and micropyle to those characteristics. Little is done on the proteinaceous matrix and the cellulosic portions of the cells. Powrie _et al_. (1960) raised the question of a possible relation between these structures and seed hardness. There is no report on the effect of proteinaceous matrix on the hardness of legume seeds but work done on wheat indicates that there may be such an effect. Scanning electron microscopes has commonly been used in such studies. In general, the primary determinant of wheat hardness was shown to be genetically controlled and appeared to relate to factors influencing the degree of compactness of endosperm cell components (Moss _et al_., 1973; Greenaway, 1969; Stenvert and Kingswood, 1977).

Much more work needs to be done on the structural differences of different varieties of legume seeds in order to be able to show different water absorption and hardness properties.

Texture Measurement Means

Texture measuring instruments can be divided into two classes (Voisey, 1971a).

a) Special purpose to perform a particular type of test such as tension, compression, shear etc. on either several products, or a specific type of product.

b) General purpose to test a wide range of products using a diverse range of methods. To name a few:

- Brine flotation test - USDA method (Anon. 1945)
- Size of peas (Boggs *et al*., 1942, 1943)
- Tenderometer (Martin, 1937)
- Texturometer (Lee, 1941)
- Penetrometer (Anon., 1938; Boggs *et al*., 1942)
- Specific gravity and density (Lee, 1941)
- Alcohol-insoluble solids (Kertesz, 1934)
- Total solids (dry matter) (Strasburger, 1933)
- Starch (Nielson et al., 1947)
- Sugars (Lee, 1941)
- Refractive index (Walls, 1936)

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Most of those methods used were not reliable for measuring tenderness and maturity of seeds (Makower, 1950). He considered the tenderometer to be probably the best means of determining the maturity of peas.

For research, the general purpose type is the logical choice because of operational flexibility.

- Kramer Shear Press (Kramer et al., 1951)
- Instron Universal Testing Machine (Bourne et al., 1966)
- General Food Texturometer (Friedman et al., 1963)
- Ottawa Texture Measuring System (Voisey and deMan, 1976)

These have basic components common to all such devices:

a) a mechanism for deforming the sample

b) a system for recording force, deformation and time

c) a test cell to hold the sample (Voisey, 1971b).

The test cell should subject the sample to appropriate forces to determine the textural characteristics of interest. All these instruments measure texture based on the response of multi-bean sample.

The shear press was first used by Kramer and Asmlid (1953) to test peas. Other scientists have used the same instrument to measure texture of vegetables (Ang et al., 1963) and beans (Rockland, 1964; Sanchez and Woskow, 1964; Hoff and Nelson, 1964; Rockland, 1966; Luh et al., 1975; and Quast and de Silva, 1977a, 1977b). According to Voisey a

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and Nonnecke (1973) the Kramer Shear Press is not entirely suitable for use in a production environment because safety features and fully automatic operation are not incorporated. The advantages associated with the instrument is that the force gauge, cell and shearing blades come as separate attachments which can be easily transported for calibration and it is suitable for testing a wide range of foods.

The Instron Universal Testing Machine measures compressive and tensile properties of materials and it has become one of the most widely used texture measuring instrument because the working parts of most texture measuring devices used in the food industry can be used in that machine (Sefa-Dedeh, 1979). Several scientists have used also the instrument on different types of food products (Bourne et al., 1966; LaBelle et al., 1969; Lee, 1970; Voisey and Larmond, 1971).

The Ottawa Texture Measuring System (O.T.M.S.) was designed for testing foods to combine the advantages of Kramer Shear Press and Instron machines and to eliminate some disadvantages (Voisey and deMan, 1976). A wide range of test cells and attachments can be used in this machine depending on the food being tested.

Other investigators have measured texture based on the response of single beans (Malcom et al., 1956; Ismael, 1976; Steinkraus et al., 1964; Burr and Morris, 1968; Molina et al., 1976; Bourne, 1972; Ige, 1977). The methods used include a penetrometer, alcohol-insoluble solids, brine flotation and starch measurement. Recently, Hudson (1982), at the USDA Regional Plant Introduction Station at Washington State University, has developed a new method for measuring cooking based on the response of single beans. His apparatus consists of 100 test points in a 10 x 10 array. Each point has a cup holding a single bean seed. The method is as described in the Materials and Methods of this dissertation and it is based on time to breakdown (TTB) system with each individual best looking bean seeds.

Sensory analysis and objective measurement are also used to conduct research into textural characteristics and for production quality control but using taste panels is cumbersome, time consuming, expensive and requires sufficient quantity of sample.

Relationship of Texture with Hard-to-cook Beans

Texture expresses three parameters (Adams, 1975):

a) Firmness: measured by the force required to penetrate a substance. Perceived on first bite.

b) Gumminess: measured by the force required to disintegrate a substance. Perceived during chewing.

c) Adhesiveness: measured by force required to remove the material from the mouth. Perceived during chewing.

These parameters could well be evaluated with reasonable accuracy by a sensory panel and also measured quantitatively by quite a variety of methods as discussed above. Those methods have been used by various investigators to study some basic quality characteristics of beans.

Powrie _et al_. (1960) raised the question of possible relation between the seed structure and hardness. Rockland and Jones (1974) suggested that the separation of bean cells during cooking may be related to the transportation or removal of divalent cations, particularly calcium and magnesium, from bridge positions within the pectinaceous matrix of the middle lamella. They also showed that there is no breakdown of the cell wall of cooked bean. Rather they found that cooked or partially cooked bean cells separated readily along the surface of individual intact cell walls. Short heat treatment loosened the intercellular matrix of the middle lamella sufficiently to allow separation of individual cells without rupture of cell walls.

Other scientists (Linehan _et al_., 1969; Huges _et al_., 1975) attributed the hard-to-cook phenomena to the solubilization and diffusion of starch from cells while plant tissues are being cooked. According to Hahn _et al_. (1977), starch granules in lima beans maintained slight birefringence after cooking. Varriano-Martson and de Omona (1979) indicated that many of the starch granules of black beans

(*Phaseolus vulgaris* L.) exhibited some birefringence even after long cooking periods. Voisey (1971) working on the measurement of baked bean texture found that large differences in texture are evident from year to year and between recipe used. It was also suggested that development of hard shells can be the product of a chemical or enzymatic process in the seed (Burr *et al*., 1968; Kon, 1968; Morris and Wood, 1956; Muneta, 1964; Ruiloba, 1973).

Mattson (1946) reported that the cookability of different dry pea varieties is related to their contents of phytic acid content and calcium. He suggested that when the phytic acid content is low, the pectin in the middle lamella formed insoluble calcium and magnesium pectates, causing the poor cooking quality.

In general there is no clear understanding at the present time of the mechanism that governs the water uptake in beans and the factors that affect the tenderness or softness of the canned product.

From that point, knowledge of the chemical composition and characteristics of some major constituents in beans, and also of the physical and structural properties of the seeds and flours is necessary to help in the understanding of the hard-to-cook phenomena. That could also be effectively used in breeding to improve yield of food and feed associated with quality of the grain.

Scanning Electron Microscopy (SEM)

The concept of a SEM is credited to Knoll (1935) who suggested that characteristics of a sample surface could be observed by focusing a scanning electron beam on the surface and recording the emitted current as a function of beam position. Unlike the TEM, which uses ultra-thin sections, the SEM samples would not be sectioned at all (Rasmussen and Hooper, 1974). The first functional SEM was constructed by von Ardenne (1938) based on Knoll's concept but the first commercial instrument became available in 1965. Today's models provide images with resolution limits of 6 - 10 nm.

Buono (1982) stated the principle of SEM as:

1. A concentrated beam of electrons is focused on the surface of a sample (Figure 2A). Electron beam size vary from 15 to 10,000 Å and energy from 1 to 40 Kv.

2. The electron beam penetrates the sample to depths of 0.2 to 2 μm, depending on the energy of the incident electrons and the atomic number (Z) or density of the sample. The incident beam electrons are called the primary electron beam. Multiple scatterings of the primary electrons by the beam interaction with the atoms of the sample surface causes the beam to widen in diameter as it penetrates into the sample (Figure 2B). The width of this scattering is on the order of 0.2 to 1 μm. The combination of lateral beam spreading with depth produces an interaction volume within the sample.

3. The interaction of the primary electrons with the atoms of the sample produces a variety of signals including secondary electrons, backscattering electrons, Auger electrons, X-rays and light (Figure 2C).

4. The signals generated within the interaction volume have different escape depths which can be used to tailor the analysis to specific regions of the

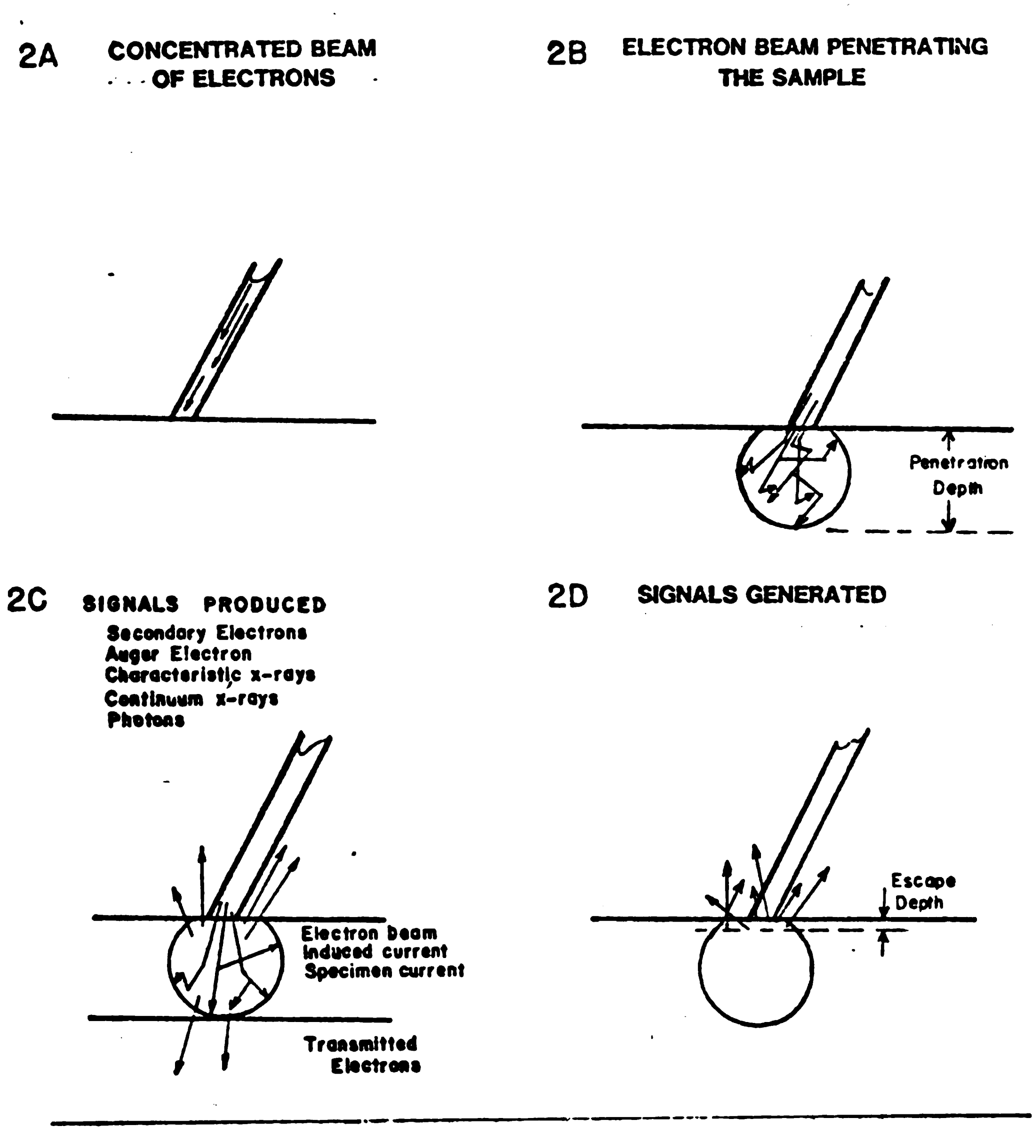

Figure 2. The Principle of Scanning Electron Microscopy.
Source: Buono (1982)

> sample by judiciously choosing the output signal and by varying the energy of the primary beam (Figure 2D). The escape depths for secondary electron is 1 to 10 nm, the depth of backscattered electron is 0.1 to 1 μm and the escape depth for X-rays is 0.5 to 2 μm.

Based on those principles, the SEM is rapidly becoming necessary to plant studies along with light microscopy (LM) and Transmission Electron Microscopy (TEM). A basic difference between SEM and other microscopes is the use of scanning coils to drive the beam in Y direction while being deflected in the X direction and the detection and display of low energy secondary electrons (Rasmussen and Hooper, 1974).

SEM technology is progressing toward greater system automation to the point of using stepper motor stages that provides images and analyses of selected areas on a large sample without the presence of the operator (Buono, 1982).

The essential features of the SEM are shown in Figure 3. The instrument contains: (1) an electron gun which provides an electron beam capable of being accelerated from 1 to 40 Kv; (2) a system of magnetic lenses to provide a means of focusing a tiny spot of electrons from the source on the specimen; (3) a specimen stage for holding the sample; (4) a scan generator to provide a means of scanning the spot of electrons across the specimen; (5) an electron collector coupled with a photomultiplier and amplifier to provide a means of detecting the response from the specimen; and (6) a cathode ray tube (CRT) display system capable of being

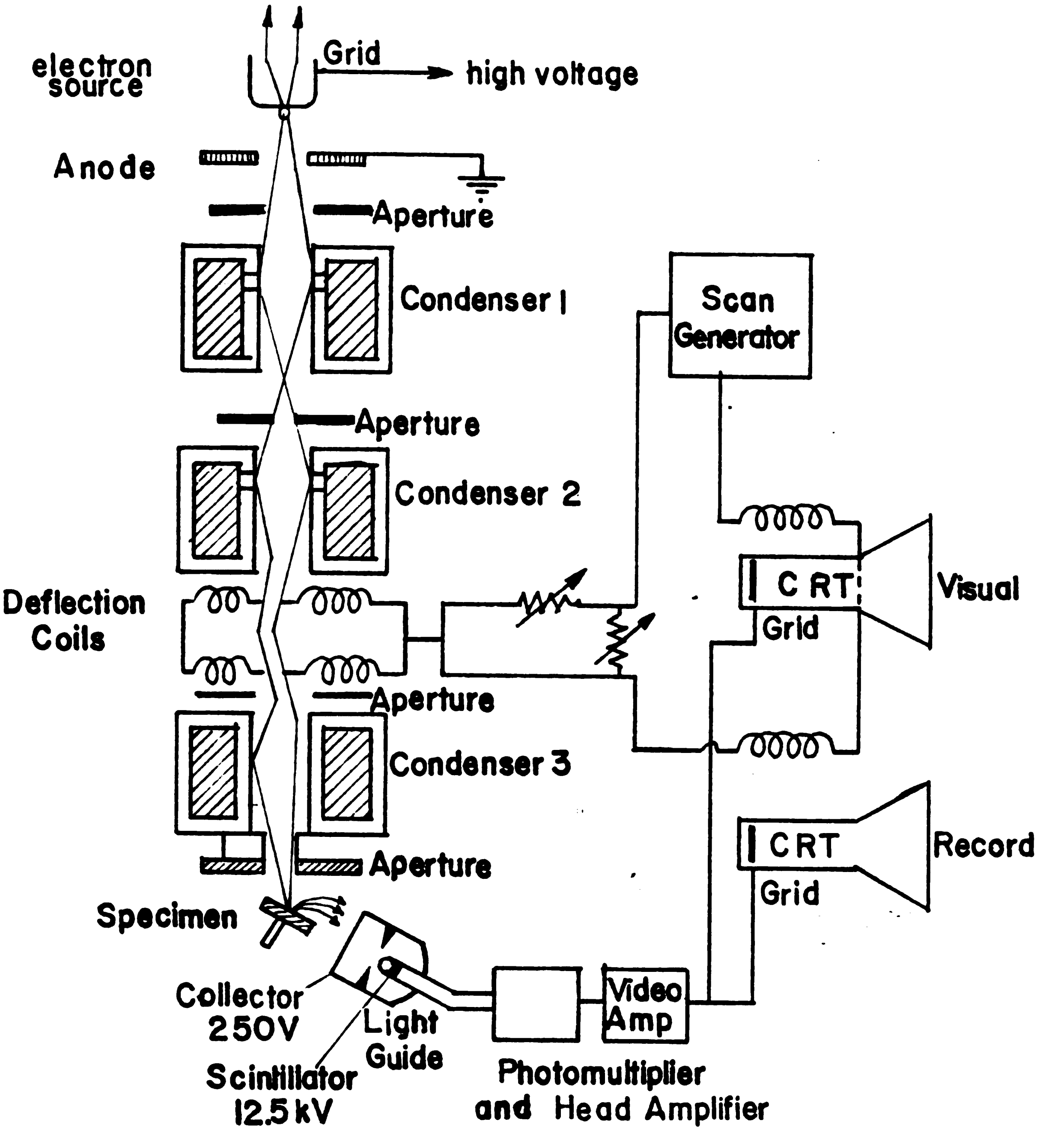

Figure 3. Essential Features of SEM.
Source: Mee, cited by Kassel and Shih, 1976

scanned in register with the incident scan.

SEMs are practical tools that have moved out of the laboratory into the production and quality control areas. They have become easier to operate and more cost effective in providing information about surface and subsurface properties of microelectronic devises.

In early 1970, Hearle (1972) provided a comparison information between optical, direct electron and Scanning Electron Microscopy (Table 1). The advantages of SEM given are:

a) great depth of focus
b) the possibility of direct observation of the external form of real objects, such as complex fracture surfaces, at high magnification thus avoiding the necessity to make thin replicas for use in direct transmission electron microscopy
c) the ability to switch over a wide range of magnification, so as to zoom down to fine detail on some part identified in position on the whole object
d) the ease of operation, and the large space available for dynamic experiments on the specimen
e) the use, besides the secondary electrons, of scattered primary electrons, light emission, X-ray emission, current in the emission, and many other responses to generate an image and obtain useful information about the specimen.

Table 1. Comparison information between optical, direct electron and scanning electron microscopy (SEM).

	Optical	Scanning electron	Direct electron
Resolution - easy	5 μm	0.2 μm	100 Å (10 nm)*
- skilled	0.2 μm	100 A (10 nm)	10 A (1 nm)*
- special	0.1 μm	5 A (0.5 nm)	2 A (0.2 nm)*
Depth of focus	poor	high*	moderate
Mode - transmission	yes	yes	yes
- reflection	yes	yes	not satisfactory
- diffraction	yes	yes	yes
- other	some	many*	no
Specimen - preparation	usually easy	easy*	skilled, liable to artefacts
- range and type	versatile real or replica	versatile real or replica	only thin, or replica
- maximum thickness for transmission	thick*	medium	very thin
- environment	versatile*	usually vacuum but can be modified	vacuum
- available space	small	large*	small
Field of view	large enough	large enough	limited
Signal	only as image	available for processing*	only as image
Cost	low*	high	high

*Advantages over others; disadvantages are underlined.
Source: Hearle, J.W. 1972.

f) the information can be processed in various ways to present images in different forms.

Some of the disadvantages are:

a) high cost

b) lack of high resolution

c) the vacuum environment of the specimen

d) inability to show up internal detail visible in optical microscopy

e) lack of color response

Because of the advantages enumerated above, scientists have used SEM in many areas (biology, medicine, horticulture, food science, microbiology...etc.) to understand basic principles involved in many materials and products. Many cereal scientists abandoned light microscopy techniques because SEM samples could be prepared easily.

In the food area, although microscopy has been used for many years, research articles on food microstructure attempting to relate structural and functional characteristics of food have become common only within the past 10 - 15 years. As mentioned by Hansen and Flink (1976), the structure of individual food components, as well as of the finished product, plays an important role in determining appearance, flavor, rheological properties and keeping qualities. To study these interrelationships, the microscope in its various modes is being used to allow visualization of the different heterogeneities in the structure of food systems.

On the use of cereals and oilseeds for food, SEM has been used extensively (Pomeranz and Sachs, 1972; Stanley _et al_., 1976; Sullins and Rooney, 1974; Wolf, 1970). Hall and Sayre (1971a) used SEM to show the surface characteristics of starch granules from tender white, pinto and lima beans. They also worked on root and tuber starches, cereal starches, legumes starches and other foods and reported much information on the sizes, shapes and surface details of these starches (Hall and Sayre, 1969; 1970a, 1970b). Wolf and Baker (1972) used to investigate the cotyledon interior of water-soaked soybeans and revealed that protein bodies of 1 - 10μ in diameter exhibited a covering spongy network. McEwen _et al_. (1974) used SEM to examine the cotyledon and seed coat of faba bean seed. They revealed that there was no discontinuity in the thick seed coat. Cross section of the seed coat showed characteristic palisade, parenchyma, tracheid, and hour-glass cells, similar to those of other legumes. Hahn _et al_. (1977) used SEM to characterize intracellular configurational changes of starch granules during gelatinization of standard and quick-cooking lima bean cotyledon. Rockland and Jones (1974) studied the effect of cooking on water-soaked and salt water-soaked beans. Other studies on foods using SEM include those of Vix _et al_. (1971) on cottonseed, Van Hofsten (1972), Gill and Tung (1976), Stanley _et al_. (1976) on rapeseed. On food starches, reports were published from Hood _et al_. (1974), Shetty *et al*. (1974), da Silva and Luh (1978), Dronzek *et al*. (1972), Robutti *et al*. (1973), Hill and Dronzek (1973), Crozet (1977), Agbo *et al*. (1979), Saio *et al*. (1977), Bernardin and Kasarda (1973), Orth *et al*. (1973), Watson and Dikeman (1977), Badi *et al*. (1976), and Chabot *et al*. (1976). The food items involved were cereals, roots or tubers, legumes and banana. Other food systems such as meat and related products and dairy products have also been studied with SEM (Schaller and Powrie, 1971, 1972; Stanley and Geissinger, 1972; Jones *et al*., 1977; Buma and Henstra, 1971; Eino, 1974; Kalab and Emmons, 1974; Stanley and Emmons, 1977).

The above reports are few among the numerous reports on the use of SEM in food research. That technique which is progressing toward greater system automation is presently used extensively   and it will continue to be so because of the advantages it has over other microscopy techniques. But in general, the key to obtaining useful information from the microscopes is the scientist's ability to recognize which microscope, if any, to use (Varriano-Marston, 1981).

Legume Seed Anatomy

Corner (1951) in his classification of leguminous seeds specified that legume seeds are generally of medium or large size, more or less compressed and exalbuminous, with large embryo and a hard, dry, generally smooth testa (Figure 4). He indicated that two microscopic characteristics distinguished the leguminous testa: 1) the external palisade, developed from the outer epidermis of the outer integument, and 2) the hour-glass cells, developed from the outer hypodermis of the outer integument and, in some cases, from its inner epidermis. He stated that any microscopic particle with these features is apparently identifiable as leguminous.

The different features of the testa found in various legume genera and species are: cuticle, palisade, hypodermal hour-glass cells, mesophyll, inner hypodermal hour-glass cells, vascular bundles and subhilar tissue (Corner, 1951; Chowdhury and Buth, 1970). The position and characteristics of these features are reported in Table 2. The seed coat surrounds two cotyledons which are the most important component of the bean seed. They are composed of parenchyma cells containing starch granules embedded in protein matrix (Bagley _et al_., 1963; Harris _et al_., 1975; Opik, 1966, 1968; Powrie _et al_., 1960; Yatsu, 1965) and vascular bundles (Opik, 1966) and protein bodies forming the protein matrix. Many scientists report that textural characteristics and nutritive value of processed bean presumably are influenced to a large extent by the size and shape of cells, dimensions of the cell walls and the localization of chemical constituents in the cotyledon. These factors and a few definitive

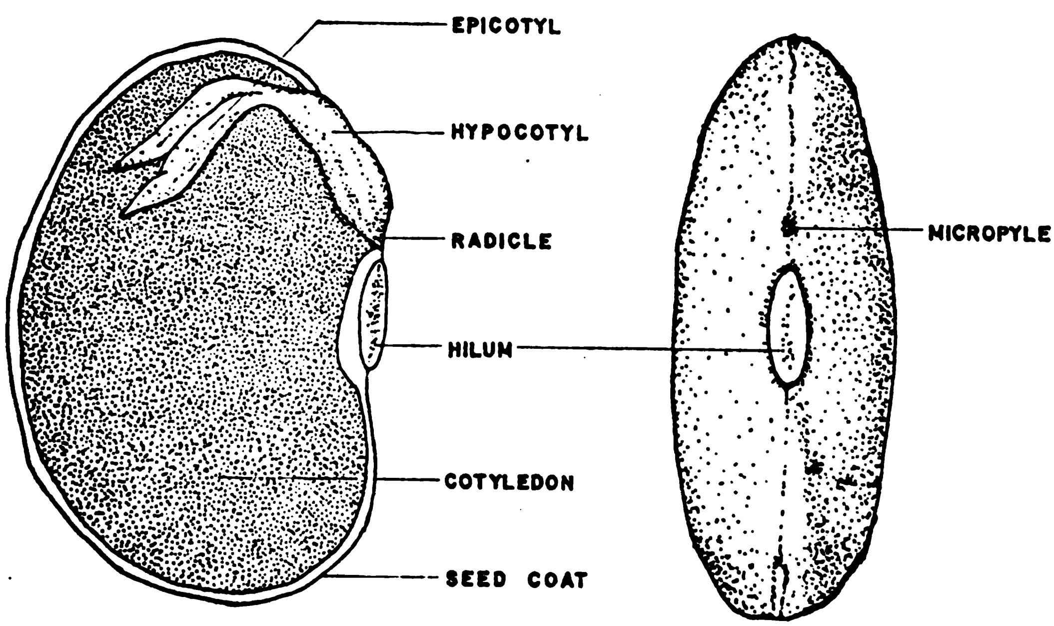

Figure 4. Visual observation of legume seed structure.

Table 2. Legume testa feature characteristics.

Feature	Position	Characteristic	Reference
Cuticle	First outer layer	-Thin and smooth -Smooth and rough	Corner (1951) Chowdhury and Buth (1970)
Palisade Cells	Second outer layer	-Irregular shape and height	Chowdhury and Buth (1970)
Hour-glass Cells	Third outer layer next to palisade	-Shape varies from species to species and within species some legumes have single hour-glass but few have two	Chowdhury and Buth (1970)
Mesophyll Cells	The remainder of the seed tests		Chowdhury and Buth (1970)

answers have been obtained to relate the ultra-structure of legume seeds to the textural characteristics of the processed product. Further research in this area is warranted.

Starch and Viscosity

Starch is one of the most abundant naturally occurring organic compounds. It is found in almost all plant tissues, where it functions as a source of reserve energy, which can be utilized gradually through enzyme actions. Starch accumulates to high concentration (20-70%) in the roots, tubers, fruits and seeds of many plants. Chemically, starches from all sources are carbohydrates, polymers of α-D-glucopyranose units linked primarily by 1,4- and 1,6-glucosidic bonds. Each glucose unit contains one primary and two secondary hydroxyl groups which are responsible for the hydrophilic properties of the starch. Starch is made up of two types of polymer chains: amylose, the linear fraction (1,4 bonds) and amylopectin, the branched chain fraction (1,4 bonds and 1,6 bonds). The ratio of amylose and amylopectin in starches also provides some indication of their origin. The exact structural relationship between the two components in the starch grain is not known, but the molecules are linked together to form starch granule by hydrogen bonds (Hahn, 1969; Wurzburg, 1968).

Starch granules are insoluble in cold water. In a humid environment, starches will take up moisture, but the swelling which occurs is reversible. When starch granules in water are heated past a critical temperature, in the range 60 - 70^{o}C (gelatinization temperature, characteristic of a specific starch), the hydrogen bonds which hold the granules together begin to weaken, allowing the granules to swell. Since the gelatinization begins at the hilum, the first indication of swelling is the loss of birefringence. Then, as the amylose fraction is dissolved and leached out, the granules begin to take in water and clarity and the viscosity of the slurry begins to increase. Eventually, the granules, having become completely hydrated, may collapse and break down. The resulting starch "paste" is composed of granule fragments and molecules in solution. The viscosity of the paste decreases as a result of this granule breakdown. On cooling the paste usually increases in viscosity and decreases in clarity. This results from retrogradation of the linear (amylose) molecules, a process whereby the amylose molecules tend to form rigid gels by hydrogen bonding. The degree of retrogradation is dependent on amylose content and the degree to which the amylose has been solubilized during the heating cycle. Cross-bonded starches have their granule structures reinforced by covalent bonds and the granule shows less tendency to swell and retrograde (Wurzburg, 1968; Leach, 1965; Smith, 1964;

Collison, 1968; Mazurs et al., 1957; Hoseney et al., 1978). Effective control and utilization of retrogradation is necessary to obtain food products of good quality after transport and storage. Since each raw starch has its own characteristic viscosity/temperature profile as a result of its particular granular composition and structure, some degree of control can be achieved simple by selection of the correct starch (Sanderson, 1981).

The suitability of starch, natural or modified, for a specific use depends on the functional properties. The functional properties would include, for example, ease of cooking (temperature, and stirring energy to cook through the swelling region), thickening power (final viscosity of the paste after cooking and cooling, as a function of concentration), and stability (resistance to thinning resulting from stirring, pH or temperature change).

Because of the variety of changes which may occur in starch paste during processing, many different methods have been developed for following these changes in the paste and estimating the functional properties of the starch from them. These methods fall into two categories, those involving direct microscopic observation such as monitoring granule swelling, loss of birefringence or staining reactions (Collison, 1968; McMaster, 1964), and those involving measurement of physical properties such as swelling power, solubilization, sedimentation rate or viscosity (Collison, 1968; Schoch, 1964; Smith, 1964). The most useful methods measure the viscosity of the paste continuously during the standardized-cooking and cooling cycle which stimulates a wide variety of processing conditions (Smith, 1964). The most common instrument used for this purpose is the Brabender Amylograph. This machine continuously measures the viscosity of starch pastes and flours while they are stirred and heated at a constant rate, held at 95^{o}C for 30 minutes and held at 30^{o}C for 30 to 60 minutes (these methods have also been developed for relating the viscosity data to the functional properties of the starch) (Mazurs et al., 1957). These kinds of data are available for most food starches such as cereal, roots, tubers, banana and legumes (Mazurs et al., 1957; Kite et al., 1957; Hahn et al., 1977; Carson, 1972; Berry et al., 1979; Rodriguez-Sosa and Gonzales, 1975; Agbo et al., 1979).

Legume starches which are of interest in this dissertation have been studied by several scientists for their incorporation into bread, cookies and other product formulations. Hall and Sayre (1971a), Kawamura et al. (1955), Lineback and Ke (1975), McEwen et al. (1974), Rockland and Jones (1974) found good agreement among various legume starch granules of the same species. They reported that legume starch granules were ellipsoid, kidney-shaped, or irregularly swollen, with an elongated hilum and smooth surface with no evidence of fissures. The sizes were determined as diameter width and length ranged from 12 to 36 μm and 12 to 40 μm for navy bean and from 16 to 18 μm and 16 to 48 μm for pinto bean respectively.

Brabender hot-paste viscosity pattern of various starches appears to be determined not only by the extent of swelling of the starch granules and the resistance of the swollen granules to dissolution by heat and fragmentation by shear (Lineback and Ke, 1975), but also by the presence of soluble starch, which is leached from the granule structure (Allen et al., 1977; Miller et al., 1973) and the interaction or cohesiveness between the swollen granules (Leach, 1965). Schoch and Maywald (1968) classified the viscosity patterns of "thick-boiling" starches into four types.

Type A: High-swelling starches, e.g., potato, tapioca, the waxy cereals, and ionic starch derivatives. The granules of these starches swell enormously when cooked in water, and the internal bonding forces become tenuous and fragile toward shear. Hence the Brabender shows a high pasting peak followed by rapid and major thinning during cooking.

Type B: Moderate-swelling starches, e.g., normal cereal starches. Because the granules do not swell excessively to become fragile, these starches show a lower pasting peak and much less thinning during cooking.

Type C: Restricted-swelling starches, especially chemically cross-bonded products. Cross-linkages within the granule markedly reduce swelling and solubilization, and stabilize the swollen granule against mechanical fragmentation. Hence the Brabender curve shows no pasting peak, but rather a very high viscosity which remains constant or else increases during cooking.

Type D: Starches with highly restricted swelling, especially "high-amylose" corn starches containing 55 - 70% linear fraction. Because of the internal rigidity imparted by the high content of associated linear molecules, the granules of the starches do not swell sufficiently to give a viscous paste when cooked in water at normal concentrations. Hence, the amount of starch must be increased two- or threefold to give a significant hot-paste viscosity of Type C. However, such high-amylose starches give a Type A or B viscosity pattern when cooked in media which cause greater granule swelling, e.g., 0.1 N sodium hydroxide.

The above two scientists found that navy bean, lentil, yellow pea and garbanzo gave Type C Brabender curves. Mung bean starch showed a mixed viscosity pattern - Type C at low concentration and Type B at high concentration. Navikul and D'Appolonia (1979) studying navy beans, pinto beans, faba beans and mung beans reported that the amylogram curves of the legume starches showed higher initial pasting temperature and higher viscosity than did wheat starch, which would indicate a higher resistance to swelling and rupture. No peak viscosity during the hold period at 95^{o}C occurred with any of the legume starches, indicating that the paste was relatively stable and that the granules did not rupture during stirring, which is not the case with wheat starch. Lineback and Ke (1975) reported that the Brabender hot-paste viscosity patterns for chick pea starch at concentrations of 5, 6, 7, 8 and 9% are similar to those reported by Schoch and Maywald (1968). They also indicated that the Brabender hot-paste viscosity patterns for horse bean starch are virtually identical to those obtained for chick pea starch at the same concentration. The gelatinization temperature ranges were 63.5 - 65 - 69^{o}C for chick pea and 61 - 63.5 - 70^{o}C for horse bean starches. Vose (1977) working on smooth-seeded field peas reported that pasting curves of smooth pea starch showed restricted-swelling characteristics similar to those shown by chemically cross-linked starches. Retrogradation of the cooked pastes resulted in rigid, opaque, friable gel with a firmer texture than corn gels. The gels occurred in either acidic or basic solutions, demonstrating characteristic differences when compared with corn or wheat pastes.

Because of the importance of legume seed in food supplementation and new product development, the pasting characteristics and functional properties of several other varieties and species of beans still need to be investigated.

Indigestible Bean Sugars Problem

Although legume seeds have high nutritional value among vegetable proteins, they contain a considerable amount of oligosaccharides which have been implicated as factors responsible for flatulence (Steggerda, 1968; Steggerda and Dimmick, 1968; Hellendoorn, 1969). Flatulence is a common complaint even among healthy individuals and is one of the most common causes of abdominal discomfort. Flatus generation has been associated with the absence of α-galactosidase activity in the upper intestinal tract of humans and animals and the fermentation process of the oligosaccharides by microorganisms with production of gas. It has been attributed also to swallowed air, inhibition of intestinal anhydrase, production of carbon dioxide from pancreatic bicarbonate (Rockland, 1969). Rackis *et al.* (1970) reported that the flatus produced by fermentation of dietary carbohydrate contained in the lower intestine may cause nausea, dyspepsia, constipation, cramps, diarrhea and discomfort. He specified that the flatus-forming factor is mainly found in the low molecular weight carbohydrate fractions of legumes which contain primary sucrose, raffinose and stachyose. Takana _et al_. (1973) and Cerning _et al_. (1975) reported that legumes contain appreciable amounts of α-galactosides of sucrose, particularly raffinose, stachyose and verbascose. Akpapunam and Markakis (1979), Cerning-Beroard and Filiatre (1976, Navikul and D'Appolonia (1978), Olson _et al_. (1975), Rackis (1975) found appreciable amounts of those oligosaccharides in mature seed such as beans - California small white, Great Northern, navy, pinto, kidney, soy, faba, lima, field, and mung; peas, cowpeas; chick peas; pigeon peas; horse gram; lentils; and lupines. Tanusi (1972) reported that mung bean, broad bean and smooth pea have small amounts of ajucose; reducing sugar ranges from 0.06 to 0.10% and nonreducing sugar from 5 to 7.0% in broad bean (_V. faba_), mung bean (_Phaseolus aureus_) and kidney bean (_P. vulgaris_). Navikul and D'Appolonia (1978) showed that the total sugar content is higher in all legume flours than in wheat flour. The legume flours contain high levels of sucrose, stachyose and verbascose, but navy and pinto bean flours contain small amounts of verbascose. Reddy and Salunkhe (1980) presented the chemical structure of those sugars containing α-galactosido-glucose and α-galactosido-galactose bonds as nonreducing sugars shown in Figure 5. When those sugars are ingested by humans, two enzymes (invertase and α-galactosidase) are required for complete hydrolysis. Gitzelmann and Auricchio (1965) explained that because the human gastrointestinal tract does not possess an α-galactosidase enzyme and because mammalian invertase is an α-glucosidase (Reddy and Salunkhe, 1980), the metabolic fate of raffinose family sugars is uncertain.

Schweizer *et al*. (1978), working with twelve mature leguminous seed crops, reported in their study using 70% ethanol extraction procedure and gas chromatography techniques, a new compound pinitol (3-O-methyl-D-chiro-inositol) and three isomers of the new disaccharide, α-D-galactopyranosyl-pinitol, for which the name galactopinitol was proposed. The chemical structures are presented in Figure 6. These sugars isolated from soya beans (Glycine max) were also identified in chick peas (*Cicer aristinum* L.), lentils (*Lens esculenta*) and beans (*Phaseolus vulgaris* L.). Their amounts ranged from 0.2 to 0.9% and 0.03 to 0.8% of dry weight respectively. Schweizer *et al*. (1978) stated that being α-galactosides, the new disaccharides must be taken into account when considering flatulence problems. Wursch (1977) proved the galactopinitols being indigestible to human intestinal enzymes. Schweizer *et al*. (1978) specified that it is probable that gas chromatography peaks

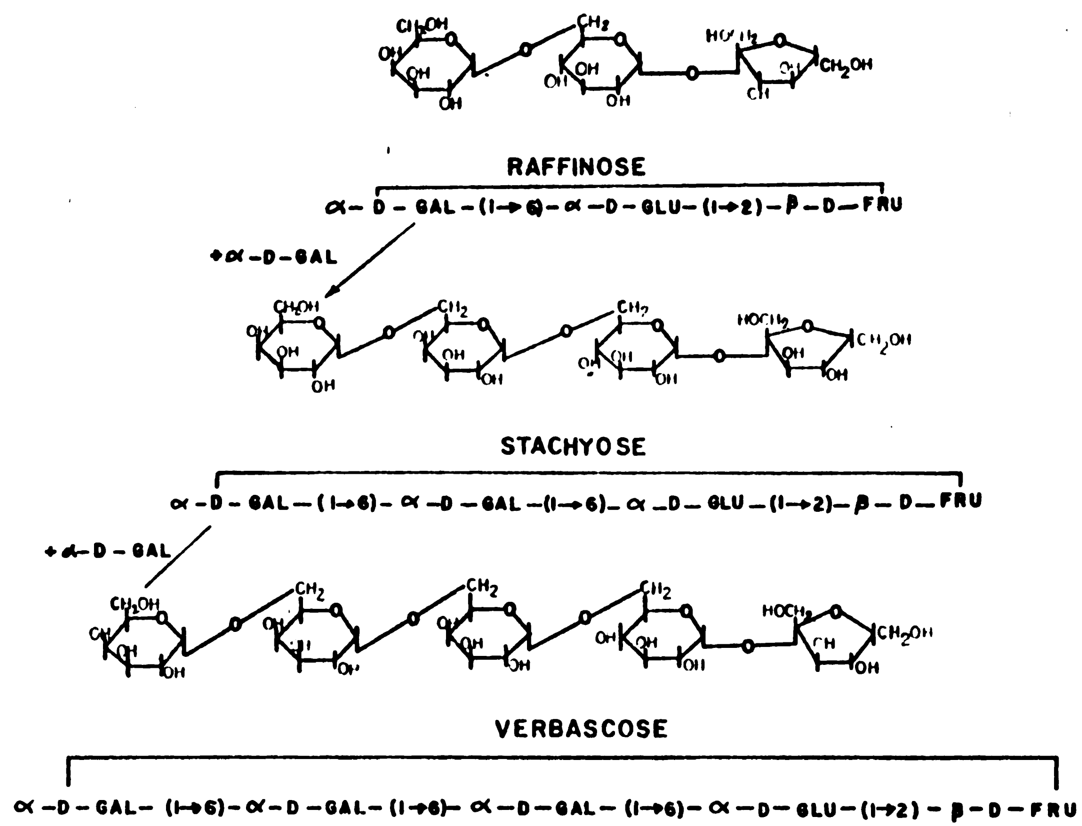

Figure 5. Structure relationships of the raffinose family oligosaccharides. Source: Reddy, N.R. and Salunkhe, D.F. 1980.

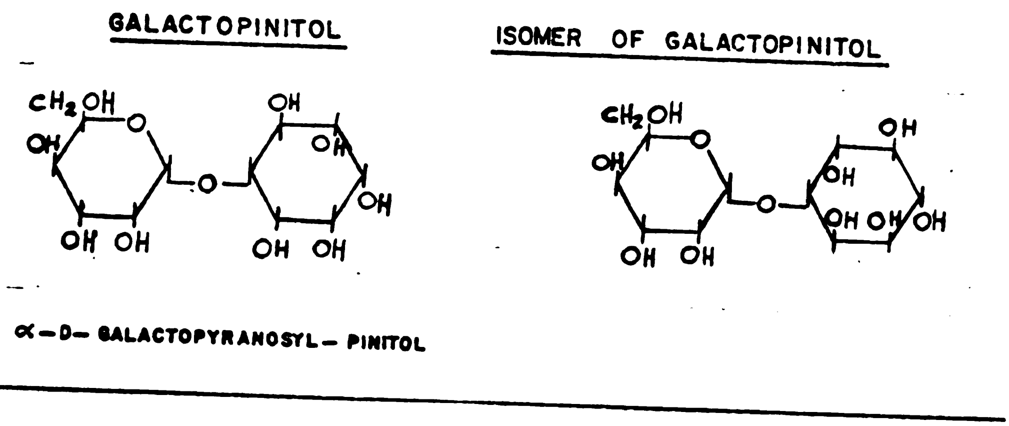

Figure 6. Structure of galactopinitol.
Source: Schweizer et al., 1978.

from non-fermented soya bean identified as melibiose in the literature were in fact a galactopinitol.

Although the incidence of flatulence in humans is unpredictable - depending on the psychological and physical state of the subjects and the type of diet, oligosaccharides are generally considered undesirable and many attempts have been made by food scientists to treat beans or their products to remove or degrade them. Mital and Steinkraus (1975) attempted to degrade oligosaccharides with lactic acid bacteria. Sugimoto and Van Buren (1970) used commercial β-galactosidase to hydrolyze oligosaccharides to their component sugars. Thanamunkul et al. (1976) and Smiley et al. (1976) used β-galactosidase in a hollow fiber reactor to hydrolyze oligosaccharides with varying degree of success. Rackis et al. (1970) and Steggerda (1967), working on experiment in vivo and in vitro, suggested that antibodies and certain phenolic acids can inhibit flatus activity.

Many workers have investigated the removal of oligosaccharides during soaking and germination (East et al., 1972; Hand, 1967; Iyengar and Kulkarni, 1977; Kawamura, 1966; Kim et al., 1973; Ku et al., 1976; Rao and Balavady, 1978; Reddy et al., 1980). Applications of ultrafiltration techniques in the removal of oligosaccharides had also been used (Omosaiyre et al., 1978).

Bean breeders are also attempting to identify genetic variants possessing low levels of oligasaccharides which may be used to biologically remove the flatulence factors of beans. Hymowitz et al. (1972) have indicated that the removal of oligosaccharides by plant breeding does not look promising. But because removal of oligosaccharides by 1) soaking results in loss of water soluble vitamin, minerals and digestible sugars, 2) germination shows alteration of the carbohydrate content of the seeds (Bond and Glass, 1963; Linko et al., 1960; Dubois et al., 1956), 3) chemicals need economic feasibility and approval for human use, the general potential for eliminating flatulence factors still lies in both biological and technological areas. Bean breeders can therefore still include that type of research in their programs.

The quantitative determination of oligosaccharides has been the subject of many investigations using either paper chromatography, thin layer chromatography, column chromatography or gel filtration (Kawamura, 1967a, 1967b; Hardinge et al., 1965; De Stafanis and Gonte, 1968). Delente and Ladenburg (1972) used gas-liquid chromatography to quantitate oligosaccharides in defatted soybean meal. Kim et al. (1973) employed liquid chromatography for the rapid determination of monosaccharides, disaccharides, stachyose and raffinose in soybeans, but absolute values for the quantity of the different sugars were not obtained by this method. Takana et al. (1975) used an analytical procedure based on thiobarbituric acid reaction (Percheron, 1962) to determine sucrose, raffinose and stachyose in whole legume seeds. All of these methods are time consuming and require some skill and experience to produce reliable results. Newly developed column packings for HPLC in the recent years have greatly simplified sugar analysis in foods and other plant materials.

Quality Evaluation of Processed Products

In the early history of breeding, the plant breeder who was the cereal breeder and particularly the wheat-breeder himself made his selections on the basis of agronomic performance and disease resistance. The main quality test that he used was the "chewing test", usually applied out in the plots after hand threshing the grain of two or three heads. To an experienced person, the chewing test gave all the quality information needed for early generation selection. The pressure applied by the jaws to crack the wheat gave a good, albeit subjective, measure of grain hardness. As the crushed grain was masticated in the mouth into a dough, the starch and other solubles, due to salivary amylase, were gradually dissolved and swallowed, or spit out, depending on the number of samples tested. Experience showed that the size of the remaining gluten ball was indicative of both protein content and gluten quality (Bushuk, 1982).

Gradually, as the cereal chemist's knowledge of the fundamentals of milling and baking quality expanded, screening of varieties for quality became much more sophisticated. Today as many as 26 different quality tests are applied to material in the final stages of development (Bushuk, 1982). Those tests are applied now not only to cereals but to many other food materials such as tubers, roots and legumes. Legume quality evaluation has been of interest in the recent years by bean breeders because of its role as a high protein source and supplement to cereal products. Adams and Bedford (1975) stated that the bases for selection of improved breeding lines require additional expenditures to conduct the various quality tests on the canned product besides the quality tests on the dry seeds. They mentioned the more subjective evaluations of quality:

1) Wholeness - the tendency of the legume seed to remain whole throughout the processing operations - not to break apart, burst, or disintegrate.
2) Consistency - the fluid is slightly viscous and clear, not cloudy or grainy; it separates or drains readily from the beans.
3) Freedom from defects - no extraneous material, loose skins, or mashed beans.
4) Flavor - must be scored by a taste panel.

5) Color - pigments in the seed coat that escape detection in the dry seed may impart an off-color to the cooked product.

6) Texture - the measure of firmness, gumminess and adhesiveness evaluated with reasonable accuracy by a sensory panel and by machines such as Kramer Shear Press, Instron and Ottawa Texture Measuring System.

Adams and Bedford (1975) stated also that there are no comprehensive studies on the source and nature of variation in the quality factors referred to above. From limited studies of special factors such as texture, and on the basis of experience, the assumption has emerged that at least a portion of the variability observed among lines depends on genetic differences although it is clear that the length of processing, the temperature of soak, the hardness of the water, and the character of the added fluid all play an important role. The above two authors recognize that, in practice, in order to assure acceptance of a new selection by growers, the breeder generally will find it necessary to compromise between the best level of quality possible in a particular program and the levels of disease resistance and agronomic performance that must be maintained. They are right to say that there is no escape from that because consumer choice for a food product is primarily good aesthetic appearance and qualities of the prepared food except that now he or she becomes more aware of nutritional evaluation of the food. Hence quality evaluation of processed foods becomes progressively important to plant breeders in order to meet the consumer requests and provide nutritious foods with great acceptability.

MATERIALS AND METHODS

Sample Source

Dry beans (*Phaseolus vulgaris* L.) used in this study were: Sanilac, a standard commercial bean; Nep-2 (Nuclear Experimental Project-2), a mutant; and San-Fernando, a tropical genotype (*Phaseolus vulgaris* L.). They were obtained from Saginaw Valley Bean and Sugar Beet Research Farm near Saginaw, Michigan, through the Department of Crop and Soil Science, Michigan State University, East Lansing, by the courtesy of Dr. G.L. Hosfield. They were delivered in paper bags to the Food Science Building and stored at room temperature in the laboratory where the experiments were carried out.

Seed Coat Separation

A certain amount of the seed from each type of bean was immersed in warm tap water for about 5 minutes. Coats were then carefully removed because they were still attached to the cotyledon outer sides. The soaking period was short in order to avoid diffusion of cell constituents such as sugars into the water. Each separated fraction was left at room temperature for three days to dry.

Grinding of Sample

Cyclose Sample Mill, U.D. Corporation, was used to grind into flour, whole seed, cotyledon (coatless) and coat from each type of bean.

Moisture

Moisture was determined by oven drying the sample at 80 ± 2°C until weight remained constant in order to avoid browning or caramelization reactions taking place when drying at 130°C for 1 hr (Hosfield and Uebersax, 1979).

Crude Fat

Crude fat was determined using A.O.A.C. method (1975) based on extracting the fat from the bean flour with petroleum ether in a Goldfisch extractor.

Ash

The A.O.A.C. method (1975) was used for ash determination. The method involved oxidizing all organic matter in a weighed sample by incineration at 550°C until the ash was gray-white and determining the weight of the ash remaining.

Protein

The Kjeldahl semi-micro-method based upon the determination of the amount of reduced nitrogen present was used.

The method consisted of: first, the wet oxidation of the sample and the conversion of protein nitrogen into ammonium sulfate; second, the decomposition of the ammonium sulfate with 10 N sodium hydroxide and the distillation of the ammonia evolved into saturated boric acid solution, and finally, the determination of the nitrogen content by titrating the ammonia with standard hydrochloric acid, 0.1 N.

Sugars

Extraction and Injection

The simple and oligosaccharide sugars (glucose, sucrose, inositol, raffinose and stachyose) commonly found in beans (*Phaseolus vulgaris* L.) were determined using High Pressure Liquid Chromatography (HPLC).

The method consisted of: first, an extraction of the sugars in a 80% ethanol:water (v/v) using a technique developed in our laboratory (Figure 7); second, precipitation and centrifugation of protein materials by a solution of lead acetate (10%, w/v); third, precipitation of excess lead in the extract using an oxalic acid solution (10%, w/v), and removal of the lead oxalate by centrifugation fourth, bringing-up of extract to volume in a 25 ml volumetric flask; fifth, filtration of the prepared sample extract through Waters Associates Sep-Pak C_{18} cartridges having the same separation properties as the separation column C_{18} where the polar compounds elute before the

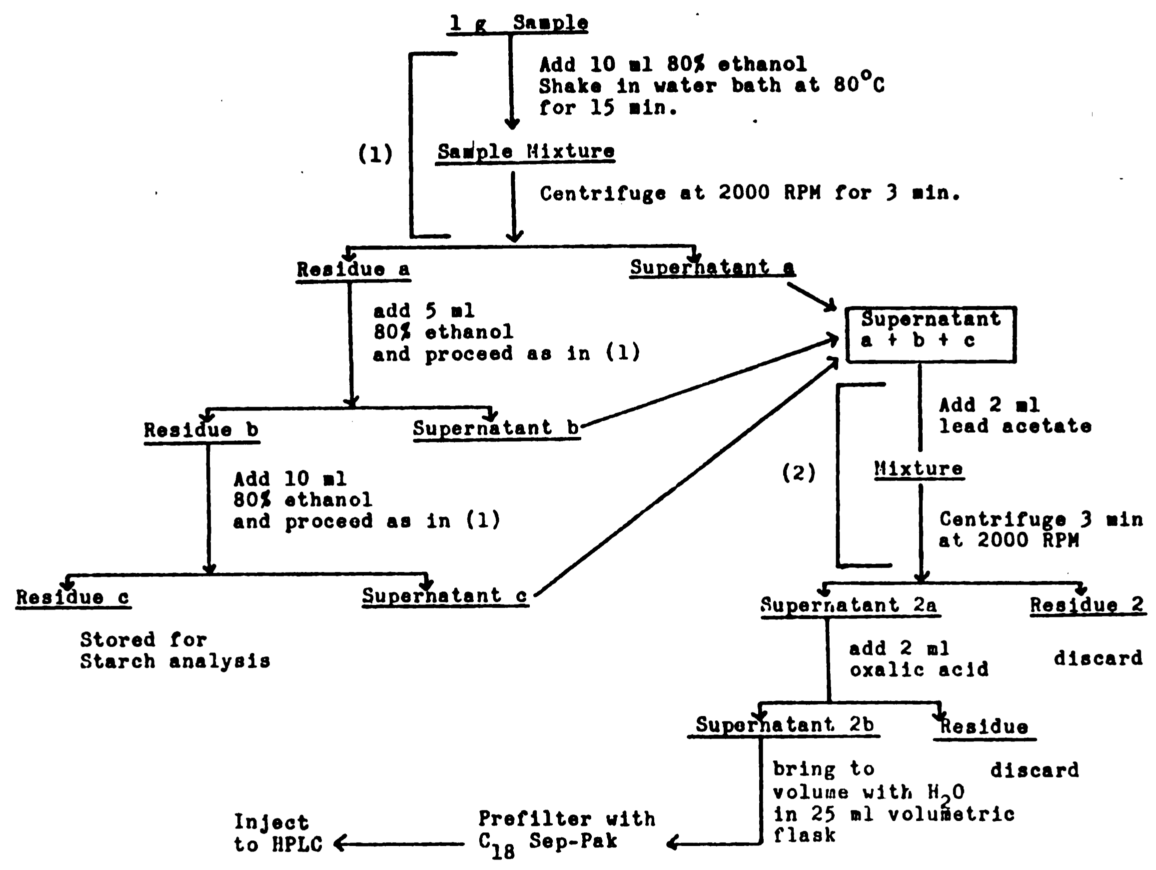

Figure 7. Flow diagram of sugar analysis.

non-polar ones. Finally, the clear extract sample was injected onto the C_{18} carbohydrate column (30 cm x 3.0 mm I.D. plates/column N/A 3000, Waters Associates, Milford, MA) for quantitation. The elution solvent was filtered acetonitrile:water (70:30, v/v, pH = 4.0) with a pump rate of 2.0 ml/min. Thirty microliter samples of the extracts were injected using a 100 µl pressure-lock microsyringe into a model U6K injector which allows the sample to be put on a bypassed injection part (no pressure exists in the bypassed state). After injection of the sample into the U6K injector loop, the bypass valve on the Waters Associates pump system Model 6000A was then switched to introduce the sample to the pressurized column held at room temperature. The refractive index detector Waters Associates Model R401 was used.

Methods of Quantitation

External standard and repetitive injection techniques (Waters Associates 1980) were used. They consisted of: first, preparing a mixed standard solution containing a known amount (1 mg/ml) of sugars to be quantitated; second, injecting a sample of the standard solution to find out the retention times of each individual known sugar appearing on the peaks of a chromatogram through a Data Module computerized machine (Waters Associates, Milford, MA); third, the retention times of the sugars were put in calibration into the computer Data Module according to the techniques of Waters Associates (1980); fourth, the unknown samples injected onto the HPLC column were then quantitated by comparison with the standard sugars put in calibration previously.

The percentage of the individual sugar extracted from the beans used (Sanilac, Nep-2 and San-Fernando) were calculated according to the following formula.

Percentage sugar =

$$\frac{\text{Amount shown on data module (mg/ml)} \times \text{original sample value (ml)}}{\text{sample weight (mg)}} \times 100$$

Starch

A new technique using HPLC was developed (Figure 8) and used in this determination. The technique consisted of a) solubilization of the sample; b) hydrolysis of the sample with amyloglucosidase enzyme to obtain glucose molecules; c) injection of the hydrolysis product to the HPLC. The amount of glucose obtained is relative to the amount of starch hydrolyzed.

Sample Preparation

Reagents

sodium hydroxide (NaOH)	0.50 N
acetic acid	0.50 N

Starch Pellet
($\sim$1.000 g)

↓

Disperse with 0.50 N NaOH (10 ml)

↓

Neutralize with 0.50 N acetic acid (10 ml)

↓

Neutral colloidal suspensions
Let stand few min. (not necessary)

↓

Aliquot A
(1 ml)
Starch-Glucose assay

↓

Add 3 ml amyloglucosidase (5 mg/ml)

↓

Incubate 30 min/55^{o} 2^{o}C
continuous shaking

↓

Remove tubes

↓

Let cool (room temperature)

↓

Filter through amberlite

↓

Injection to HPLC

Figure 8. Flow diagram of starch dispersion, hydrolysis and analysis.

The samples were the dry residue pellets obtained from extraction techniques developed in this same laboratory. 10 ml of 0.50 N sodium hydroxide was added to the centrifuge tube containing the pellet (1.000 g). With a rod, the pellets were colloidally dispersed into the 10 ml NaOH solutions (0.50 N) by continuous crushing and stirring.

After all the pellet was completely dispersed, 10 ml of acetic acid solution (0.50 N) were added into the tube to neutralize the sodium hydroxide used for dispersion.

The neutral colloidal suspensions were allowed to stand for a few minutes in order to allow unsolubilized particles, mainly cellulose, and pectic substances, to settle before using the sample for injection (this step is not obligatory).

Buffer and Enzyme Solutions

0.2 M Acetate Buffer Stock Solution

0.2 M acid: 12 ml glacial acetic acid was transferred to 1000 ml volumetric flask and diluted to volume with distilled water.

0.2 M Sodium Acetate: 16.408 g of anhydrous sodium acetate were dissolved in 800 ml of distilled water, transferred to a 1000 ml volumetric flask and diluted to volume.

Stock Solution: Three volumes of 0.2 M sodium acetate with two volumes of 0.2 M acetic acid were mixed. Volumetric flasks were used. The pH was

4.9 (theoretically 4.92). The solution was stored in a well-stoppered colored bottle under refrigeration; it could be stable for at least a month.

0.02 M Acetate Buffer Working Solution

To one volume of the 0.2 M acetate buffer stock nine volumes of distilled water were added and shaken to mix. This dilution was carried out using pipette and volumetric flask and prepared each time immediately before each analysis.

Enzyme Solutions

5 mg/ml amyloglucosidase (500 mg/100 ml, w/v) solutions were prepared in 0.02 M acetate buffer working solution.

Enzyme Characteristics

Amyloglucosidase (glucoamylase 1,4-α-D-glucan glucohydrolase; EC 3.2.1.3 from rhizopus genus mold 10,000 units/g solids).

The enzyme was purchased from Sigma Chemical Company, St. Louis, MO.

Hydrolysis

1 ml of sample was mixed with 3 ml of the enzyme amyloglucosidase (5 mg/ml) solution in a test tube. The samples were placed in a water bath at 55 ± 2^{o}C (optimum temperature for amyloglucosidase) and continuously shaken for 30 min. At the end of the time period, the test tubes were removed from the water bath and filtered through a Sep-pak to

remove the acetate (Lester, 1980) prior to the injection to the HPLC column.

Injection

Injections proceeded as described above for the introduction of sugars onto the C_{18} carbohydrate column.

Methods of Quantitation

Same as in the above quantitation of sugars with exception that only glucose was used as standard solution.

Calculation

The percentage of starch was calculated according to the following formula:

Percent starch =

$$\frac{\text{Amount shown on data module (mg/ml)} \times 10 \times \text{Original volume (ml)} \times 100 \times 0.9}{\text{sample weight (mg)}}$$

where:

0.9 (factor to account for the water gained during hydrolysis)

10 (equation correction factor)

Triplicate determinations were performed on samples for all above methods.

Water Absorption in Beans

The hydration properties of beans were determined by soaking a 10 g sample of dry beans (Sanilac, Nep-2 and San-Fernando) contained in cylindrical grids in tap:distilled water (1/1) at room temperature for 0, 15, 30, 45, 60, 75, and 90 minutes. After soaking, the cylindrical grids were removed from the water and allowed to stand on laboratory paper towel in order to remove surface water. The beans were then weighed and the increase in weight taken as the amount of water absorbed. Triplicate determinations were performed.

Water content was calculated using the following formula (Hosfield and Uebersax, 1980).

$$100 - \left(\frac{\text{wt. of solids (g) in dry beans}}{\text{wt. (g) of soaked beans}}\right) \times 100$$

Canning

Beans used for canning evaluations were adjusted rapidly to 16.0 ± 0.02% moisture content in a controlled air circulating humidity chamber prior to soaking and processing. This was done in order to eliminate any effect differential seed moisture might have on cotyledonary tenderization during soaking and cooking and to insure that each sample lot of beans contained a constant level of total solids (TS).

The soaking and processing procedures developed by Hosfield and Uebersax (1980) were applied in this process (Figure 9). The parameters considered in the evaluation of the beans used in this experiment were: color of dry and canned beans by Hunter colorimeter; drained weight by decanting contents of cans on a number 8 mesh sieve, rinsing in 21^{o}C tap water to remove adhering brine and draining for 2 minutes on the sieve positioned at a 15^{o} angle; texture using a Kramer Shear Press (KSP) fitted with a standard multiblade shear compression cell No. C338 (Food Technology Corp., Reston, VA). One hundred grams of washed processed beans were placed in the compression cell and force was applied until blades passed through the bean sample while the instrument was set at range 10 (300 lb of force full scale). Water content of canned beans (final moisture) was determined from 100 gm texture samples. These were oven dried at 80^{o} ± 2^{o}C to a constant weight (Table 3).

Subjective bean quality evaluation was made on contents of all processed cans while beans were drained on the mesh screens. The degree of packing (clumping) was rated on a 3 point scale. Overall bean appearance was evaluated to measure the suitability of beans to commercial processing. Criteria included examining beans for loose or free coats ("free skins"), individual bean integrity, and fluid consistency (Table 3).

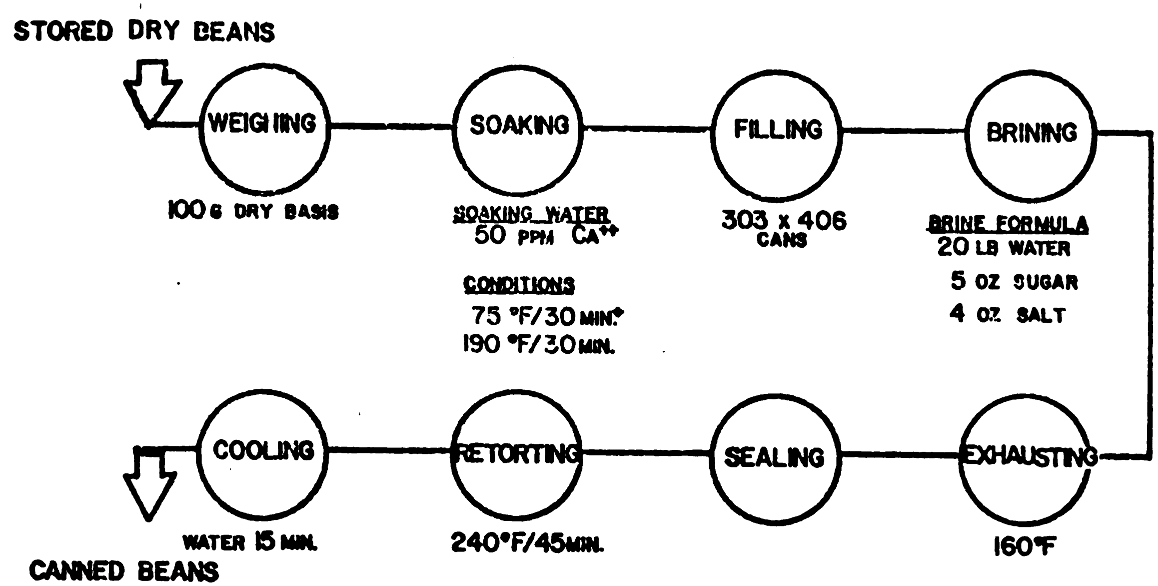

Figure 9. Schematic diagram unit operations for dry bean processing procedures.

Table 3. Bean processing evaluation and calculations.

Character	Description
<u>Soaking</u>	
Hydration Coefficient	Ratio of $\frac{\text{wt of soaked beans (g)}}{\text{wt of dry beans (g)}}$
Water content (%)	100- $\left(\frac{\text{wt of solids (g) in dry beans}}{\text{wt (g) of soaked beans}} \times 100\right)$
<u>Canning</u>	
<u>Objective</u>	
Drained weight (g)	Weight of rinsed beans drained for 2 minutes on a number 8 mesh screen (0.239 cm) positioned at a 15^{o} angle. One determination per can was made.
Texture (Kg force/100 g)	Determined by placing 100 g of washed processed beans into a standard shear-compression cell of a Kramer Shear Press and applying force with a dynamic hydraulic system. Values reported indicate the Kg force required to sheat 100 g of beans. Three determinations per can were made.
Water content (%)	Determined by oven drying each texture evaluation at $80 \pm 2^{o}C$ until weight remained constant. $\% = \frac{\text{initial wt - dry wt}}{\text{initial wt}} \times 100$
<u>Subjective</u>	
Degree of packing (1-3)	Extent of packing (clumping) of beans in can. 1 = no clumping; 2 = bean clumping but easily decanted from can; 3 = beans clumped or packed solidly in bottom of can. One determination per can was made.

Table 3. (cont'd.).

Overall Appearance (1-5)	Evaluation for general suitability of commercial processing made on each can. Criteria included examination for loose (free) seed coats (split), bean integrity, and brine consistency. Low values indicate poor appearance; high values indicate excellent appearance.

Source: Hosfield, G.L. and Uebersax, M.A. 1980.

All data were subjected to an analysis of variance appropriate to a completely randomized design. Duplicate readings were taken on all cans for texture and final moisture content.

Texture Measurement Based on Single Bean

To accomplish this, some samples of our bean varieties under investigation were sent to Dr. L.W. Hudson at the Regional Plant Introduction Station, Washington State University, Pullman, Washington. The tests were performed using the apparatus developed at that station.

The apparatus consists of 100 tests points in a 10 x 10 array. Each point has a cup holding a single bean seed. Resting on each bean is a 1.47 mm diameter pin attached to the end of a length of tubing. The tube contains enough No. 8 shot so each of the 100 pins bears 90.6 grams on each bean. The cooking vessel contains about 180 liters of water heated by steam-coils to 93.3^{o}C. A Taylor controller keeps the water at a very constant temperature (± 0.25^{o}C). When the water is at the prescribed temperature, the testing device is put in the water and the time recorded as "Beans in". Simultaneously a timer is started and in due course, as each bean reaches the proper state of "cook" the pin penetrates and the time is noted in the square corresponding to the test point.

The conditions of the test were: a) the moisture content of the seeds was adjusted by humidifying the seeds for 6 hrs; b) the beans were scarified by clipping away a small portion of the pericarp on the edge of the seed opposite the hilum and then c) soaked for 12 hours using distilled water kept at room temperature.

Tests have shown that with the apparatus, significant differences can be shown with ten seeds per lot. In this study, the test was performed four times using best looking bean seeds (twice with seed coat intact and twice with seed coat partially removed). The results were reported as time to breakdown (TTB) in minutes with standard deviation and coefficient of variation.

Gelatinization Characteristics

A sample of 50 g of whole bean and cotyledon flour (adjusted to 14% moisture) from each type of bean was used with 400 ml of phosphate buffer, pH 5.30 to make the slurry ready for heating. The functional properties of the flours were evaluated according to the AACC methods using Brabender Amylograph (1969). The method of graphic analysis for functional properties are as follows (Table 4) (Mazurs *et al*., 1957; Kite *et al*., 1957):

The Peak Viscosity or Pasting Peak

This is the highest viscosity which is reached during the gelatinization of the starch. The temperature where

Table 4. Functional and molecular properties associated with pasting characteristics of starches.

Paste Properties (Experimentally Determined)	Functional Properties	Molecular Properties
Rate of increase in viscosity when heated to 95°C (Region prior to Point A)	Ease of cooking	Rate of granules swelling
Viscosity peak (Point A)	Maximum thickness on cooking	Extent of granule swelling
Viscosity changes (after reaching maximum viscosity) during heating and 95°C holding cycles (region of Points A to C)	Stability during cooking	Granule fragility and degree of solubilization
Increase in viscosity during cooling (region of Points C to D)	Set-back on cooling	Regragradation of linear molecules
Changes in viscosity during holding at 50°C (region of Points D to E)	Resistance to shear	Granule rigidity
Final viscosity after holding at 50°C (Point E)	Thickening power or thickening efficiency	Granule rigidity extent of maintained swelling

Source: Mazurs et al., 1957.

the viscosity begins to increase, and the rate of increase are also considered. Together these three factors indicate the ease of cooking and the pasting peak provides an estimation of the power requirements for stirring the starch paste during gelatinization.

Some starches do not have a distinct peak. The viscosity simply increases during heating and tends to remain relatively constant during the holding cycle at 95°C.

<u>The Viscosity at the End of the Heating Cycle as Sample Reaches 95°C</u>

This gives an indication of stability during cooking when related to peak viscosity. A sharp drop in viscosity from the viscosity peak indicates granule fragility and solubilization.

<u>The Viscosity at the End of the 95°C Holding Cycle</u>

This indicates the degree of fragility or stability of the hot paste. A drop suggests additional breakdown of granules or solubilization due to stirring.

<u>The Viscosity at the End of the Cooling Cycle, When the Paste Reaches Again 50°C</u>

This is a measure of the thickening or "set-back" of the paste when cooling. It arises from retrogradation of the linear molecules and is a serious obstacle during processing.

The Viscosity at the End of the 50^{o}C Holding Cycle

This indicates the stability of the paste to stirring in the form in which it will most likely be used by the industry. It is a good indication of granule rigidity and resistance to shear. The actual viscosity at this point may also be considered as a measure of thickening power or thickening efficiency of starch.

Table 4 summarizes the relationships between the Brabender viscosity curves and the functional properties. These functional properties are the basis for determining the usefulness of a good starch. Table 4 also indicates the molecular events which are believed to be responsible for the observed changes (Mazurs _et al_., 1957; Carson, 1972). Medcalf and Gilles (1966) and Schoch and Maywald (1968) also described the terminology used to express the amylogram results. The temperature of initial pasting for this work is defined as the temperature at which the viscosity curve starts rising during the heating period. Perpendicular rising temperature is the temperature at which the C-shape curve starts rising and a peak reading is taken just at the top of that first curving point. The normal peak height is taken at the second curving point of the C-shape curve. In the cases where a definite sharp peak is not obtained, no value is given because height values at 95^{o}C are reported. The peak after 15 min is the viscosity of the sample after 15 min holding period at 95^{o}C. The peak drop is the height obtained at the drop point of the curve during the cooling period. The height is the viscosity of the sample after it has cooled to 25^{o}C. Peak on cooling is the height of the curve obtained during the 15 min cooling period. After 15 min at 25^{o}C is the height reached after the 15 min cooling period.

Scanning Electron Microscopy

Dehydration

Bean samples (dry, soaked, canned) were freeze-dried in a freeze dryer Unitrap II (Virtis, Gardiner, NY, 12525), 4 to 8 hrs respectively.

Coating and Viewing

Dried beans were dry-fractured by hand or using blade to "open" tissues and cells, mounted on stubs with colloidal finger polish and coated with an approximately 20 nm layer of gold using a sputter coater. The coated samples were viewed and photographed in a Philips Super III Scanning Electron Microscope (SEM) at an acceleration voltage of 15 Kv with a Polaroid P/N film type 665.

Freeze-Drying

↓

Dry-fracturing

↓

Coating

↓

Viewing

Figure 10. Flow diagram of sample preparation for SEM.

Statistical Analysis

The "Statistical Package for Social Science" computer programs described by Nie *et al*. (1975) for use on the CDC 6500 computer operated by Michigan State University Computer Laboratory was used to assist statistical analyses.

Multivariate analyses of variance and covariance were determined using subprogram ANOVA. Mean values were reported after roundings. Single classification analyses of variance, Tukey mean separations were determined using subprogram ONEWAY.

Tukey separations were presented such that treatments which were not significantly different ($p \geq 0.05$) were indicated with like letters.

Bean quality parameters and quality evaluation were subjected to an analysis of variance appropriate to a completely randomized design. Mean squares with significant F ratios were used to determine significant probability level of p 0.05.

RESULTS AND DISCUSSION

Proximate Composition

The proximate composition of the three dry bean cultivar, Sanilac, Nep-2 and San-Fernando over three crop years is shown in Tables 5, 6, 7, 8, 9, 10, 11, 12, 13 and 14.

Moisture content was higher in the 1978 beans than in the other two crop years (Table 5). Few significant differences were obtained within the crop years among the three cultivars of whole bean. The combined result indicated no significant differences among the cultivars for the three portions of the seed.

The seeds have similar high crude protein contents as obtained in other <u>Phaseolus vulgaris</u> L. beans (Ruth <u>et al</u>., 1979; Pedro and Ladermiro, 1979; Shalini <u>et al</u>., 1968; Valdemiro <u>et al</u>., 1979). There were no significant differences in the whole bean and seed coat protein for the three cultivars over the three crop years except San-Fernando which showed significant differences for the 1979 and 1980 beans (Table 7). In cotyledon alone no singificant differences were observed among the varieties except Nep-2 and Sanilac cotyledons which possessed significant differences in the 1978 and 1980 beans, respectively. The

Table 5. Percent moisture content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) by crop years (1978, 1979 and 1980) and seed portions (whole bean, cotyledon and seed coats.

Cultivars	Crop Years								
	1978			1979			1980		
	Seed Portions			Seed Portions			Seed Portions		
	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat
Sanilac	8.7^{a}	7.2^{b}	8.3^{e}	6.8^{a}	6.3^{b}	6.5^{c}	6.3^{a}	5.9^{b}	5.4^{d}
Nep-2	8.7^{a}	9.2^{c}	8.3^{e}	6.7^{a}	6.2^{b}	6.7^{c}	6.3^{a}	6.0^{b}	5.2^{d}
SF	8.6^{a}	9.9^{d}	9.0^{f}	6.6^{a}	6.3^{b}	7.3^{d}	6.5^{a}	5.3^{c}	5.6^{d}

n = 3 (3 replicates/seed portion/cultivar.
Like letters in column denote non-significant differences ($p>0.05$) among cultivars and within crop years and seed portions.

Table 6. Percent moisture content (dry basis) of bean cultivars (Sanilac, Nep-2, San-Fernando) for three crop years (1978, 1979 and 1980) by seed portions (whole bean, cotyledon and seed coat).

Cultivars	Seed Portions		
	Whole	Cotyledon	Seed Coat
Sanilac	7.3^a	6.5^b	6.8^c
Nep-2	7.2^a	7.2^b	6.7^c
SF	7.2^a	7.2^b	7.3^c

n = 9 (3 replicates/seed portion/cultivar x 3 years).
Like letters in column denote non-significant differences ($p \leq 0.05$) among cultivars and within seed portions.

Table 7. Percent protein content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) by crop years (1978, 1979 and 1980) and seed portions (whole bean, cotyledon and seed coat).

Cultivars	Crop Years								
	1978			1979			1980		
	Seed Portions			Seed Portions			Seed Portions		
	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat
Sanilac	23.0^{a}	24.8^{b}	5.8^{d}	21.3^{a}	25.2^{c}	7.5^{d}	27.6^{a}	27.3^{c}	9.8^{e}
Nep-2	23.1^{a}	22.5^{c}	6.5^{d}	20.1^{a}	24.8^{c}	7.3^{d}	28.1^{a}	22.6^{d}	8.8^{e}
SF	21.6^{a}	25.1^{b}	6.5^{d}	23.9^{b}	25.2^{c}	7.3^{d}	26.0^{b}	23.8^{d}	6.2^{f}

n = 9 (3 replicates/seed portion/cultivar x 3 injections/replicate).
Like letters in column denote nonsignificant differences ($p \geq 0.05$) among cultivars and, within crop years and seed portions.

Table 8. Percent protein content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) for three crop years (1978, 1979 and 1980) by seed portions (whole bean, cotyledon and seed coat).

Cultivars	Seed Portions		
	Whole	Cotyledon	Seed Coat
Sanilac	24.0[a]	25.8[b]	7.7[d]
Nep-2	23.8[a]	23.3[c]	7.5[d]
SF	23.8[a]	24.7[bc]	6.7[d]

n = 9 (3 replicates/seed portion/cultivar x 3 years). Like letters denote nonsignificant differences ($p \geq 0.05$ among cultivars and within seed portions.

Table 9. Percent fat content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) by crop years (1978, 1979 and 1980) and seed portions (whole bean, cotyledon and seed coat)

Cultivars	Crop Years								
	1978			1979			1980		
	Seed Portions			Seed Portions			Seed Portions		
	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat
Sanilac	1.3^{a}	1.7^{b}	0.8^{c}	1.4^{a}	1.4^{c}	0.4^{e}	1.1^{a}	1.1^{b}	0.5^{d}
Nep-2	1.6^{a}	1.8^{b}	0.6^{c}	1.5^{a}	1.8^{d}	0.6^{f}	1.2^{a}	1.4^{c}	0.5^{d}
SF	1.2^{a}	1.7^{b}	0.7^{cd}	1.2^{b}	1.9^{d}	0.4^{e}	1.2^{a}	1.1^{b}	0.2^{e}

n = 3 (3 replicates/seed portion/cultivar).
Like letters in column denote nonsignificant differences ($p \geq 0.05$) among cultivars and, within years and seed portions.

Table 10. Percent fat content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) for three crop years (1978, 1979 and 1980) by seed portions (whole bean, cotyledon and seed coat).

Cultivars	Seed Portions		
	Whole	Cotyledon	Seed Coat
Sanilac	1.3[ab]	1.4[c]	0.6[d]
Nep-2	1.4[b]	1.7[c]	0.5[d]
SF	1.2[a]	1.6[c]	0.4[d]

n = 9 (e replicates/seed portion/cultivar x 3 years).
Like letters in column denote nonsignificant differences ($p>0.05$) among cultivars and within seed portions.

Table 11. Percent ash content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) by crop years (1978, 1979 and 1980) and seed portions (whole bean, cotyledon and seed coat).

Cultivars	Crop Years								
	1978			1979			1980		
	Seed Portions			Seed Portions			Seed Portions		
	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat
Sanilac	5.2^{a}	4.0^{c}	6.1^{e}	4.1^{a}	3.9^{c}	6.7^{e}	4.9^{a}	4.6^{c}	6.1^{f}
Nep-2	4.2^{b}	4.1^{d}	5.4^{f}	3.9^{b}	4.0^{cd}	4.7^{f}	4.5^{b}	4.4^{d}	5.2^{f}
SF	4.1^{b}	3.9^{c}	3.9^{g}	4.1^{a}	4.1^{d}	3.7^{f}	4.4^{b}	4.3^{e}	3.9^{g}

n = 3 (3 replicates/seed portion/cultivar)
Like letters in column denote nonsignificant differences ($p>0.05$) among cultivars and, within crop years and seed portions.

Table 12. Percent ash content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) for three crop years (1978, 1979 and 1980) by seed portions (whole bean, cotyledon and seed coat).

Cultivars	Seed Portions		
	Whole	Cotyledon	Seed Coat
Sanilac	4.7^{a}	4.2^{c}	6.3^{d}
Nep-2	4.2^{b}	4.2^{c}	5.1^{e}
SF	4.2^{b}	4.1^{c}	3.8^{f}

n = 9 (3 replicates/seed portion/cultivar x 3 years)
Like numbers in column denote nonsignificant differences ($p \geq 0.05$) among cultivars and within seed portions.

Table 13. Percent starch content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) by crop years (1978, 1979 and 1980) and seed portions (whole bean, cotyledon and seed coat).

Cultivars	Crop Years								
	1978			1979			1980		
	Seed Portions			Seed Portions			Seed Portions		
	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat
Sanilac	45.4^{a}	41.6^{c}	4.1^{d}	52.0^{a}	39.4^{c}	Trace	45.5^{a}	47.0^{b}	Trace
Nep-2	49.5^{ab}	47.2^{c}	2.7^{e}	41.7^{b}	50.3^{c}	6.2^{d}	37.3^{a}	42.4^{c}	6.7^{d}
SF	56.6^{b}	48.7^{c}	3.2^{de}	40.0^{b}	43.2^{c}	4.8^{d}	47.9^{a}	47.7^{b}	Trace

n = 3 (3 replicates/seed portion/cultivars).
Like letters in column denote nonsignificant differences ($p \geq 0.05$) among cultivars and, within crop years and seed portions.

Table 14. Percent starch content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) for three crop years (1978, 1979 and 1980) by seed portions (whole bean, cotyledon and seed coat).

Cultivars	Seed Portions		
	Whole	Cotyledon	Seed Coat
Sanilac	47.6[a]	42.3[b]	1.5[c]
Nep-2	42.8[a]	46.7[b]	5.2[d]
SF	48.1[a]	46.5[b]	2.7[cd]

n = 27 (3 replicates/seed portion/cultivar x 3 injections x 3 years).
Like letters in column denote nonsignificant difference ($p > 0.05$) among cultivars and within seed portions.

combined three crop years results showed significant differences among the cultivars for cotyledon protein content ranging from 23.3% (Nep-2) to 25.8 (Sanilac (Table 8).

Fat content constitutes a relatively small amount of the overall bean composition like in most low fat legumes (Table 9). There were no significant differences in the whole seed fat content among the cultivars except that San-Fernando showed a slight difference in the 1979 beans. Significant differences in fat were obtained in the cotyledon from the 1979 and 1980 beans but not in those of 1978 (Table 9). Seed coat fat content was less than one percent. Nep-2 and San-Fernando showed no significant differences in their seed coat fat content for the year 1978. Sanilac and Nep-2 showed no significant differences for 1980 beans (Table 9). Combined fat content from the three crop years results indicated significant differences in the whole bean among the cultivars but not in the beans cotyledons and seed coats (Table 10).

Ash content falls into the range reported for legumes (Phaseolus vulgaris L.) by Ruth et al. (1979), Pedro and Ladermiro (1979) (Table 8). The ash content did not show a significant difference between the two isolines 1978 and 1980 whole bean samples (Table 11). Within the years, significant differences were obtained for cotyledons and seed coats among the three cultivars. The combined percent ash content revealed no significant differences between the two isolines in whole beans and cotyledons however, in the seed coat a significant difference was obtained (Table 12).

The starch content in Table 13 is similar to other legumes *Phaseolus vulgaris* L. reported by Navikul and d'Appolonia (1978), Cerning and Aliette (1979), Roberto and Eidiomar (1971), and Theravuthi *et al*. (1974). There was no significant differences between the two isolines for the three seed portions (whole, cotyledon and seed coat) for the three crop years (Table 14), except San-Fernando which showed some difference in the cotyledon with the 1980 beans.

In general, the statistical analysis of the proximate composition revealed, except for fat content, no significant differences for the protein, ash and starch contents in the whole beans among the three cultivars although differences were obtained in some cotyledon and seed coats. In this case, we can not rely on the proximate composition to explain the textural behavior differences of the beans used in the experiment.

Sugars

Sugars commonly found in legumes (*Phaseolus vulgaris* L.) are reported in Table 15A, B, and C. Hexose represents a mixture of D-glucose and D-galactose, and probably D-fructose because in this study, these three reducing sugars appear at the same retention time from a standard "cocktail"

Table 15. Percent sugar (hexose, sucrose, inositol, raffinose and stachyose) content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) and seed portions (whole bean, cotyledon and seed coat) by crop year (1978, 1979 and 1980).

	Hexose			Sucrose			Inositol			Raffinose			Stachyose		
	Seed Portion			Seed Portion			Seed Portion			Seed Portion			Seed Portion		
	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat
							A. Crop Year 1978								
Sanilac	.9^{a}	.9^{b}	.2^{c}	2.1^{a}	2.5^{b}	.5^{c}	.5^{a}	.6^{b}	.1^{c}	.3^{a}	.3^{b}	ND	1.4^{a}	1.1^{b}	ND
Nep-2	.8^{a}	.9^{b}	.1^{d}	2.3^{a}	2.8^{b}	.2^{d}	.4^{a}	.4^{b}	ND	.3^{a}	.4^{b}	ND	1.5^{a}	1.1^{b}	ND
SF	.9^{a}	1.0^{b}	.2^{c}	2.0^{a}	2.5^{b}	.3^{cd}	.4^{a}	.4^{b}	.1^{cd}	.3^{a}	.5^{b}	ND	1.4^{a}	1.2^{b}	ND
							B. Crop Year 1979								
Sanilac	1.7^{a}	1.4^{b}	2.0^{c}	1.6^{a}	2.9^{b}	.2^{c}	.7^{a}	.6^{d}	.1^{f}	.3^{a}	.5^{b}	ND	2.0^{a}	2.7^{b}	ND
Nep-2	1.9^{a}	1.6^{b}	1.8^{c}	1.8^{a}	3.3^{b}	.2^{c}	.2^{b}	.3de	.1^{f}	.4^{d}	.4^{b}	ND	2.0^{a}	3.0^{b}	ND
SF	2.3^{a}	1.3^{b}	1.5^{c}	2.0^{a}	2.9^{b}	.1^{d}	.4^{c}	.2^{e}	ND	.5^{a}	.5^{b}	ND	2.2^{a}	2.8^{b}	ND
							C. Crop Year 1980								
Sanilac	.8^{a}	.8^{d}	ND^{g}	1.9^{a}	2.2^{c}	.2^{d}	.6^{a}	.5^{b}	ND	.8^{a}	.5^{c}	ND[1]	2.7^{a}	3.5^{d}	ND
Nep-2	1.1^{b}	1.3^{e}	.1^{g}	2.6^{b}	1.9^{c}	.2^{d}	.5^{a}	.4^{b}	ND	ND	ND	ND	3.0^{b}	2.6^{e}	ND
SF	1.8^{c}	1.9^{f}	.1^{g}	2.2^{ab}	2.3^{c}	.2^{d}	.7^{a}	.8^{c}	ND	.3^{b}	.3^{c}	ND	2.4^{c}	2.3^{f}	ND

n = 9 (3 replicates/seed portion/cultivar x 3 injections/replicate)

[1]ND = Nondetectable

Like letters in column denote nonsignificant differences ($p \geq 0.05$) among cultivars and within seed portions.

mixture of these sugars. It has previously been reported that anomeric forms of reducing sugars such as D-glucose and D-galactose are not resolved in HPLC (Palmer, 1975; Eileen et al., 1979). Two flatulent sugars raffinose and stachyose, which are of concern to consumers, were eluted. The stachyose content was higher as compared to raffinose. In general, no significant differences for each sugar were shown in the whole bean, cotyledon and seed coat of the three cultivars for the three crop years (Appendix Table 1A). Hexose and sucrose did not show any significant differences in the whole bean and cotyledon for the crop years 1978 and 1979 for the three cultivars. The combined results were similar although they differ in the 1980 bean samples (Tables 15A, B, and Appendix Table 1A). Higher hexose contents ranging from 1.7 to 2.3% were obtained in 1979 whole beans (Table 15B) and lower values in those of 1978 (Table 15A). Stachyose showed its highest content (2.4%, 2.7% and 3.0%) in 1980 whole beans (Table 15C) and lowest (1.4%, 1.4% and 1.5%) in 1978 beans (Table 15A). Nep-2 and Sanilac had the highest (3.0%) and lowest (1.4%) stachyose values respectively (Table 15C and 15A). The 1980 beans revealed no detectable amount of raffinose in the entire beans (Table 15C). The inositol content was less than 0.7% in the beans used and, in the three crop years combined results, it did not show any significant difference at the three portions among the isolines (Appendix Table 1A). The seed coat present no detectable raffinose and stachyose. Combined results are presented in Figures 11, 12 and 13.

All the results obtained on sugars fall in the range of values reported in legumes by Navikul and d'Appolonia (1978), Quemener and Mercier (1980), Cerning et al. (1975), Akpapunam and Markakis (1979), and Munehiko et al. (1975).

The variation in the content of the individual sugars from year to year could be related to soil conditions, weather, nutrient availability. Nep-2, which showed no detectable amount of raffinose in the 1980 whole beans, possessed the highest value of stachyose (Table 15C). This implies a compensation process which could be important to plant breeders in the selection of cultivars leading to elimination of flatulent factors in beans.

Scanning Electron Microscopy Examination of Dry Beans

Examination of the seed coat outer surface showed both Sanilac and Nep-2 to possess a highly rough and wrinkled outer surface with pores and sinking holes while San-Fernando revealed only a highly rough and convoluted structure (Figure 14A_1, B_1, C_1). The coat inner surface views showed Sanilac and Nep-2 with similar structure, wide "hills" and narrow "valleys" (Figure 14A_2, B_2, C_2).

The seed coat of Sanilac was not difficult to remove compared to the two isolines Nep-2 and San-Fernando. A

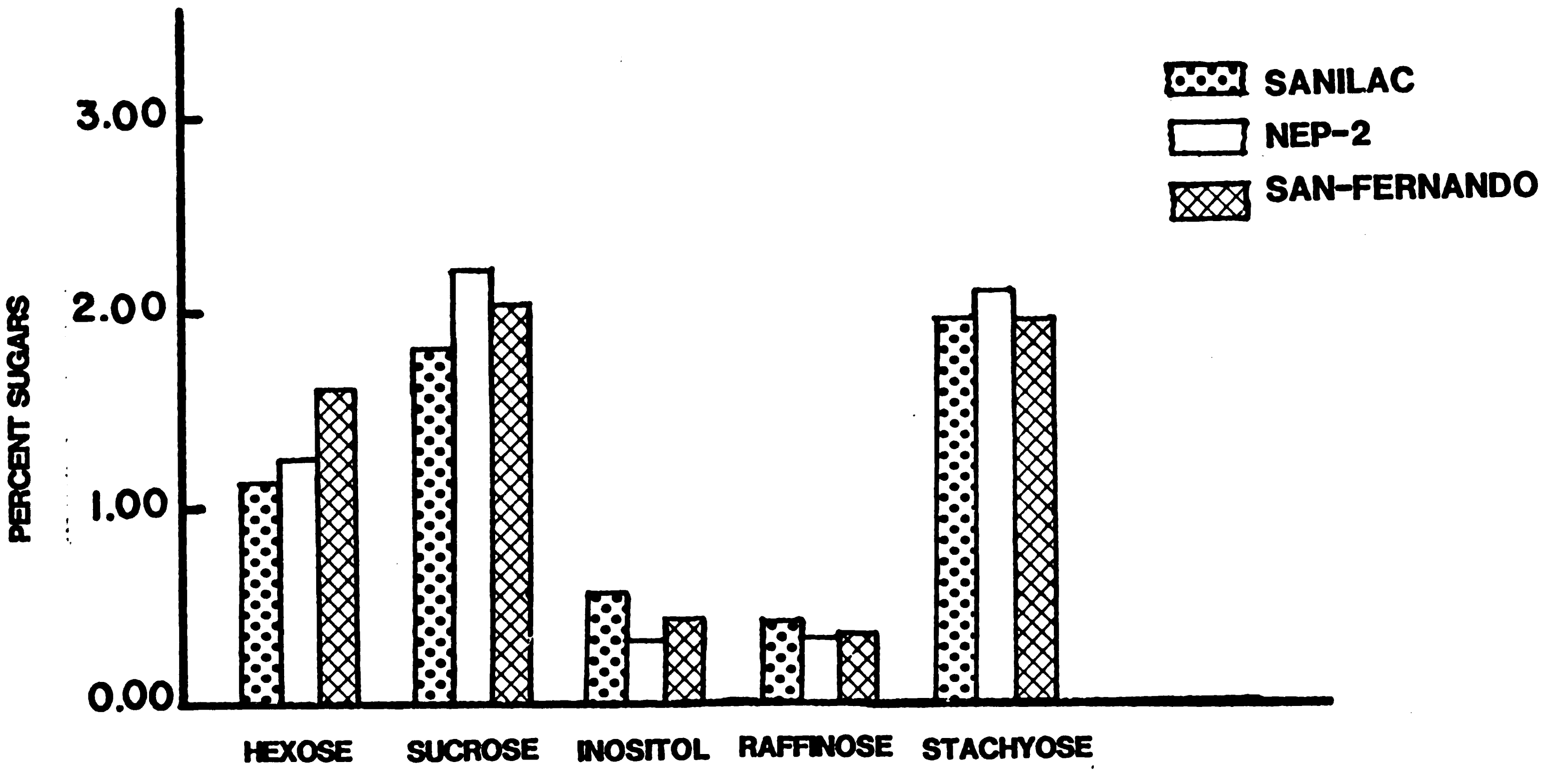

Figure 11. Mean sugars (hexose, sucrose, inositol, raffinose and stachyose) content values over three crop years (1978, 1979 and 1980) for whole bean cultivars (Sanilac, Nep-2 and San-Fernando).

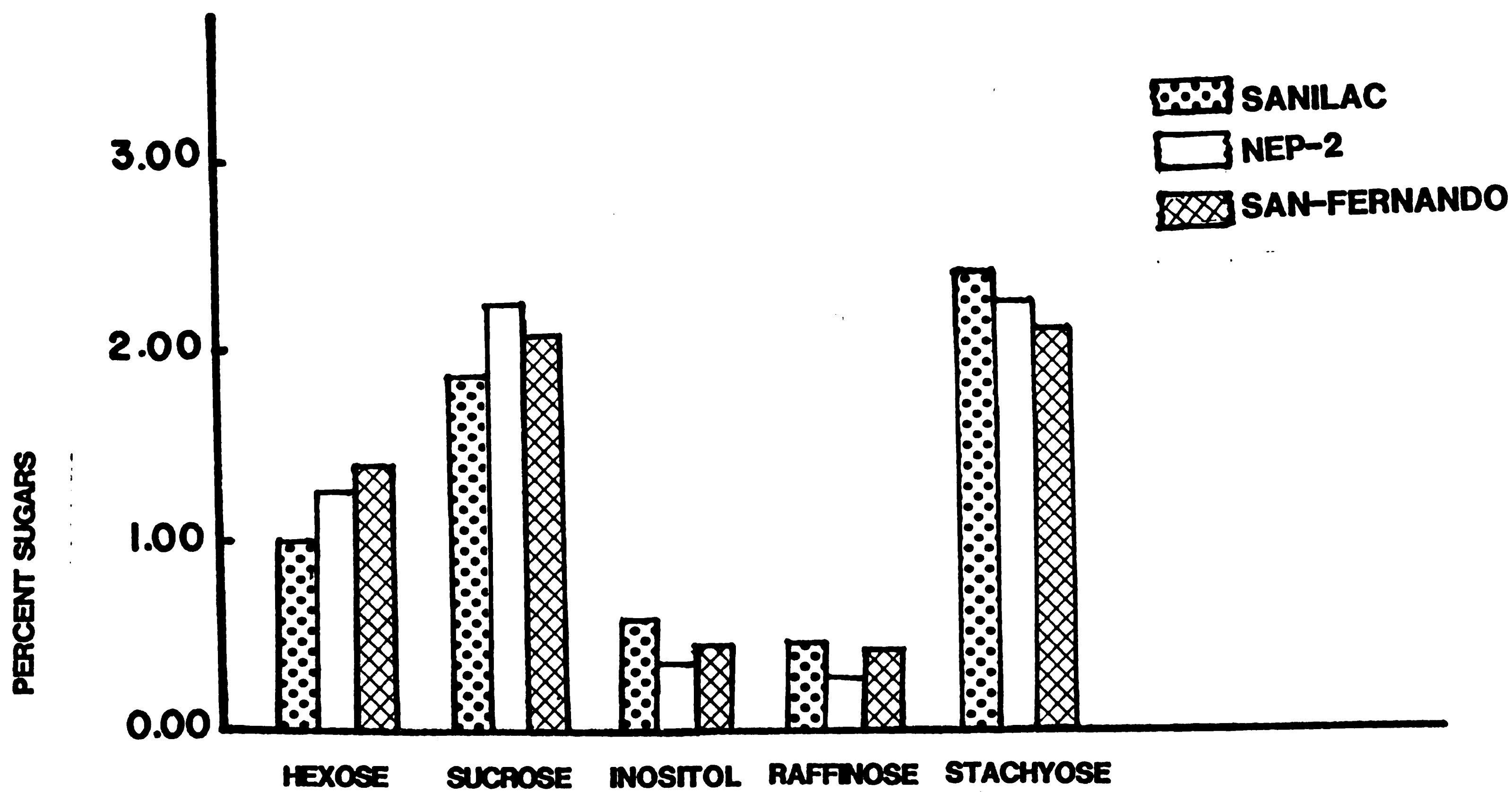

Figure 12. Mean sugars (hexose, sucrose, inositol, raffinose and stachyose) content values over three crop years (1978, 1979 and 1980) for bean cultivar (Sanilac, Nep-2 and San-Fernando) cotyledons.

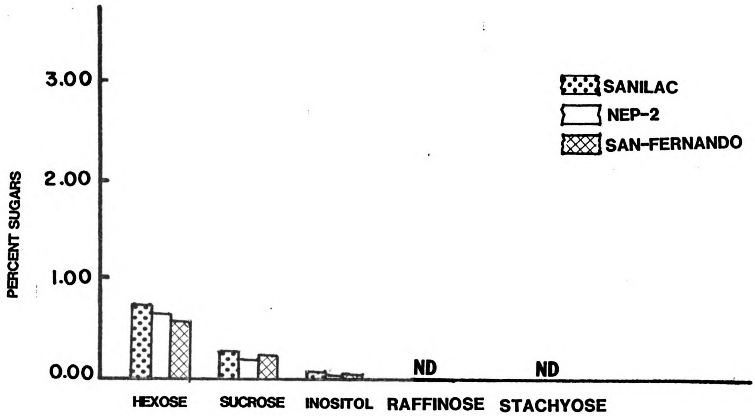

Figure 13. Mean sugar (hexose, sucrose, inositol, raffinose and stachyose) content values over three crop years (1978, 1979 and 1980) for bean cultivars (Sanilac, Nep-2 and San-Fernando) seed coat.

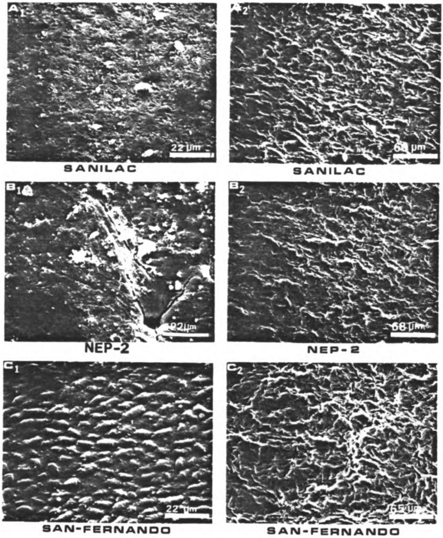

Figure 14. Scanning electron micrographs of dry bean seed coat outer (1000x) and inner (1000x) surfaces structures; A_1 = Sanilac coat outer surface; A_2 = Sanilac coat inner surface; B_1 = Nep-2 coat outer surface; B_2 = Nep-2 coat inner surface; C_1 = San-Fernando coat outer surface; C2 = San-Fernando coat inner surface.

sticky membrane frequently remained on the cotyledon of Nep-2 and San-Fernando when the seed coats were removed. A larger membrane was always observed on the San-Fernando cotyledon than on Nep-2 (Figure $15B_1$ and C_1). Higher magnification (Figures $15A_2$, B_2, C_2) revealed Sanilac and Nep-2 with similar structure compared to San-Fernando. Contrary to the inner surface of the seed coat, cotyledon surfaces were rough and covered by wide "hills" with narrow "valleys" (Figure $15A_2$, B_2, C_2), whereas the inner surface of the seed coat was covered with narrow "hills" and wide "valleys" (Figure $14A_2$, B_2, C_2) as was reported by Sefa-Dedeh for cowpea cultivar Adua Ayers (1978). As reported by Sefa-Dedeh (1978) in cowpeas, the surface topography suggested also that Sanilac, Nep-2 and San-Fernando cotyledon surface and seed coat inner surface may be of complementary structures, the "hills" of the cotyledon fitting into the "valleys" of the seed coat. The protein membrane found on the cotyledon surface of Nep-2 and San-Fernando shows a tighter interlocking structure of the cotyledon and seed coat in those two isolines. This prevents a rapid translocation of water between the cotyledon and the seed coat; hence slow absorption of water into the parenchyma cells of the cotyledon of those isolines. San-Fernando cotyledon covered with a larger portion of the membrane showed the slowest water absorption pattern.

In external topography, distinct hypocotyl area, elliptical hilum, and micropyle are visibel at low magnification

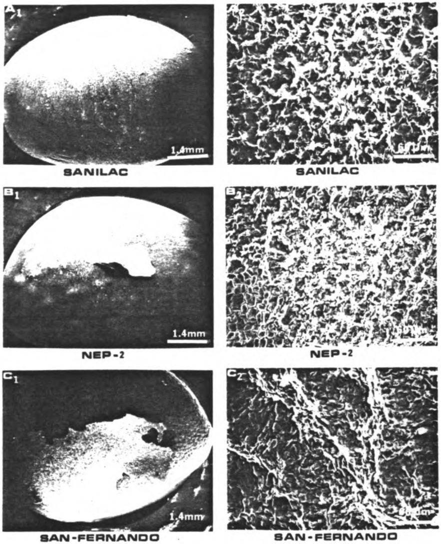

Figure 15. Scanning electron micrographs of dry bean seed cotyledon outer surface structures (30x and 700x); A_1 and A_2 = Sanilac cotyledon outer surface; B_1 and B_2 = Nep-2 cotyledon outer surface; C_1 and C_2 = San-Fernando outer surface.

in all three cultivars as commonly found in legumes (Figures 16A_1, B_1, C_1). Micropyle just below the hilum indicated specific differences by being heart-shaped open, Y-shaped open and Y-shaped closed for Sanilac, Nep-2 and San-Fernando, respectively (Figure 16A_2, B_2, C_2).

Sanilac showed a flat hypocotyl area, Nep-2 a slightly elevated one, and San-Fernando a highly elevated area (Figure 17A_1, B_1, C_1). This is a meaningful difference among the three cultivars because all individual seeds from each cultivar showed this characteristic. The hilum structure consists of a double layer of palisade cells like in soyabean reported by Kondo (1913) and Wolf and Baker (1972); the upper layer gives the hilum a meshlike structure (Figure 17A_2, B_2, B_3). Double layers of palisade cells are seen transversely through the hilum (Figure 17A_2, B_2, C_2). Immediately below the hilum groove are the tracheids which are similar in all three cultivars except that those of San-Fernando are narrower than the other two (Figures 17A_2, B_2, C_2).

The seed testa showed all the major anatomical characteristics of legume seeds as reported by Corner (1951). Longitudinal and transverse cross-sections of the bean seed coats show similar structure (Figure 18).

The palisade thickness were about 23 μm, 30 μm and 32 μm for Sanilac, Nep-2 and San-Fernando, respectively. Only a single layer of hour-glass cells was present and was thinner

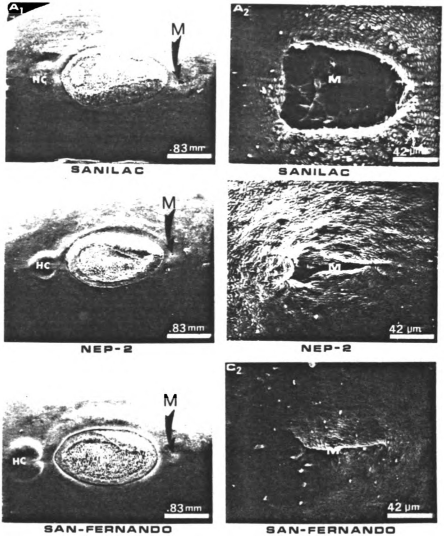

Figure 16. Scanning electron micrographs of dry bean seed hilum area structures.
A_1 = Sanilac hilum area (50x); A_2 = Sanilac micropyle (1000x); B_1 = Nep-2 hilum area (50x); B_2 = Nep-2 micropyle (1000x); C_1 = San-Fernando hilum area (50x); C_2 = San-Fernando micropyle (1000x); HC = hypocotyl area (50x); H = hilum; M = micropyle.

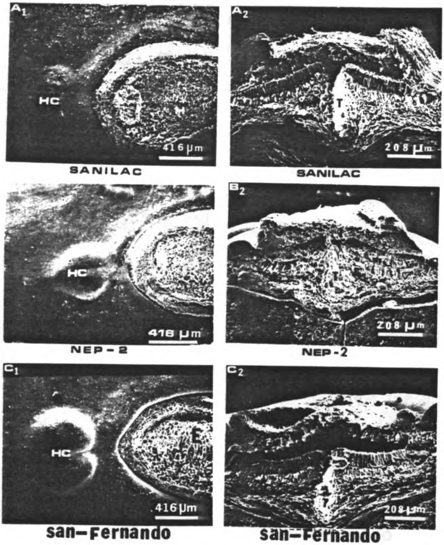

Figure 17. Scanning electron micrographs of dry bean seed hypocotyl area (100x) and hilum transverse cross-section (200x) structures. A_1 = Sanilac hypocotyl area; A_2 = Sanilac hilum transverse section; B_1 = Nep-2 hypocotyl area; B_2 = Nep-2 hilum transferse section; C_1 = San-Fernando hypocotyl area; C_2 = San-Fernando hilum transverse section; HC = hypocotyl area; H = hilum; T = tracheid

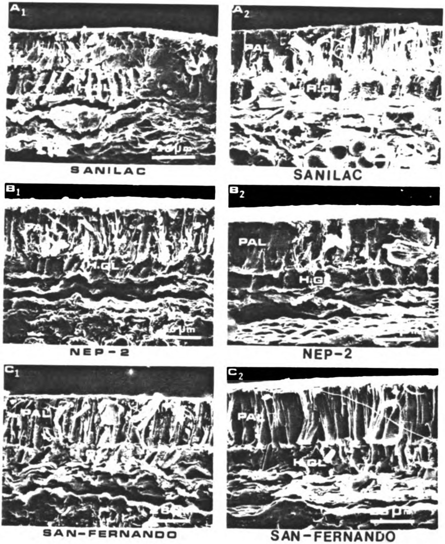

Figure 18. Scanning electron micrographs of dry bean seed coat cross-section structures (1600x).
A_1 = Sanilac longitudinal cross-section;
A_2 = Sanilac transverse cross-section;
B_1 = Nep-2 longitudinal cross-section;
B_2 = Nep-2 transverse cross-section;
C_1 = San-Fernando longitudinal cross-section;
C_2 = San-Fernando transverse section; PAL = Palisade cells; HGl = Hour-glass cells

than the palisade with thickness of 21 μm, 17 μm and 13 μm for Sanilac, Nep-2 and San-Fernando, respectively. Hour-glass cells appear more organized and rigid in structure for each bean cultivar. San-Fernando has the thinner (13 μm) hour-glass and thicker (32 μm) palisade size. Ratio of palisade to hour-glass indicates about 1.1, 1.8, and 2.5 fold thickness in Sanilac, Nep-2 and San-Fernando, respectively. This high palisade thickness could be a factor in the slow absorption of water through the San-Fernando seed coat compared to the other two cultivars. The combined palisade and hour-glass thicknesses were approximately the same for the three cultivars by being 45 μm in Sanilac, 47 μm in Nep-2 and 45 μm in San-Fernando. Amorphous mesophyll layers were seen in all the cultivars next to the hour-glass cells (Figure 18). The presence of a single layer of hour-glass cells has been reported in most bean species by Chowdhury and Buth (1970).

Figure 19 presents the seed cotyledon inner wide surface structure of the three cultivars under investigation. Nep-2 and San-Fernando cotyledon inner side surfaces are similar in structure with well organized protein film bundles covering the entire inner surface while Sanilac shows a protein film with thinner bundles. The effect of the film bundles on the water absorption process is not known, however it may be a barrier to the easy penetration of water into the cotyledon parenchyma cells. The two isolines which

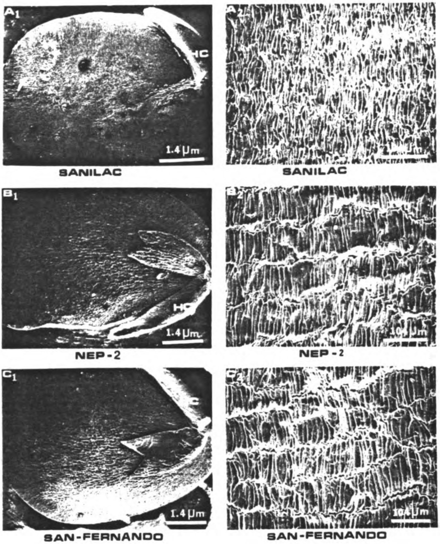

Figure 19. Scanning electron micrographs of dry bean seed cotyledon inner side surface structures (30x and 400x). A_1 and A_2 = Sanilac cotyledon inner side surface; B_1 and B_2 = Nep-2 cotyledon inner side surface; C_1 and C_2 = San-Fernando cotyledon inner side surface; HP = hypocotyl.

have different structure at the cotyledon outer side surface show a genetic similarity in structure on this side of the cotyledon (Figure 19B_2, C_2).

The 1980 Nep-2 beans revealed seed cotyledon naturally divided in sections (Figure 20A, B, C). This presentation was different from Nep-2 seeds obtained from previous crop years and also to Sanilac and San-Fernando seed cotyledons in general. The fracture lines seen clearly on the inner side surface of the cotyledon (Figure 20B, C) were observed on most of the seeds of that crop year while no such phenomena was seen in the previous crop years and the other cultivars. This natural division of the Nep-2 cotyledon explained its high split score in the processed beans of that year. Plant breeders should be aware of that phenomena because it can not be seen with seed coat intact.

Under the SEM, the cotyledon which is the major part of the seed reveals parenchymatous cells (Figures 21A_1, A_2, B_1, B_2, C_1, C_2). Irregular shapes and arrangement of the parenchyma cells with reserve materials in form of starch granules are observed in the three cultivars. The parenchyma cells length and width ranged from 39 to 94 μm and 30 to 56 μm for Sanilac, 63 to 104 μm and 31 to 57 μm for Nep-2 and 46 to 68 μm and 29 to 42 μm for San-Fernando, respectively. San-Fernando shows shorter and thinner parenchyma cells. These cells are more tightly surrounded by the middle lamella with the starch granules firmly embedded in the protein matrix (Figures 21C_1, C_2). This could imply

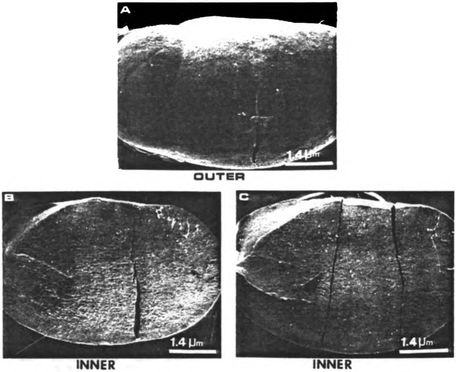

Figure 20. Scanning electron micrographs of 1980 dry Nep-2 bean cotyledon outer and inner side surfaces structures (30x); A = cotyledon outer side surface; B & C = cotyledon inner side surfaces.

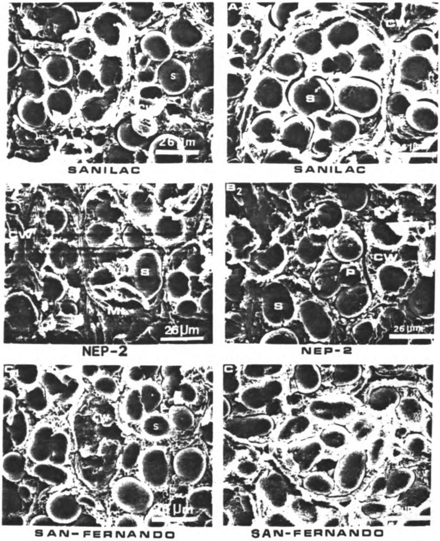

Figure 21. Scanning electron micrographs of dry bean seed cotlydon cross-section structures (1600x). A_1 = Sanilac longitudinal cross-section; A_2 = Sanilac transverse cross-section; B_1 = Nep-2 longitudinal cross-section; B_2 = Nep-2 transverse cross-section; C_1 = San-Fernando longitudinal cross-section; C_2 = San-Fernando transverse cross-section; CW = cell wall; ML = middle lamella; P = protein matrix; S = Starch granules.

that there may be more parenchyma cells in the San-Fernando than in the other two cultivars. The firmly packing of the cells is a good indication of the firmer texture of that bean cultivar. This firm packing of cells could cause increased resistance to shear.

Although Sanilac and Nep-2 have organized parenchyma cell structures which are amorphously bound to consecutive cells, Nep-2 possesses more vascular bundles scattered throughout the seed cotyledon as seen at lower magnification (Figure 22B_1, B_2). No topographical difference was observed between the longitudinal and transverse section structures of the individual bean cultivar.

The starch granules, although irregular in form and shape, ranged from 12 to 28 μm, 3 to 39 μm and 9 to 22 μm in Sanilac, Nep-2 and San-Fernando, respectively. The San-Fernando starch granules are of smaller size. This may have been caused by the firm packing of the parenchyma cells in the cotyledon during the maturation of the seed, hence preventing a full expansion of the starch granules in the process.

The boundaries between the cell wall of adjacent cells which could not be distinguished in raw lima beans by Rockland and Jones (1974) are clearly seen here as also observed in cowpea by Sefa-Dedeh (1978).

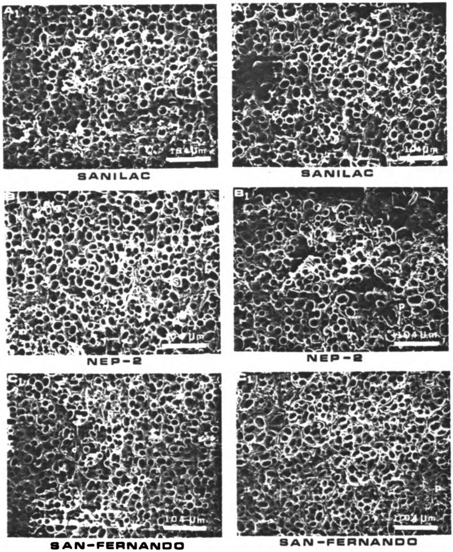

Figure 22. Scanning electron micrographs of dry bean seed cotyledon cross-section structures at lower magnification (400x). A_1 = Sanilac longitudinal section; A_2 = Sanilac transverse section; B_1 = Nep-2 longitudinal section; B_2 = Nep-2 transverse section; C_1 = San-Fernando longitudinal section; C_2 = San-Fernando transverse section; V = vascular vessels; S = starch granules; C = cell; P = protein matrix.

Water Absorption on Soaking

Dry beans from the individual year (1978, 1979, 1980) samples with their respective moisture content were submitted to cold water soaking tests (Table 16). The water absorption curve pattern is similar to those reported on black beans by Quast and da Silva (1977a, b) and cowpea by Sefa-Dedeh (1978). Sanilac and Nep-2 have the highest water absorption rate compared to San-Fernando (Appendix Figures A_1 and A_3) except in the 1979 beans where Nep-2 water absorption rate was less than that of San-Fernando (Appendix Figure A_2). There was no explanation of this from the examination of the seeds under SEM. In general, we could attribute the effect to weather, maturation and storage conditions of the seeds. But we cannot imply moisture content of the seed as a factor in the condition because there was no difference in water content within that year among the cultivars. The difference in the water absorption rate among the three bean cultivars under investigation could mainly be associated with the differences found in their structural topography. As discussed earlier, the seed coat in San-Fernando presents a resistance to water penetration because of its structure. Despite the initial moisture of the beans Sanilac achieved a more rapid uptake of water than the other two (Figure 23 and Appendix Figures A_1, A_2 and A_3). The fact that beans stored at high moisture content tends

Table 16. Percent water absorption[1] of dry beans soaked in ambient temperature water (1:1, tap water:distilled water) for up to 90 minutes by crop years (1978, 1979 and 1980).

Bean Cultivar	Sanilac			Nep-2			San-Fernando		
	Crop Years			Crop Years			Crop Years		
Soak time (min.)	1978	1979	1980	1978	1979	1980	1978	1979	1980
0	8.1	6.5	5.9	8.8	6.5	5.8	9.2	6.7	5.8
15	35.4	21.1	23.1	26.0	14.1	22.1	16.9	16.9	12.8
30	47.7	40.1	40.5	41.6	15.6	31.7	21.8	18.2	13.7
45	47.7	40.1	47.9	44.2	15.7	41.9	25.9	24.2	15.0
60	51.4	50.7	51.1	46.5	17.2	46.5	32.2	31.8	15.1
75	53.1	52.7	52.8	49.8	20.8	49.4	33.8	34.1	16.1
90	53.3	53.1	52.8	50.5	21.1	50.5	37.5	40.5	17.1

n = 3 (3 replicates/soak time/crop year)
1 = The percent water absorption was calculated according to Hosfield, L.G. and Uebersax, M.A. methods (1980).

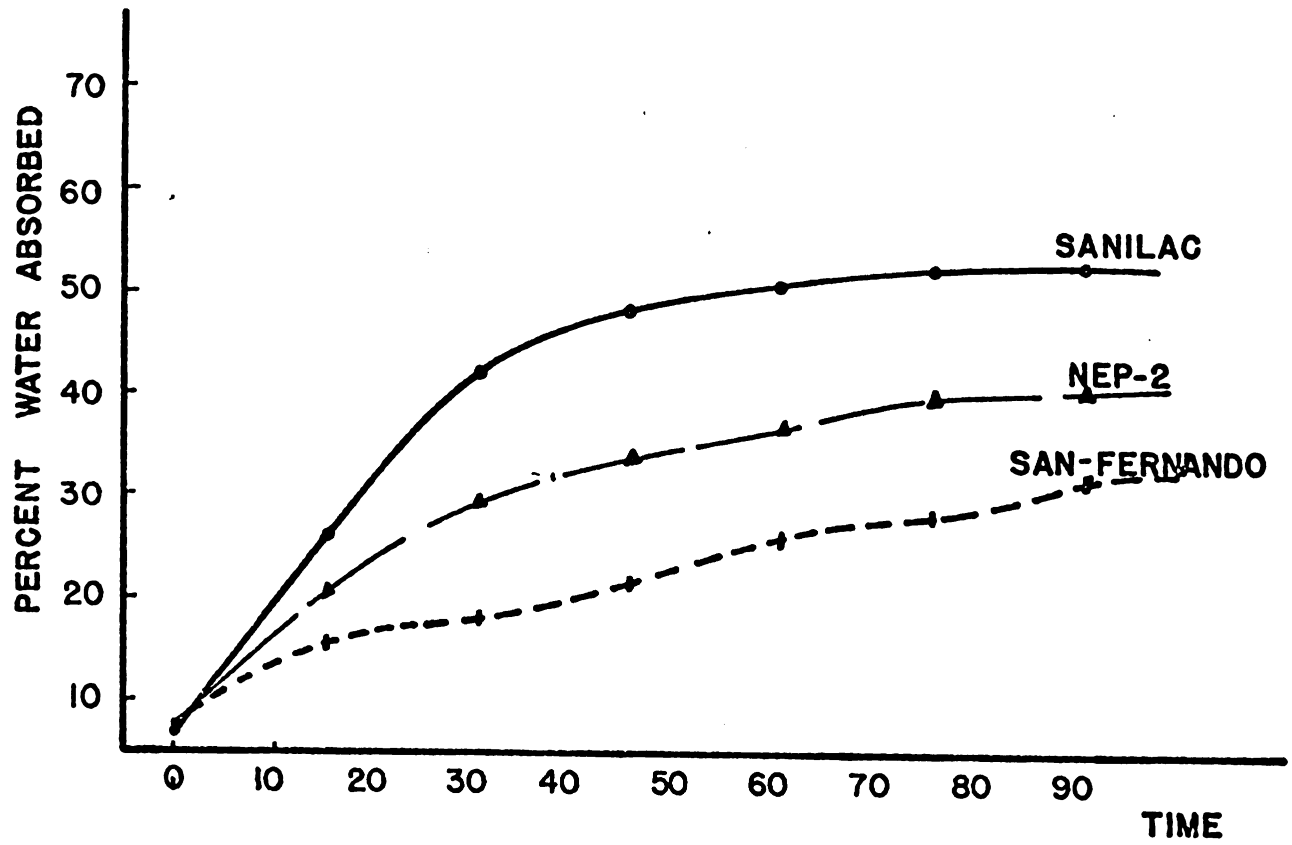

Figure 23. Mean percent water absorption pattern of dry bean cultivars (Sanilac, Nep-2 and San-Fernando) soaked in ambient temperature water (1:1, tap water:distilled water) for up to 90 minutes over three crop years (1978, 1979 and 1980).

to give a higher water uptake on soaking is not applicable in this study because less than 10% moisture content were found in the beans under investigation. This implies Sanilac's easy water absorption through the seed coat and micropyle compared to Nep-2 and San-Fernando which possess a sticky protein membrane on the cotyledon outer surface preventing the easy translocation of water between the seed coat and the cotyledon during the hydration process (Figure 15B_1, C_1). The hypothesis presented on cowpeas by Sefa-Dedeh (1978) and soybean by Mayer and Poljakoff-Mayber (1975) indicating that highest amount of water absorbed is related to high protein content is not supported here because the three cultivars used in this study did not have any significant difference in their protein content but there were differences in their water content absorption rates during soaking. If protein is the chief component absorbing water in seeds as reported by Mayer and Poljakoff-Mayber (1975), the absorption quality of the protein may be a factor to consider in the hydration of seeds. The firm packing of the parenchyma cells in San-Fernando bean could also be suggested to delay the water absorption through the entire cotyledon.

Pasting Properties

Tables 17A, B, and C and Appendix Table 1B show the Brabender Visco/amylograph pasting properties of the three cultivars.

The flours from the three bean cultivars (Sanilac, Nep-2 and San-Fernando) have Type C pasting curves with no definite peak which is commonly observed in cereals. This particular shape is commonly found in legumes (Colonna and Mercier, 1979; Lineback and Ke, 1975; Navikul and d'Appolonia, 1979; Suzuki et al., 1981; Schoch and Maywald, 1968; Vose, 1980). All three cultivars had high initial pasting temperature 72.8°C, 72.3°C and 77.3°C for Sanilac, Nep-2 and San-Fernando whole flours, respectively (Appendix Table 18). The highest value for San-Fernando indicates the slow swelling of the starch granules of this cultivar.

Higher values were also obtained with cotyledon flours with 70.6°C, 75.3°C and 75.8°C over the three crop years for Sanilac, Nep-2 and San-Fernando, respectively (Appendix Table 1B). While Nep-2 initial pasting temperature is similar to that of Sanilac in the whole bean flour, it is most similar to that of San-Fernando in the cotyledon flour alone. There was no difference in the initial pasting temperature within the crop years between the whole bean and cotyledon flours among the individual cultivar. From the whole bean flours over the three crop years study,

Table 17. Pasting characteristics of dry bean portions (whole bean and cotyledon) flours by crop years (1978, 1979 and 1980) and cultivars (Sanilac, Nep-2 and San-Fernando) in a 400 ml phosphate buffer solution at pH 5.30.

A

VARIETIES	VISCOSITY IN BU[1]												
	INITIAL PASTING TIME (min.)	INITIAL PASTING T°C.	PERPENDICULAR RISING			AT 95°C	AFTER 15min. 95°C	PEAK TIME (min.)	PEAK	PEAK DROP	AT 25°C	PEAK on cooling	AFTER 15min. at 25°C
			TIME (min.)	T°C	PEAK								
SANILAC													
whole	32	74.5	–	–	–	85	260	82	385	–	345	–	260
coatless	31	73	46	95	220	145	375	72	485	455	675	685	640
NEP-2													
whole	33	76	43	91	90	180	340	–	–	–	555	555	470
coatless	33	77	44	91	140	150	330	68	420	400	715	720	670
SAN FERNANDO													
whole	35	79	–	–	–	70	225	81	345	–	330	–	270
coatless	34	77.5	44	92	140	150	270	74	360	330	600	615	580

1=BRABENDER UNITS

Table 17. (cont'd.).

B

VARIETIES	INITIAL PASTING TIME (min.)	INITIAL PASTING T°C	VISCOSITY IN BU[1]: PERPENDICULAR RISING TIME (min.)	PERPENDICULAR RISING T°C	PERPENDICULAR PEAK	AT 95°C	AFTER 15 min. 95°C	PEAK TIME (min.)	PEAK	PEAK DROP	AT 25°C	PEAK on cooling	AFTER 15 min. at 25°C
SANILAC													
whole	28	69	42	91	115	150	280	71	350	305	540	565	510
coatless	28	69	45	93	230	110	410	69	460	405	705	720	670
NEP-2													
whole	27	66	—	—	—	230	550	—	—	—	<1000	<1000	<1000
coatless	33	75	—	—	—	85	180	—	—	—	605	605	550
SAN FERNANDO													
whole	33	77	—	—	—	35	90	—	—	—	240	265	240
coatless	30	73	—	—	—	80	160	—	—	—	560	570	480

1 = BRABENDER UNITS

Table 17. (cont'd.).

C

VARIETIES	INITIAL PASTING TIME (min.)	INITIAL PASTING T°C	VISCOSITY IN BU[1]										
			PERPENDICULAR RISING			AT 95°C	AFTER 15min. 95°C	PEAK TIME (min.)	PEAK	PEAK DROP	AT 25°C	PEAK on cooling	AFTER 15min. at 25°C
			TIME (min.)	T°C	PEAK								
SANILAC													
whole	32	75	43	92	100	120	235	—	—	—	520	525	460
coatless	28	70	50	88	160	100	210	70	230	220	580	590	560
NEP-2													
whole	32	75	44	91	120	160	345	—	—	—	610	615	530
coatless	31	74	54	95	260	80	285	68	310	265	550	570	550
SAN FERNANDO													
whole	32	76	—	—	—	55	140	—	—	—	415	420	380
coatless	33	77	54	95	180	50	220	71	245	230	455	480	480

1 = BRABENDER UNITS

1 = Brabender Units
n = 2 (2 replicates/seed portion/variety)

Nep-2 showed the highest viscosity curves and San-Fernando the lowest with the Sanilac curve intermediate (Figure 24 and Appendix Figures B_1A_1, B_2A_1 and B_3A_1). With the coatless (cotyledon) bean flours, Sanilac demonstrated higher curves followed by Nep-2 and then San-Fernando (Figures 24, A_2 and Appendix Figures B_1A_1 and B_2A_1) except in the 1980 flour samples where Sanilac showed a lower curve only during the heating period (Appendix Figure B_3A_2). Considering the individual cultivar, viscosity curves appear to be higher after removal of the seed coat in the 1978 and 1979 Sanilac and San-Fernando bean flours (Figures 25, 27 and Appendix Figures B_1A_2 and B_2A_2). In the year 1980 beans, this increase did not occur with Sanilac (Appendix Figure B_3A_2). Inversely, Nep-2 showed a loss in its viscosity strength after removal of the seed coat (Figures $24A_2$, 26 and Appendix Figures B_1A_2 and B_2A_2). The 1979 Nep-2 beans which presented a low water absorption in rate pattern (Appendix Figure A_2) revealed the highest whole bean flour viscosity of all materials evaluated (Appendix Figures B_1A_1, B_2A_1 and B_3A_1). This behavior was not explainable on the basis of structural topography, but it could be suggested that the starch granules, unable to pick up water in the cold stage, absorbed the water to a large extent during the heating period before they rupture and that made the slurry more viscous. The same behavior can not be related to starch content because the Nep-2 beans

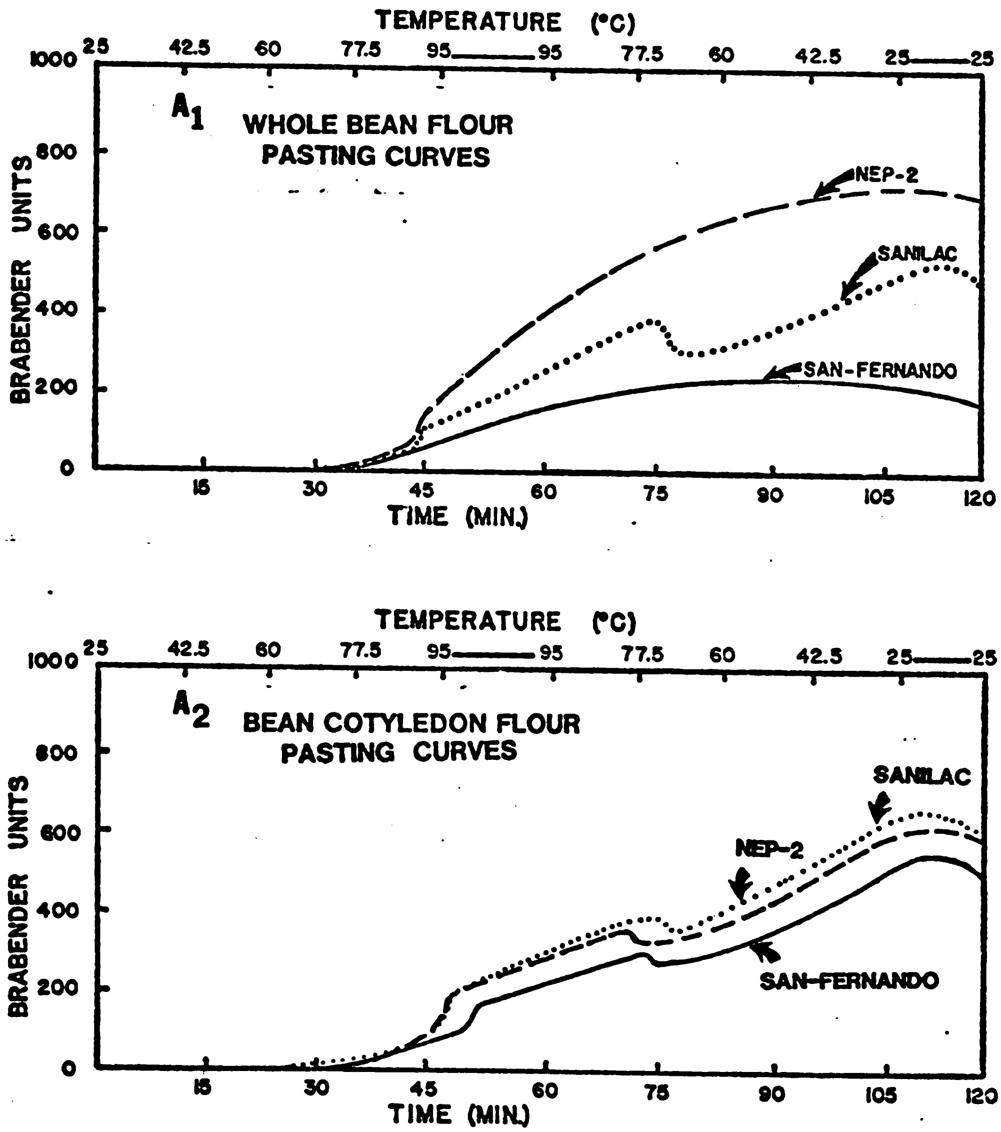

Figure 24. Mean pasting curves of dry bean portions (whole bean and cotyledon) flours by cultivars (Sanilac, Nep-2 and San-Fernando) over three crop years (1978, 1979 and 1980).

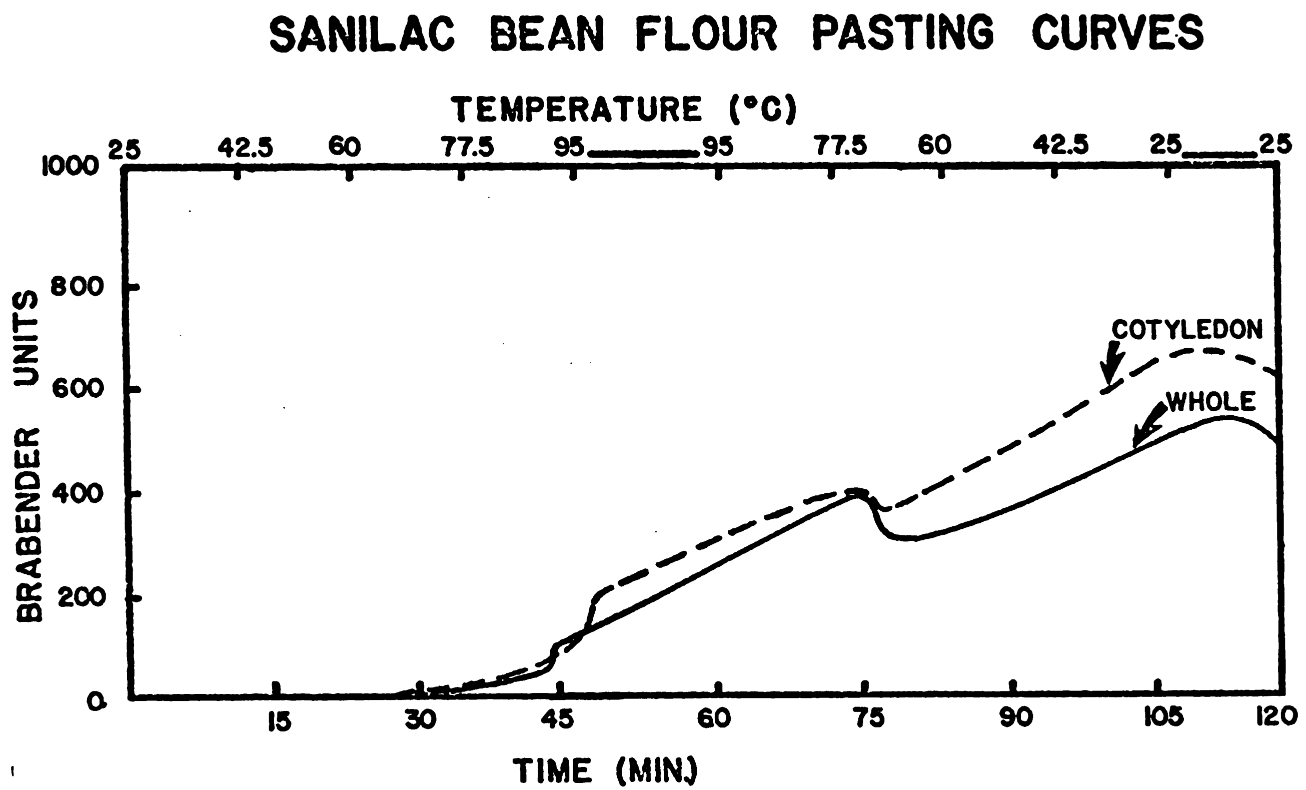

Figure 25. Mean pasting curves of dry Sanilac bean portions (whole bean and cotyledon) flours over three crop years (1978, 1979 and 1980).

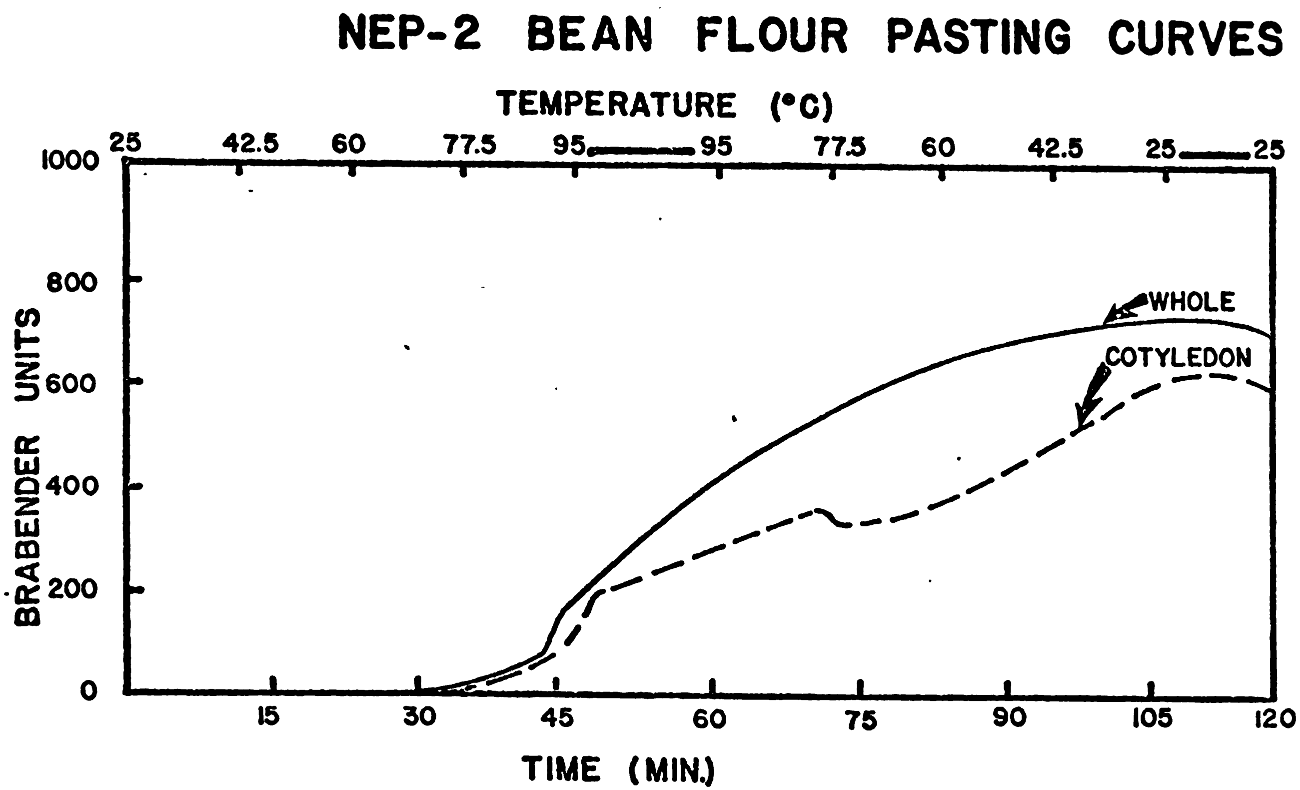

Figure 26. Mean pasting curves of dry Nep-2 bean portions (whole bean and cotyledon) flours over three crop years (1978, 1979 and 1980).

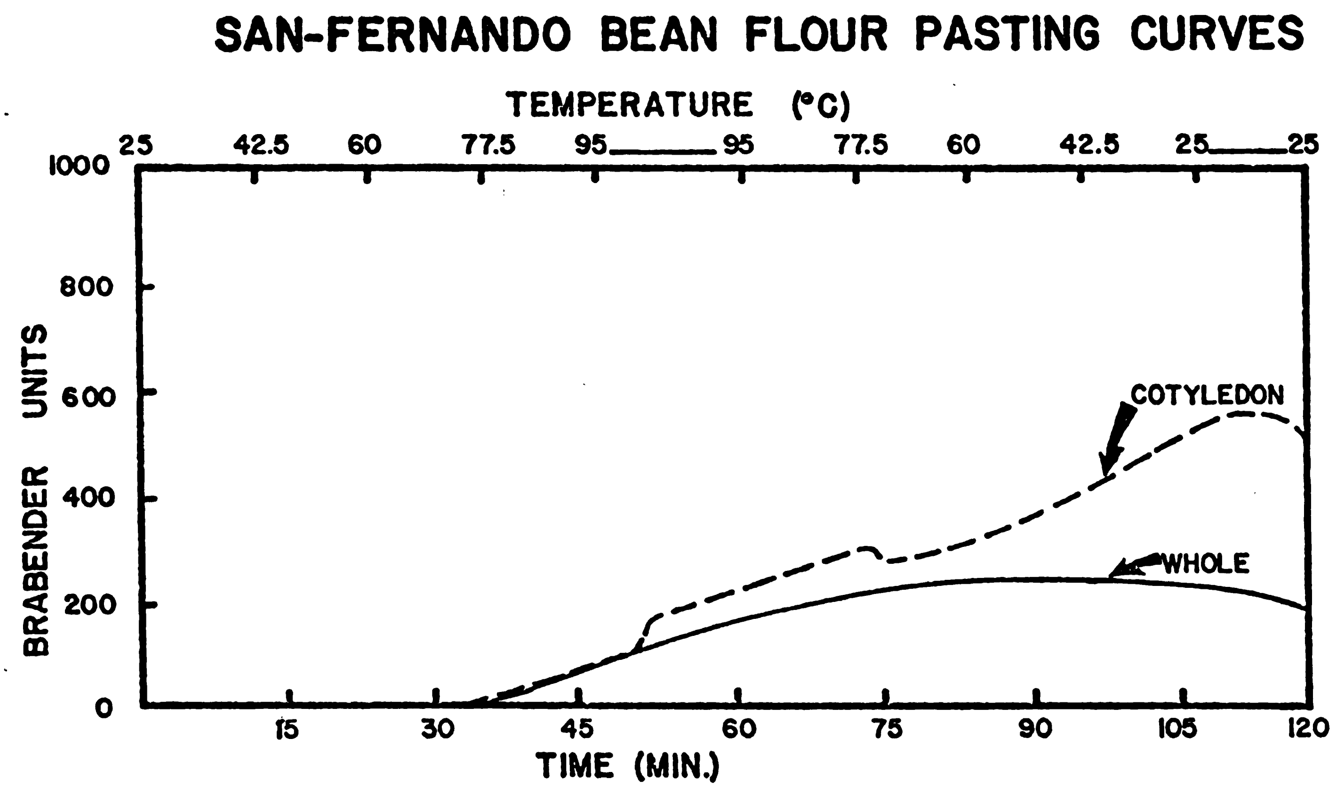

Figure 27. Mean pasting curves of dry San-Fernando bean portions (whole bean and cotyledon) flours over three crop years (1978, 1979 and 1980).

of that year did not show any significant difference compared to San-Fernando (Tables 13 and 14).

The retrogradation properties which were observed during the cooling period of the bean flour slurries showed similar patterns for the overall study, with San-Fernando always the lowest curves. This suggests that San-Fernando would not influence bread staling if used in bread making, while Nep-2 and Sanilac bean flours may influence staling due to higher viscosity curves. Similar results were reported on faba bean, lentil and mung bean starches by Navikul and d'Appolonia (1979). The continuous setting-back of the slurry implies a good retrogradation of linear molecules in the starch granules.

SEM of Soaked Beans

The soaked bean seed coat outer surface and cross-sectional structure are presented in Figure 28A$_1$, A$_2$, B$_1$, B$_2$, C$_1$, C$_2$).

Sanilac and Nep-2 showed a plastic-like rupture of the soaked seed coat while that of San-Fernando is a rigid rupture (Figure 28C$_1$). The sinking holes in the Nep-2 coat expanded during soaking (Figure 28B$_1$). The cross-sections show that the palisade cells become amorphous during soaking (Figures 28A$_2$, B$_2$, C$_2$). Rupture is observed in the Sanilac coat cross-section. This implies a "rupture through" the entire thickness of that seed coat during the soaking process differing from the two isolines seed coat.

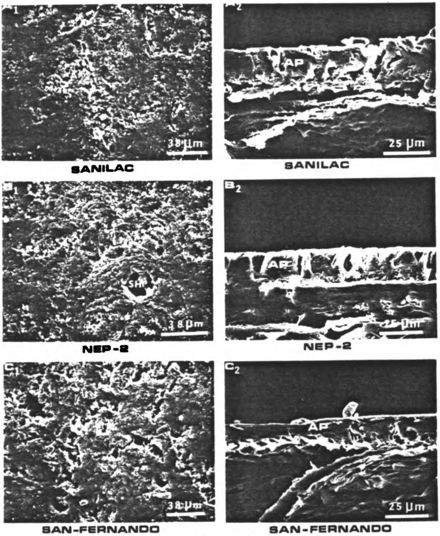

Figure 28. Scanning electron microscopy of soaked bean seed coat outer surface (1000x) and cross-section (1000x) structures. A_1 = Sanilac seed coat outer surface structure; A_2 = Sanilac seed coat cross-section structure; B_1 = Nep-2 seed coat outer surface structure; B_2 = Nep-2 seed coat cross-section structure; C_1 = San-Fernando seed coat outer surface structure; C_2 = San-Fernando seed coat cross-section structure; SH = sinking hole; AP = amorphous palisade.

The soaked bean seeds hilum area structure is shown in Figures 29A_1, B_1, and C_1.

All three cultivars present ruptured seed coat around the hilum area and expanded micropyle. San-Fernando, which showed a Y-shaped closed micropyle in the dry seed (Figures 16C_1 and C_2) reveals an open Y-shaped micropyle in the soaked seed (Figure 29C_2). This enlargement of the micropyle contributed to the maximum absorption of water by San-Fernando beans during the cold and hot soaking prior to canning. The micropyle as mentioned in the literature by different scientists plays an important role in the water absorption of beans. In this study that role is well confirmed and makes a difference between Nep-2 and San-Fernandy compared to Sanilac.

The soaked bean cotyledon structures are shown in Figures 30A_1, A_2, B_1, B_2, C_1 and C_2. Soaked Sanilac structure shows released starch granules from ruptured parenchyma cells exposing the middle lamella while soaked Nep-2 and San-Fernando present intact parenchyma cells although some starch granules were partially released. More intact parenchyma cells are present in the San-Fernando cotyledon. This implies higher potential resistance to shear for San-Fernando compared to Nep-2 and Sanilac.

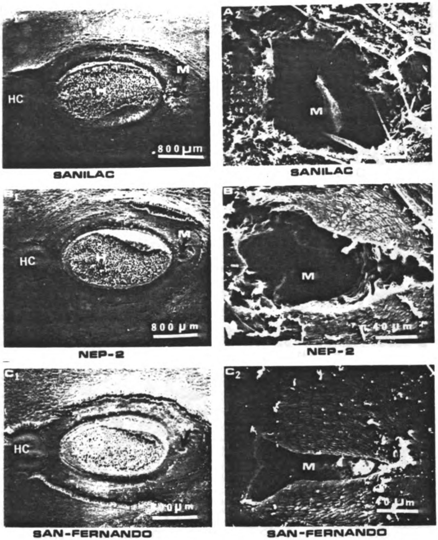

Figure 29. Scanning electron micrographs of soaked bean seed hilum area structure; A_1 = Sanilac hilum area (50x); A_2 = Sanilac micropyle (1000x); B_1 = Nep-2 hilum area (50x); B_2 = Nep-2 micropyle (1000x); C_1 = San-Fernando hilum area (50x); C_2 = San-Fernando micropyle (1000x); HC = hypocotyl area (50x); H = hilum; M = micropyle

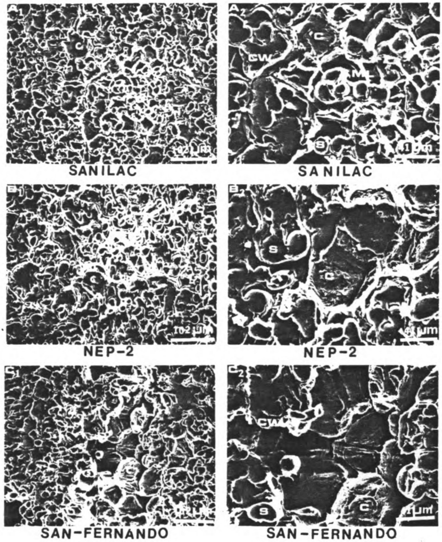

Figure 30. Scanning electron micrographs of soaked bean cotyledon structures (400x and 1000x). A_1 and A_2 = Sanilac cotyledon; B_1 and B_2 = Nep-2 cotyledon; C_1 and C_2 = San-Fernando cotyledon; C = cell; CW = cell wall; S = starch granules; ML = middle lamella; I = intercellular space.

SEM of Processed Beans

Processed Sanilac seed coat structure shows a "plastic-like" rupture of the cuticle exposing an outer surface of a swollen membrane present between the cuticle and the cotyledon (Figure 31A, B). Higher magnification shows a "hill-like" structure which might have been created by expansion of the palisade cells during canning. Similar observations are seen with the processed Nep-2 seed coat outer surface structure (Figure 32A, B, C, and D). The cuticle of both cultivars look similar (Figure 31C and 32C) although Sanil c has a more structured appearance.

The processed San-Fernando seed coat outer surface shows a rigid rupture of the seed coat with "grit like" structure observed over the three crop years (Figure 33A, B, C and D). This is a characteristic difference from the other isoline, Nep-2, which shows a clear separation of the cuticle after processing, exposing the outer surface structure of an intermembrane. This intermembrane is not the <u>linea lucida</u> or light line observed between mucilage stratum and palisade cells by various researchers (Corner, 1951; Hamly, 1932, 1934; Reeve, 1946a, 1946b; Chowdhury and Buth, 1970) because it is positioned between the cuticle and the palisade cells. This could not be seen on the dry seed coat cross-section. Processed Nep-2 seed coat outer surface structure is similar to that of Sanilac. This

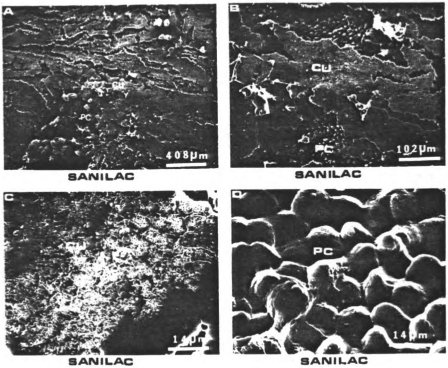

Figure 31. Scanning electron micrographs of processed Sanilac seed coat outer surface structure; A and B = cuticle and intermembrane outer surface (100x and 400x); C = cuticle cell outer surface (3000x); D = protein intermembrane cells outer surface (3000x); PC = protein intermembrane cells; CU = cuticle.

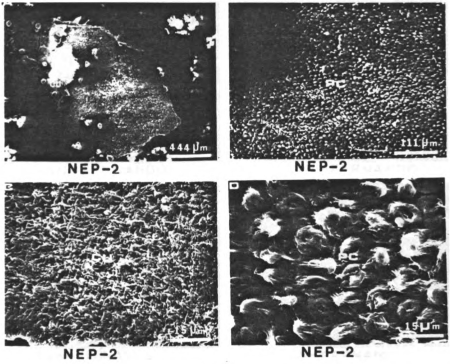

Figure 32. Scanning electron micrographs of processed Nep-2 seed coat outer surface structures; A and B = cuticle and intermembrane surface (100x and 400x); C = cuticle cell outer surface (3000x); D = protein intermembrane cells outer surface (3000x); PC = protein intermembrane cells; CU = cuticle.

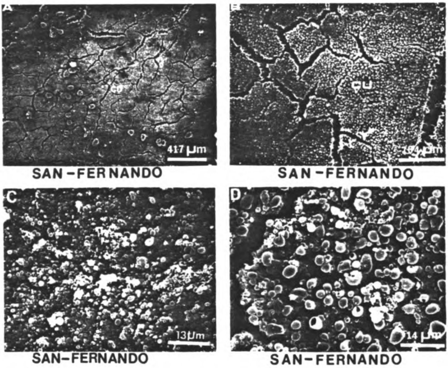

Figure 33. Scanning electron micrographs of processed San-Fernando seed coat outer surface structures; A and B (100x and 400x) and C and D (3000x) cuticle cell outer surface; CU = cuticle.

concurs with the observation made from the dry seeds of these two cultivars. San-Fernando seed coat maintains its differential characteristics throughout the processing system. This behavior could prevent rapid absorption of water.

The processed bean cotyledon parenchyma from the three cultivars show similar irregular shape and size of expanded ruptured starch granules held within fine protein filaments. The middle lamella was ruptured during the canning step, leaving the starch granules free in the cotyledon (Figures 34A_1, A_2, B_1, B_2, C_1 and C_2). This also has been observed by other scientists (Bourne, 1976; Hoseney et al., 1977; Hahn et al., 1977; Rockland et al., 1977; Rockland and Jones, 1974). No visible differences between Sanilac and Nep-2 were observed following thermal processing. Processed San-Fernando beans have some of the parenchyma cell walls still holding some starch granules together. This is a great potential of resistance to shear in the measurement of texture compared to the other cultivars.

Bean Color

The colors of dry and processed beans are presented in Tables 18A_1, A_2, A_3 and Appendix Figure 1C. The lightness of the seeds specified by "L" values is not significantly different in Sanilac and Nep-2 through the overall study (Tables 18A_1, A_2, A_3 and Appendix Figure 1C). The two

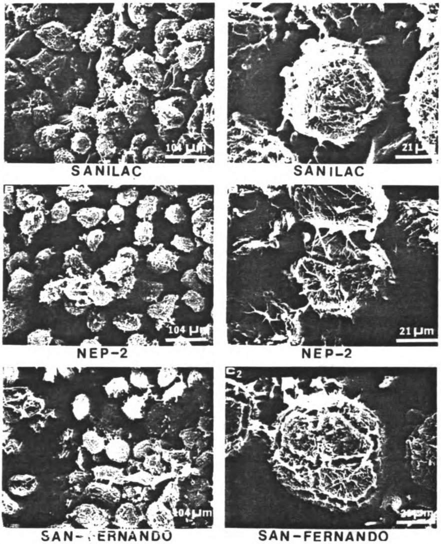

Figure 34. Scanning electron micrographs of processed bean structures; A_1 and A_2 = Sanilac cotyledon (400x and 2000x); B_1 and B_2 = Nep-2 cotyledon (400x and 2000x); C_1 and C_2 = SF cotyledon (400x and 2000x); RS = expanded rupture starch granules.

Table 18. Hunter Lab color and color differences values (L, a_L, b_L) of dry and processed bean cultivars (Sanilac, Nep-2 and San-Fernando) by crop years (1978, 1979 and 1980).

Bean	Dry Beans L	Dry Beans a_L	Dry Beans b_L	Processed Beans L	Processed Beans a_L	Processed Beans b_L
			BEAN COLOR[1]			
			A_1. Crop Year 1978			
Sanilac	64.6^a	-0.1^d	8.7^e	50.1^g	4.4^i	14.8^k
Nep-2	60.2^a	-0.1^d	11.4^e	49.7^g	3.8^j	14.8^k
San-Fernando	14.7^b	0.3^c	-0.7^f	17.3^h	4.7^i	3.2^l
			A_2. Crop Year 1979			
Sanilac	63.0^a	0.3^b	10.9^c	50.7^e	14.5^g	17.0^i
Nep-2	61.2^a	0.1^b	10.7^c	46.8^e	17.0^g	17.6^i
San-Fernando	17.2^b	-0.1^b	0.3^d	15.5^f	5.0^h	-3.0^j
			A_3. Crop Year 1980			
Sanilac	60.4^a	0.3^c	11.0^d	50.7^f	14.9^h	16.7^i
Nep-2	54.8^a	0.3^c	11.1^d	50.0^f	14.8^h	17.6^i
San-Fernando	17.8^b	-0.2^c	0.3^e	14.1^g	6.6^h	-2.9^j

n = 8 (2 replicates/sample x 2 samples/can x cans/cultivar).
[1]Hunter value L, a_L and b_L.
Like letters denote no significant differences ($p \leq 0.05$).

cultivars have the highest values above 50 (Hunter readings) while that of San-Fernando is low and below 20 (Tables 18A_1, A_2, A_3 and Appendix Table 1C, and Figure 35 and Appendix Figures C_1, C_2 and C_3). The "L" values are higher in dry seeds than in processed ones except in the 1978 San-Fernando beans where the reverse was obtained (Table 18A and Appendix Figure C_1). Greenness or redness represented by "a" values is very low in dry seeds but high in processed ones. Contrary, a reverse process is observed with "b" values which denote the yellowness or blueness (except in 1978 SF beans) (Figure 35 and Appendix Figure C_1 and C_2). This implies that when "L" values decrease the "a_L" values increase respectively in the seeds during processing. This is expected as a result of browning during the thermal processing as reported by Uebersax and Bedford (1980). Through the overall study, dry and processed San-Fernando beans showed significant differences of "L" and "b_L" values when compared to the other two cultivars (Tables 18A_1, A_2, A_3 and Appendix Table 1C).

Processed Bean Evaluation

Moisture Content

During the canning process water contents reach the optimum after the hot soaking step in each individual bean cultivar (Tables 19A, B, C and Figure 36 and Appendix Table 1D and Figures D_1, D_2 and D_3). The maximum is

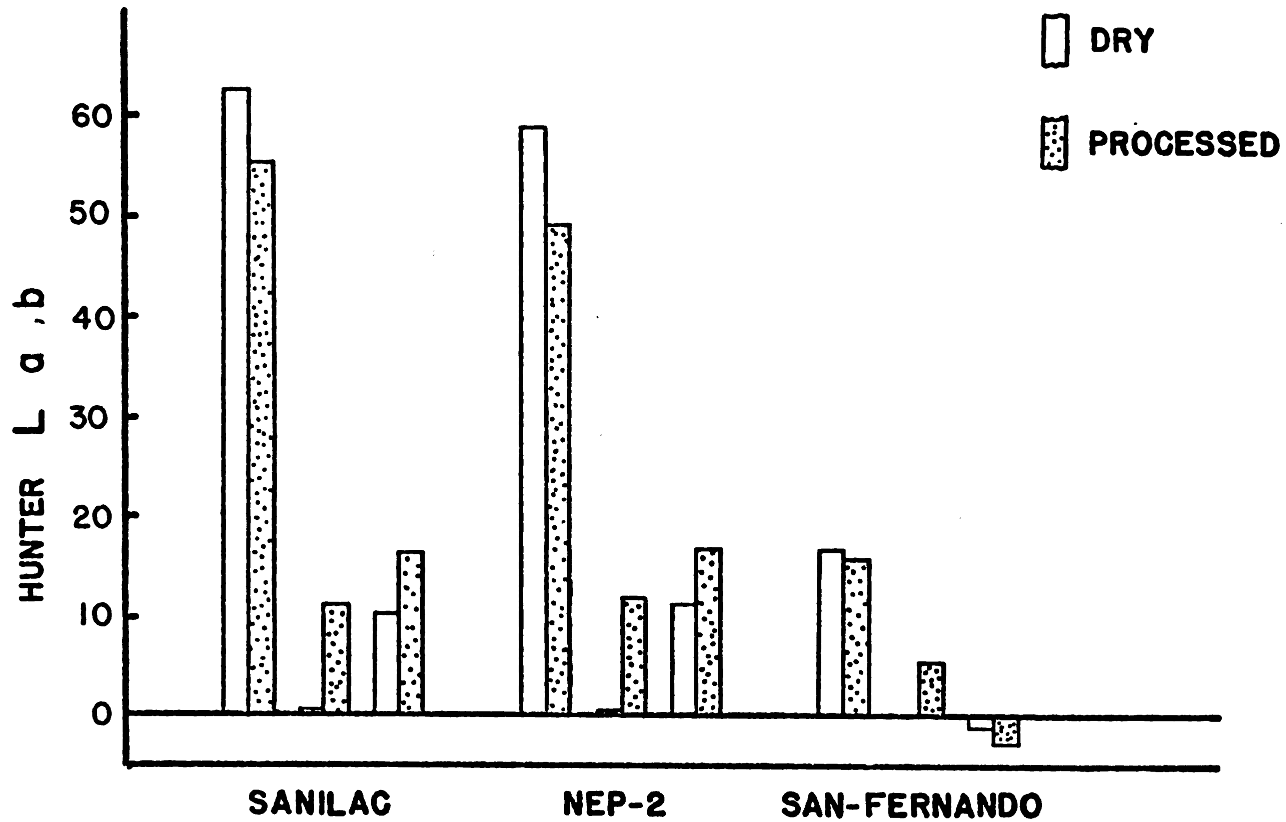

Figure 35. Mean Hunter color and color differences values of dry and processed bean cultivars (Sanilac, Nep-2 and San-Fernando) over three crop years (1978, 1979 and 1980).

Table 19. Quality parameters evaluation for processed bean cultivars (Sanilac, Nep-2 and San-Fernando) by crop year (1978, 1979 and 1980).

Legumes	Quality parameters evaluation								
	Initial Moisture[1] %	Water Content After Soaking[1] %	Water Content After Processing[2]	Drained Weight[1] g	Clump[1] Rate 1 to 3	Split[1] Rate 1 to 5	Texture[2] Force Height (Kramer units)	Texture[2] Force Kg/100 g	Soak Ratio
					A. Crop Year 1978				
Sanilac	16.1^{a}	54.9^{b}	58.6^{c}	286.6^{d}	1.2^{e}	1^{f}	47.4^{h}	64.5^{j}	1.9^{k}
Nep-2	13.7^{a}	53.8^{b}	68.2^{c}	284.9^{d}	$.6^{e}$	1^{f}	52.8^{h}	71.7^{j}	1.9^{k}
SF	15.2^{a}	54.3^{b}	68.3^{c}	286.6^{d}	1.8^{e}	1^{f}	65.3^{g}	88.7^{i}	1.9^{k}
					B. Crop Year 1979				
Sanilac	17.3^{a}	54.7^{b}	71.4^{c}	316.8^{d}	$.3^{f}$	4.5^{i}	22.5^{m}	30.6^{q}	1.8^{r}
Nep-2	15.2^{a}	51.8^{b}	69.1^{c}	316.4^{d}	$.6^{h}$	1.5^{j}	30.5^{l}	41.5^{p}	1.8^{r}
SF	15.9^{a}	52.3^{b}	68.4^{c}	283.3^{e}	1.8^{g}	3.0^{i}	48.5^{k}	66.0^{o}	1.8^{r}
					C. Crop Year 1980				
Sanilac	16.5^{a}	56.2^{b}	71.7^{d}	320.3^{e}	3.0^{f}	4.0^{g}	25.8^{i}	35.0^{k}	1.9^{l}
Nep-2	17.8^{a}	54.6^{b}	71.2^{d}	315.0^{e}	3.0^{f}	4.5^{g}	27.5^{i}	37.4^{k}	1.8^{l}
SF	15.5^{a}	47.2^{c}	67.3^{d}	288.8^{e}	2.0^{f}	3.0^{g}	53.5^{h}	72.8^{j}	1.6^{m}

[1] n = 2 (1 replicate/can x 2 cans/cultivars).
[2] n = 4 (2 replicates/can x 2 cans/cultivar).
Like letters denote no significant differences ($p \leq 0.05$).

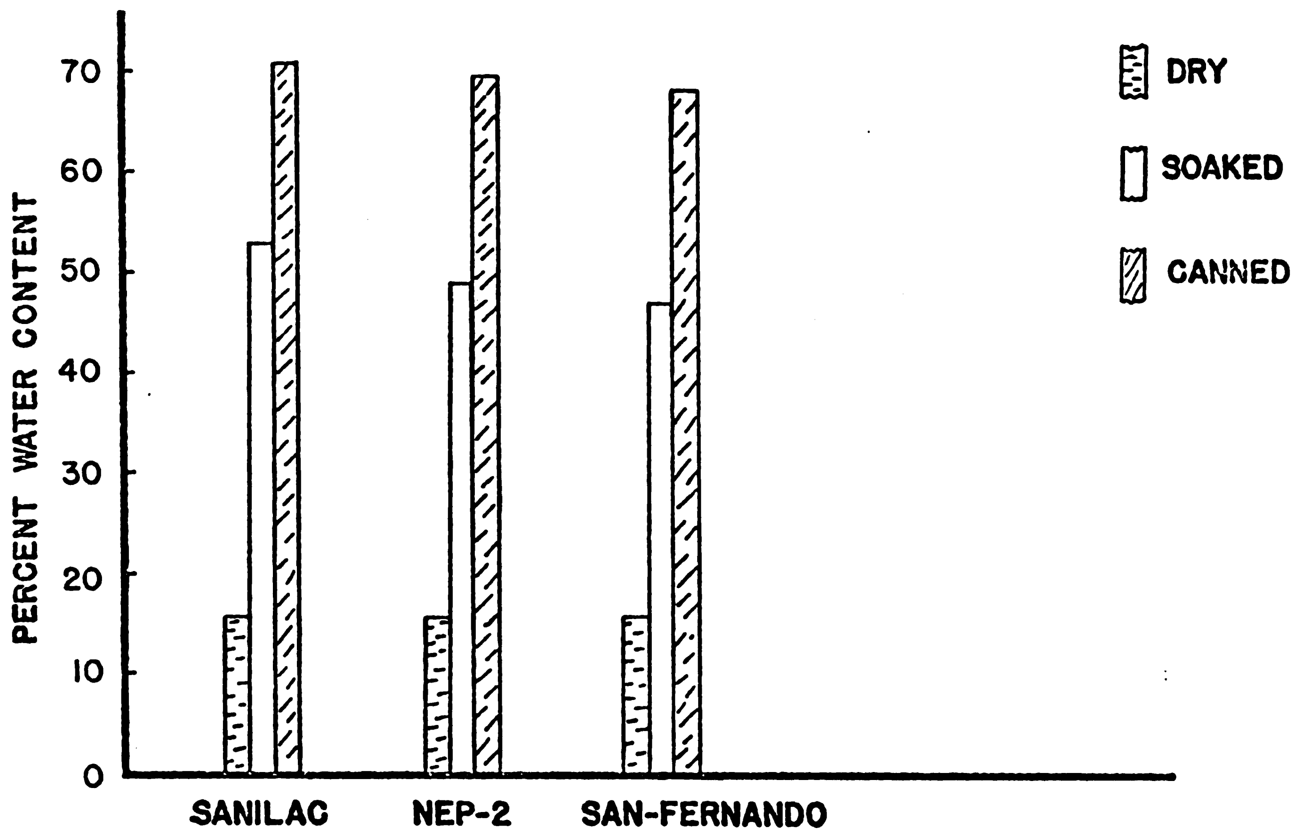

Figure 36. Percent water content for bean cultivars (Sanilac, Nep-2 and San-Fernando) in quality parameters evaluation over three crop years (1978, 1979 and 1980).

only after canning and that is clearly seen in Figure 36 and Appendix Figures D_1, D_2 and D_3. There was no significant difference between the cultivars for the moisture contents after soaking (cold and hot) prior to canning and after canning, except San-Fernando which showed some differences in the 1980 bean samples. San-Fernando beans which had a slow water absorption rate (Figure 23 and Appendix Figures A_1 and A_3) reached its optimum and maximum in absorption during the soaking and after canning process respectively (Figure 36 and Appendix Figures D_1, D_2 and D_3). This may be related to the opening of its micropyle during the soaking step and also to the heat treatment which caused rupture of the seed coat and release of the starch granules from the parenchyma cells as observed under the SEM (Figure 29). This confirms the role of seed coat (Coe and Martin, 1920; Martin and Watt, 1944; Ott and Ball, 1943; Powrie et al., 1960) and micropyle (Snyder, 1936; Kyle and Randall, 1964) in the water absorption system of beans. Although the soak ratio obtained in this study is lower than those reported in navy bean by Uebersax and Bedford (1980), there was no significant differences among the values obtained for the three cultivars (Table 19A, B, C and Appendix Table 1D).

Drained Weight

Drained weight is a function of the equilibrium of beans and brine in the can as shown in Tables 19A, B, C and Appendix Table 1D and Figures 37 and Appendix Figure E. No significant differences were found among Sanilac, Nep-2 and San-Fernando except in the 1979 beans where San-Fernando differed from the other two with the value of 283.3 g (Table 19B). The combined data showed similar results from San-Fernando beans (Appendix Table 1D) which had the lowest values 286.2 g.

Clump and Split

The 1980 beans scored higher clump and split values over the three year study. The split in Nep-2 incurred dramatically from 1 to 4.5 score in the 1978 and 1980 beans, respectively (Table 19A and C). The difference is explained by the natural division or splitting of the 1980 Nep-2 beans observed under the SEM. The overall score did not show any significant difference between the cultivars (Appendix Table 1D).

Texture

The texture evaluated using the Kramer Shear Press revealed San-Fernando with highest peaks followed by Nep-2 and then Sanilac (Figures 38 and 39 and Appendix Figures F_1, F_2 and F_3). The peak values converted into force kg/100 g sample are reported in Tables 19A, B and C, Appendix Table 1D and Figure 39 and Appendix Figure F_1, F_2 and F_3. The texture of the 1978 and 1980 beans did not differ significantly between Sanilac and Nep-2 within those years, but

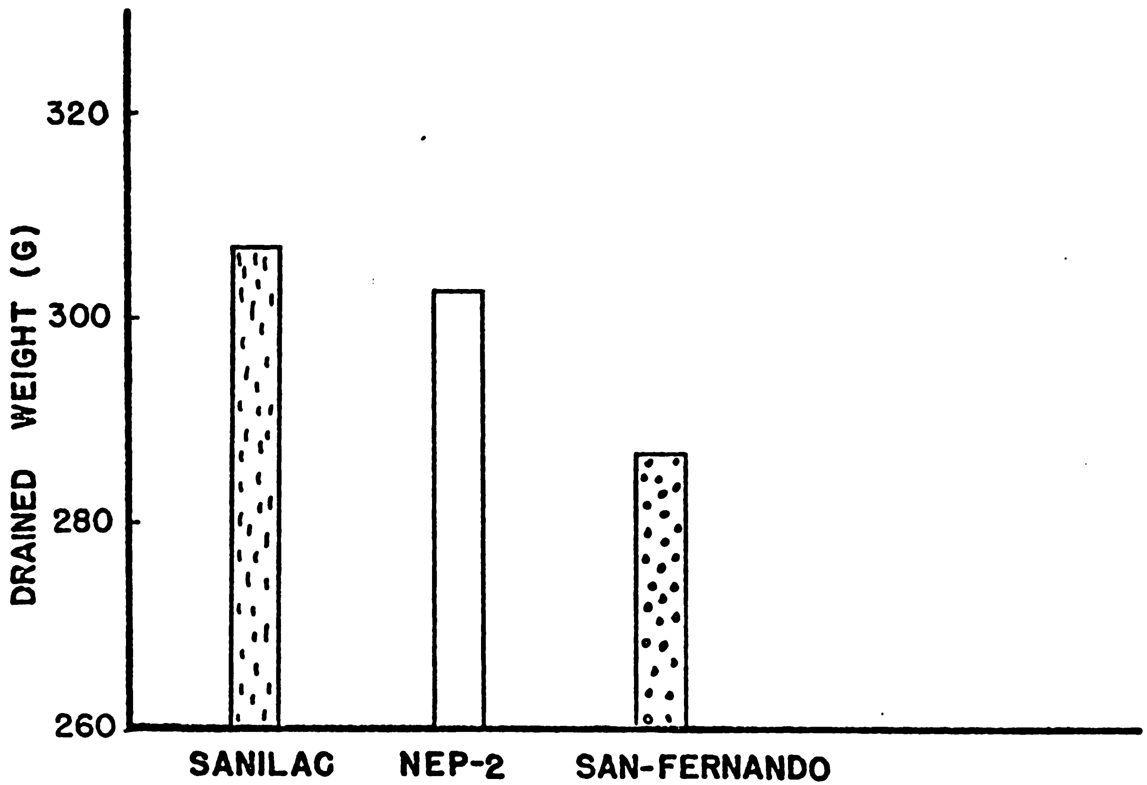

Figure 37. Drained weight for bean cultivars (Sanilac, Nep-2 and San-Fernando) in canned quality parameters evaluation over three crop years (1978, 1979 and 1980).

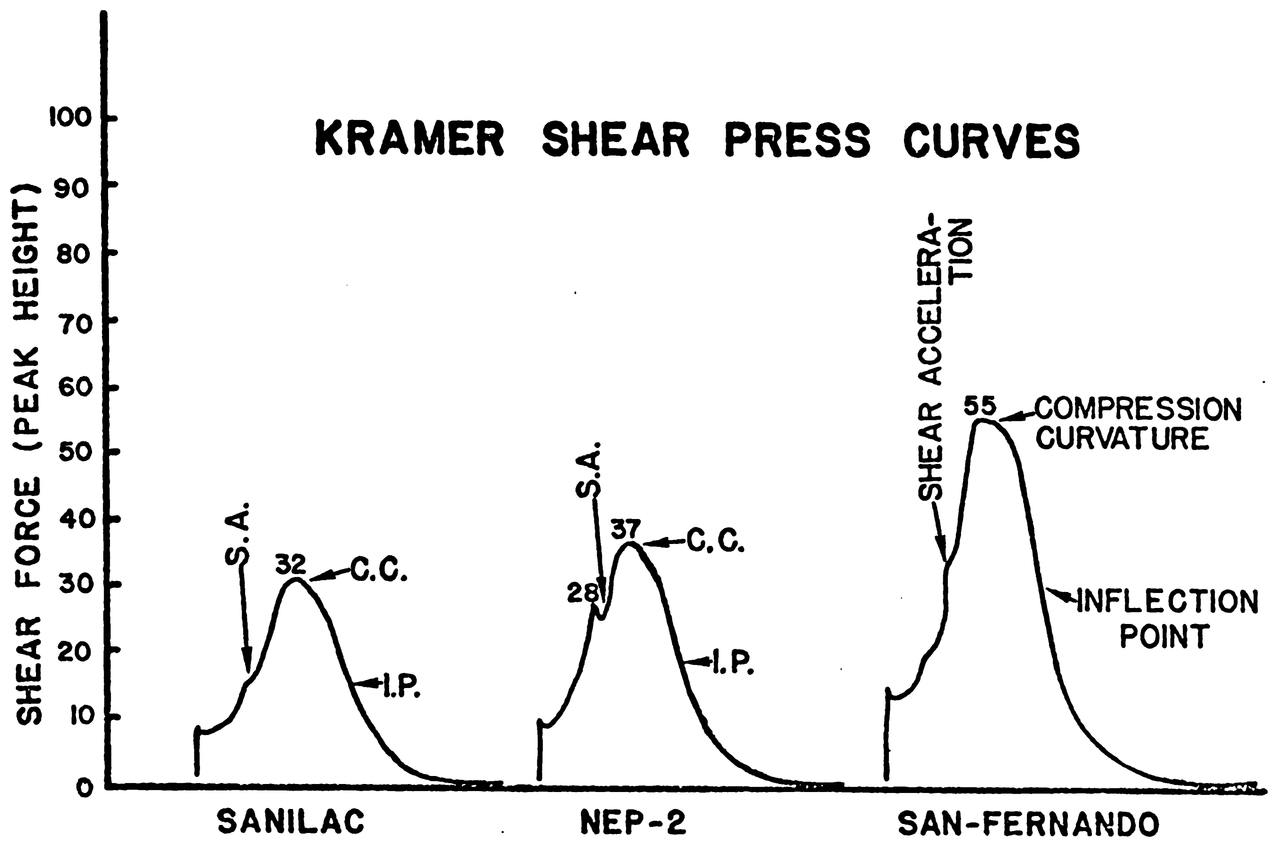

Figure 38. Mean Kramer-Shear Press curves for bean cultivars (Sanilac, Nep-2 and San-Fernando) over three crop years (1978, 1979 and 1980). Curve parameters source: Bolles, A.D. et al., 1982.

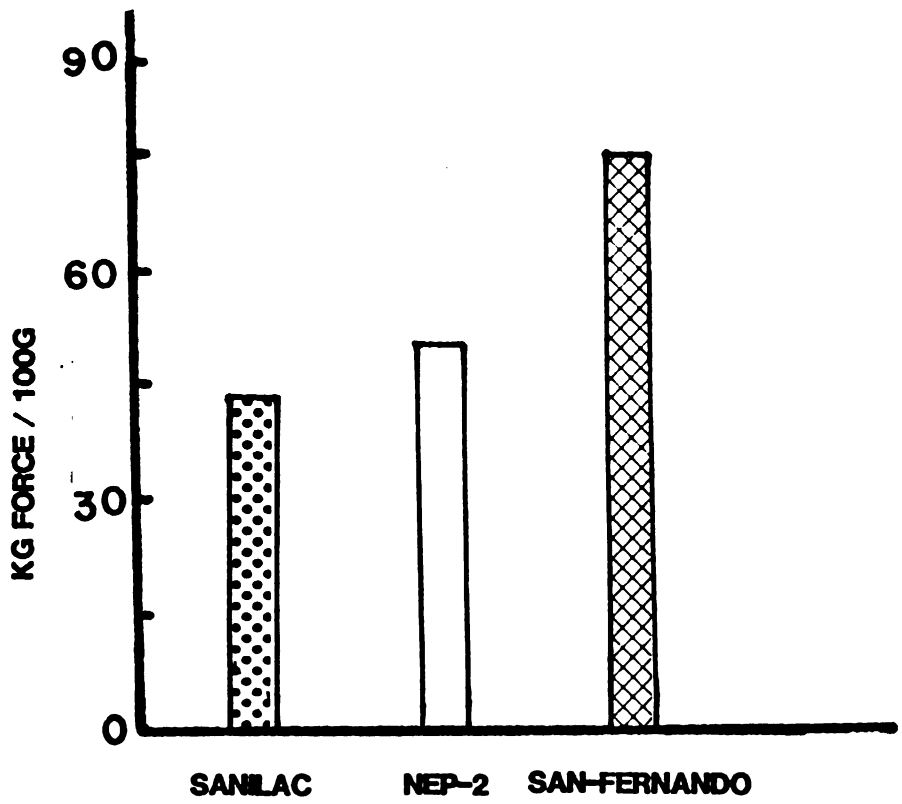

Figure 39. Texture for bean cultivars (Sanilac, Nep-2 and San-Fernando) in canned bean quality parameters evaluation over three crop years (1978, 1979 and 1980).

the 1979 beans showed differences among the three cultivars. The overall data presented a significant difference among the cultivars with Sanilac and San-Fernando having the lowest and highest texture, respectively (Appendix Table 1D). This difference in texture is a main factor in the evaluation of the seeds under investigation. The parenchyma cell packing or arrangement inside the cotyledon seen in Figures 21 and 22 is a prime factor in the process. Differences revealed by the SEM microstructural examination showing 1) firm packing of parenchyma cells in San-Fernando cotyledon and 2) non-complete liberation of the cells and starch granules from the middle lamella prior to retort treatment contributed to the higher texture of that variety when compared to the other two. That was evident when the SEM study on the processed beans revealed some starch granules still being held by the cell walls (Figure 34). San-Fernando's rigid ruptured seed coat after processing could also contribute to the resistance to shear leading to an increase in the texture measurement while the "plastic-like" seed coat in Sanilac and Nep-2 would not give such resistance. These results support the assumptions of Adams and Bedford (1975) in that at least a portion of the variability observed among lines depends on genetic differences without discounting that the harvesting and storage conditions, the initial moisture level of the seed, the length of processing, the temperature of the soak, the hardness of the water and the character of the added fluid all play an important role in that variability.

The study of texture based on single bean cooking time is given in Table 20. Results indicated that it takes more time to penetrate the whole seed than seed without coat. San-Fernando revealed the highest time required for the fall-pin to penetrate the seed. This indicates again the resistance of its seed coat to intrusion. There was no significant difference between Sanilac and Nep-2 cooking time in this experiment.

Although texture differences among the three cultivars under investigation are characterized in this study, the process of resistance to shear in legumes, in general, still needs further attention. Plant breeders and food scientists are well qualified to cooperate in this study.

Table 20. Texture based on single bean cooking time (minutes) for bean cultivars (Sanilac, Nep-2 and San-Fernando.

	MEAN COOKING TIME (minutes)	
Varieties	Seed Coat Intact	Seed Coat Partially Removed
Sanilac	43.0 ± 2.4^{a}	22.6 ± 1.2^{c}
Nep-2	41.6 ± 1.0^{a}	21.8 ± 2.6^{c}
San-Fernando	85.5 ± 6.2^{b}	31.9 ± 3.1^{d}

n = 40 (4 replicates/variety x 10 seed-cup/cultivar). Like letters in column denote no significant differences ($p \leq 0.05$) among cultivars and within seed portions.

CONCLUSIONS

The results reported in this dissertation on the proximate analysis; microstructure of dry, soaked and processed beans; texture; and other processing evaluation parameters of Sanilac, Nep-2 and San-Fernando have provided valuable information and the following conclusions can be drawn.

Proximate analysis over three years samples revealed that there were no significant differences among the cultivars under investigation with the exception of different crude fat contents in the 1979 and 1980 beans.

Sugar analyses showed high hexose and stachyose contents in all the three cultivars of whole beans. Raffinose and stachyose were not detected in the seed coat of any cultivar. This indicates that the color of the seed does not affect the sugar content in the seed coat.

The hydration study resulted in San-Fernando having the slowest absorption rate curve while its protein content, which is the major absorption fraction of a bean, did not differ significantly from the other two cultivars. Reduced hydration of San-Fernando was attributed to seed coat structural differences.

The scanning electron microscopy study on the dry seeds of the three cultivars showed highly organized structures commonly found in legume seeds. The different anatomical structures observed could well be used to explain some of the different functional property behaviors such as water absorption and texture which occur between the two isolines Nep-2 and San-Fernando. From these studies, it is concluded that San-Fernando seed coat outer surface, seed coat palisade:hour-glass thickness ratio, seed coat inner surface and cotyledon outer surface interlocking, all have significant effect on the seed water absorption.

The micropyle and the hypocotyl area can be used as indices of identification of the three cultivars because of their specificity for the individual cultivar. San-Fernando beans possess characteristic micropyle openings during soaking which contributes to its water absorption balance during canning. The non-ruptured parenchyma cells observed in the soaked San-Fernando cotyledon are an indication of increased resistance to shear in that cultivar compared to Sanilac which possessed complete release of the starch granules from these cells. In addition, the rigid seed coat and the partially intact starch granules in the San-Fernando processed beans contributed to the increased resistance to shear (increased firmness). These distinct observations could help plant breeders in the choice of their lines of selection among bean materials for a particular processing quality characteristic.

The bean lightness of color (L value) decreases during processing while redness ($+a_L$ value) and yellowness ($+b_L$ value) increase except in San-Fernando beans where there were fluctuation in values from year to year. This is an expected process which takes place under the heat treatment causing browning.

No significant differences were obtained among the cultivars for the moisture content after soaking and processing. The processed product revealed a high drained weight and low texture for Sanilac and Nep-2 but the reverse for San-Fernando.

The texture differences observed under the Kramer Shear Press between the two isolines can be attributed, when using a scanning electron microscope (SEM), to the structural differences obtained in their seed coat and parenchyma cell arrangement. Longer time is required to cook San-Fernando beans according to the single bean texture study.

The viscosity study indicated high initial pasting temperature with type C curves for all the cultivars. The removal of the seed coat increases the viscosity potential of Sanilac and San-Fernando contrary to Nep-2 which showed a decreased viscosity. Seed contribution to viscosity varied therefore with cultivar and showed a difference between the two isolines.

This work provides fundamental data regarding genetic, physico-chemical and structural characteristics of selected dry beans. These data are of significant value for inducting both genetic (biological) and technological (physical) changes to improve the quality of dry edible beans. Cooperative research efforts among plant breeders and food scientists could contribute to improved culinary and nutritional quality of cooked or processed beans and result in reduction of the energy required to prepare beans for human consumption.

RECOMMENDATIONS FOR FUTURE RESEARCH

1. The work on the proximate composition provided crude estimation of the bean constituents. Further work on the starch components (amylose and amylopectin) ratio and the protein quality may help in understanding some of the specific functional properties such as water absorption of the seeds and wettability of the flour.
2. The microstructure studies revealed information on the seed coat, micropyle, cotyledon and parenchyma cells of the dry, soak and thermally processed seeds. Work in this area on the seeds at different storage temperatures and humidities may provide information on the structural changes taking place during storage of legume seeds.
3. The processed bean evaluation indicated major texture differences using Kramer Shear Press and single bean texture measurement instruments. Further study with Instron or Wedge texture measurement instruments, may give additional information on the resistance of individual beans to shear forces.
4. The study of pasting characteristics of the bean flours showed variable behavior of the strain Nep-2 from year to year. Further work in this area is needed for the use in new product development to ascertain the effects

of rop location and season on flour pasting characteristics. In general, more investigations on the physical characteristics of dry edible bean are needed to provide information which could be used by plant breeders to develop uniformly quick cooking beans for consumers. The quick cooking character would be of most benefit to bean consumers in lesser developed countries where fuel is scarce and beans often require a prolonged cooking time to render them edible.

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APPENDICES

Table 1A. Percent sugars (hexose, sucrose, inositol, raffinose and stachyose) content (dry basis) of bean cultivars (Sanilac, Nep-2 and San-Fernando) fro three crop years (1978, 1979 and 1980) by portions (whole bean, cotyledon and seed coat).

	Hexose			Sucrose			Inositol			Raffinose			Stachyose		
	Seed Portion			Seed Portion			Seed Portion			Seed Portion			Seed Portion		
	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat	Whole	Cotyledon	Seed Coat
Sanilac	1.2^{a}	1.0^{a}	0.8^{a}	1.9^{a}	2.5^{a}	0.3^{a}	0.6^{a}	0.6^{a}	0.1^{a}	0.5^{a}	0.5^{a}	ND[1]	2.0^{a}	2.4^{a}	ND
Nep-2	1.3^{a}	1.3^{a}	0.7^{a}	2.2^{a}	2.7^{a}	0.2^{a}	0.4^{b}	0.3^{b}	0.0^{a}	0.4^{a}	0.3^{a}	ND	2.1^{a}	2.2^{a}	ND
SF	1.6^{a}	1.4^{a}	0.6^{a}	2.1^{a}	2.6^{a}	0.2^{a}	0.5^{ab}	0.4^{ab}	0.0^{a}	0.4^{a}	0.4^{a}	ND	2.0^{a}	2.1^{a}	ND

n = 27 (3 replicates/seed portion/cultivar x 3 injections/replicate x 3 years)

[1]ND = Nondetectable

Like letters in column denote nonsignificant differences ($p \geq 0.05$) among cultivars and within seed portions

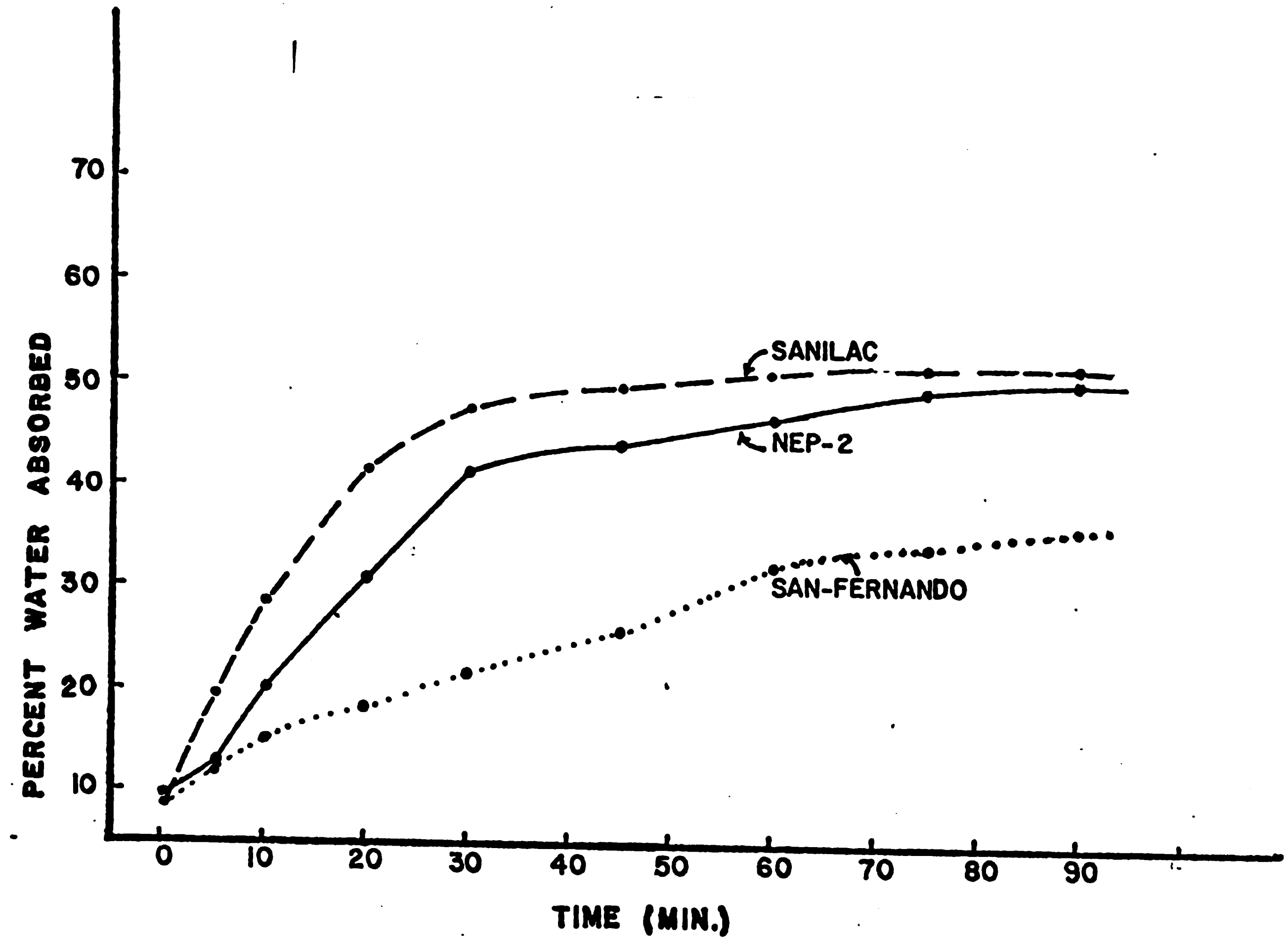

Figure A_1. Percent water absorption pattern of dry bean cultivars (Sanilac, Nep-2 and San-Fernando) soaked in ambient temperature water (1:1, tap water, distilled water) for up to 90 minutes for crop year 1978.

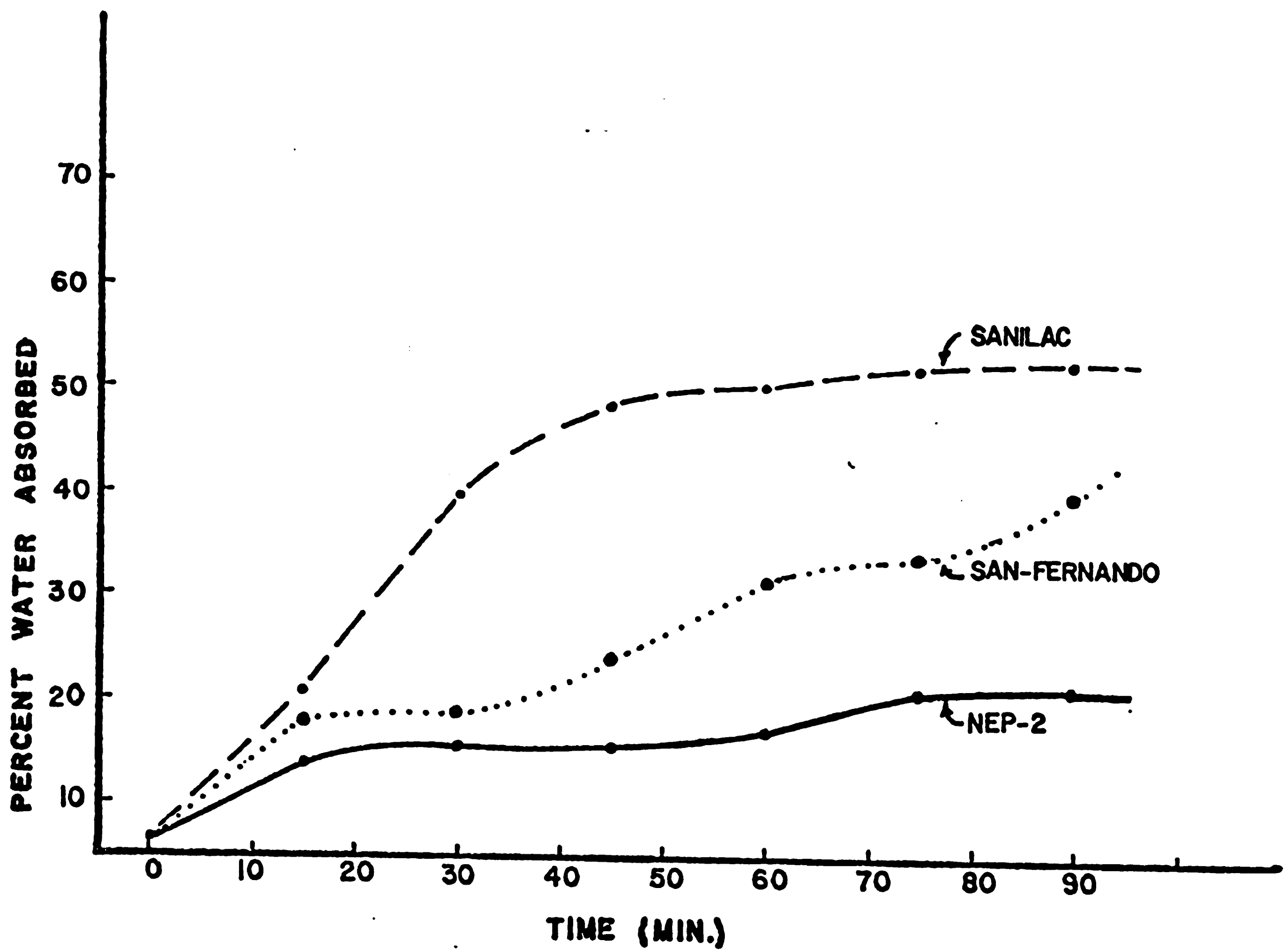

Figure A_2. Percent water absorption pattern for dry bean cultivars (Sanilac, Nep-2 and San-Fernando) soaked in ambient temperature water (1:1, tap water:distilled water) for up to 90 minutes for crop year 1979.

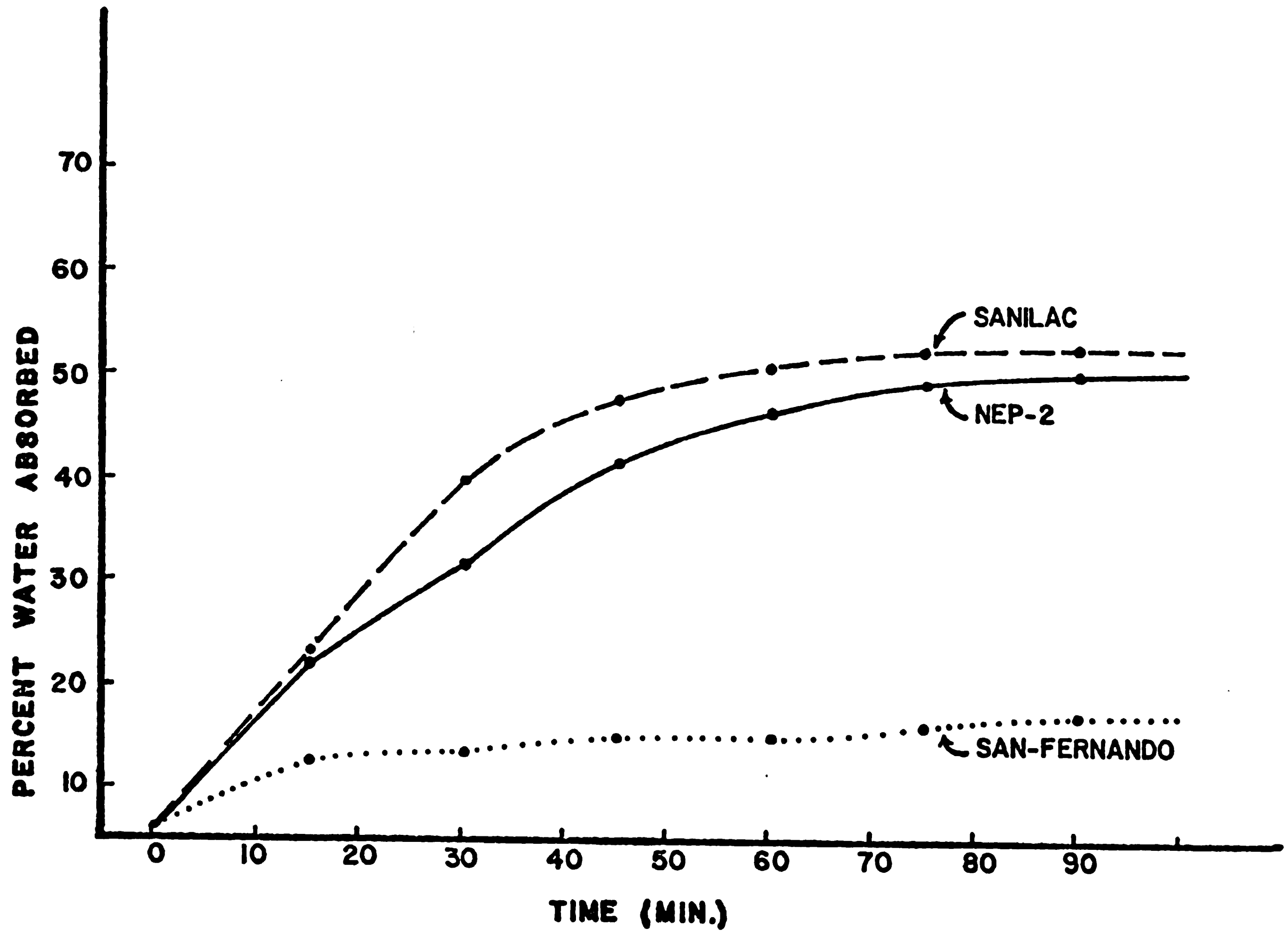

Figure A_3. Percent water absorption pattern of dry bean cultivars (Sanilac, Nep-2 and San-Fernando soaked in ambient temperature water (1;1, tap water:distilled water) for up to 90 minutes for crop year 1980).

Table 1B. Pasting characteristics of dry bean portions (whole bean and cotyledon) flours over three crop years (1978, 1979, 1980) by cultivars (Sanilac, Nep-2 and San-Fernando) in a 400 ml phosphate buffer solution at pH 5.30.

Legumes	Initial Pasting T°C	VISCOSITY IN (BU)[1]			
		at 95°C	after 15 min	at 25°C	After 15 min at 25°C
Sanilac					
Whole	72.8	118	258	468	410
Cotyledon	70.6	118	331	653	623
Nep-2					
Whole	72.3	190	411	721	667
Cotyledon	75.3	105	265	623	590
San-Fernando					
Whole	77.3	53	151	328	296
Cotyledon	75.8	93	216	538	513

n = 6 (2 replicates/seed portion/cultivar x 3 crop years)
1 = Brabender units

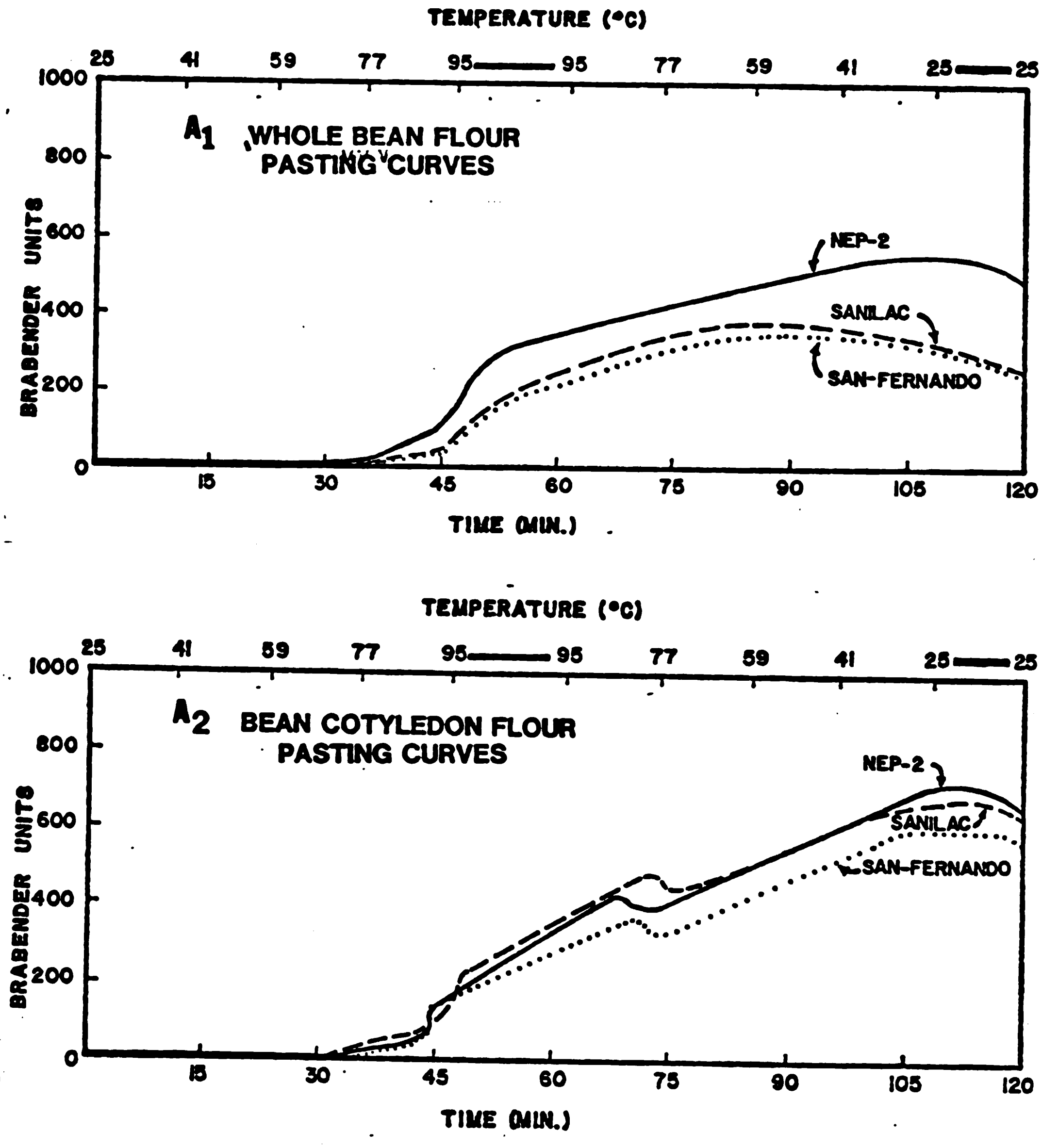

Figure B_1. Pasting curves of dry bean portions (whole bean and cotyledon) flour by cultivars (Sanilac, Nep-2 and San-Fernando) in a 400 ml phosphate buffer solution at pH 5.30 for crop year 1978.

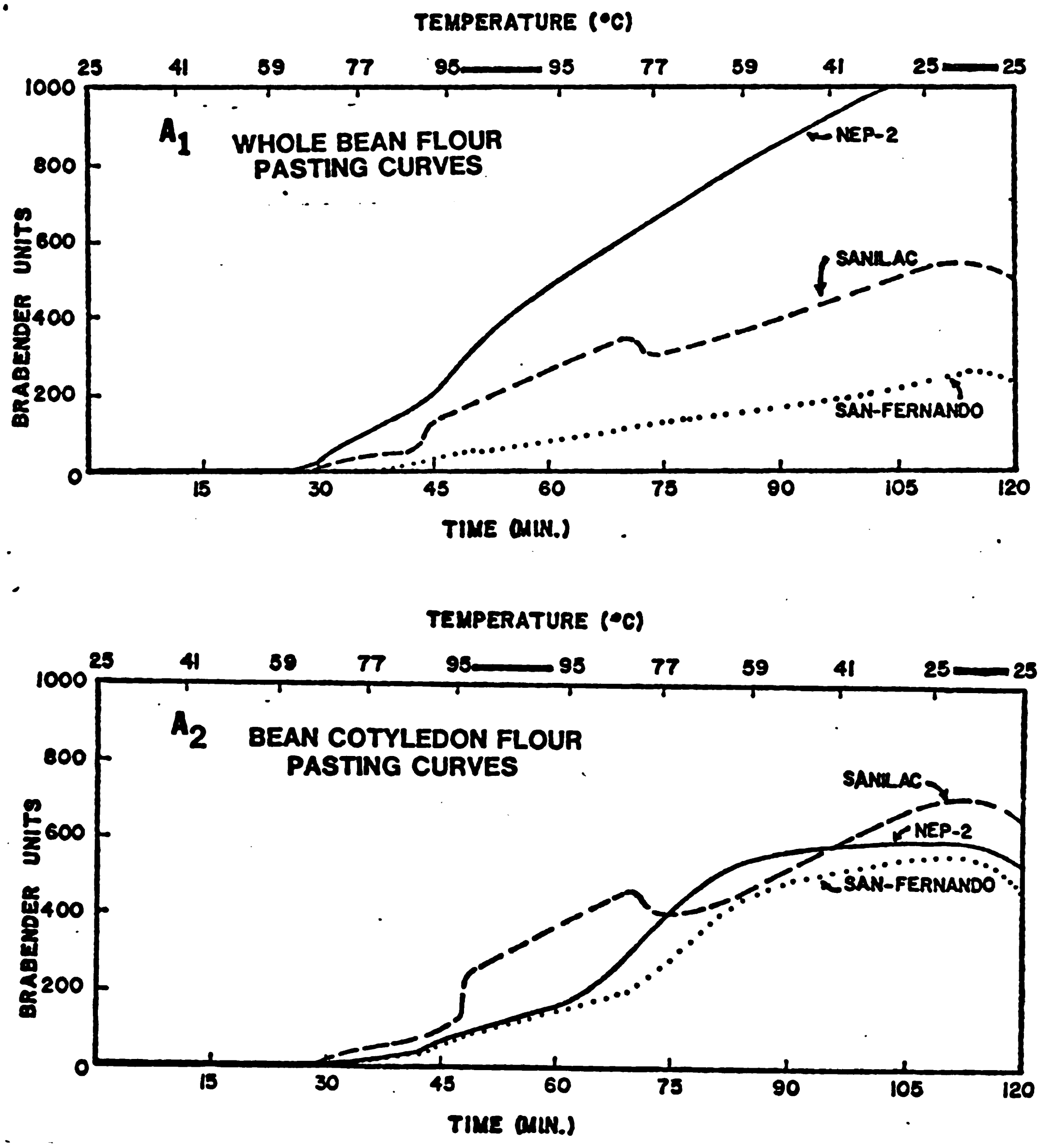

Figure B_2. Pasting curves for dry bean portions (whole bean and cotyledon) flours by cultivars (Sanilac, Nep-2 and San-Fernando) in a 400 ml phosphate buffer solution at pH 5.30 for crop year 1979.

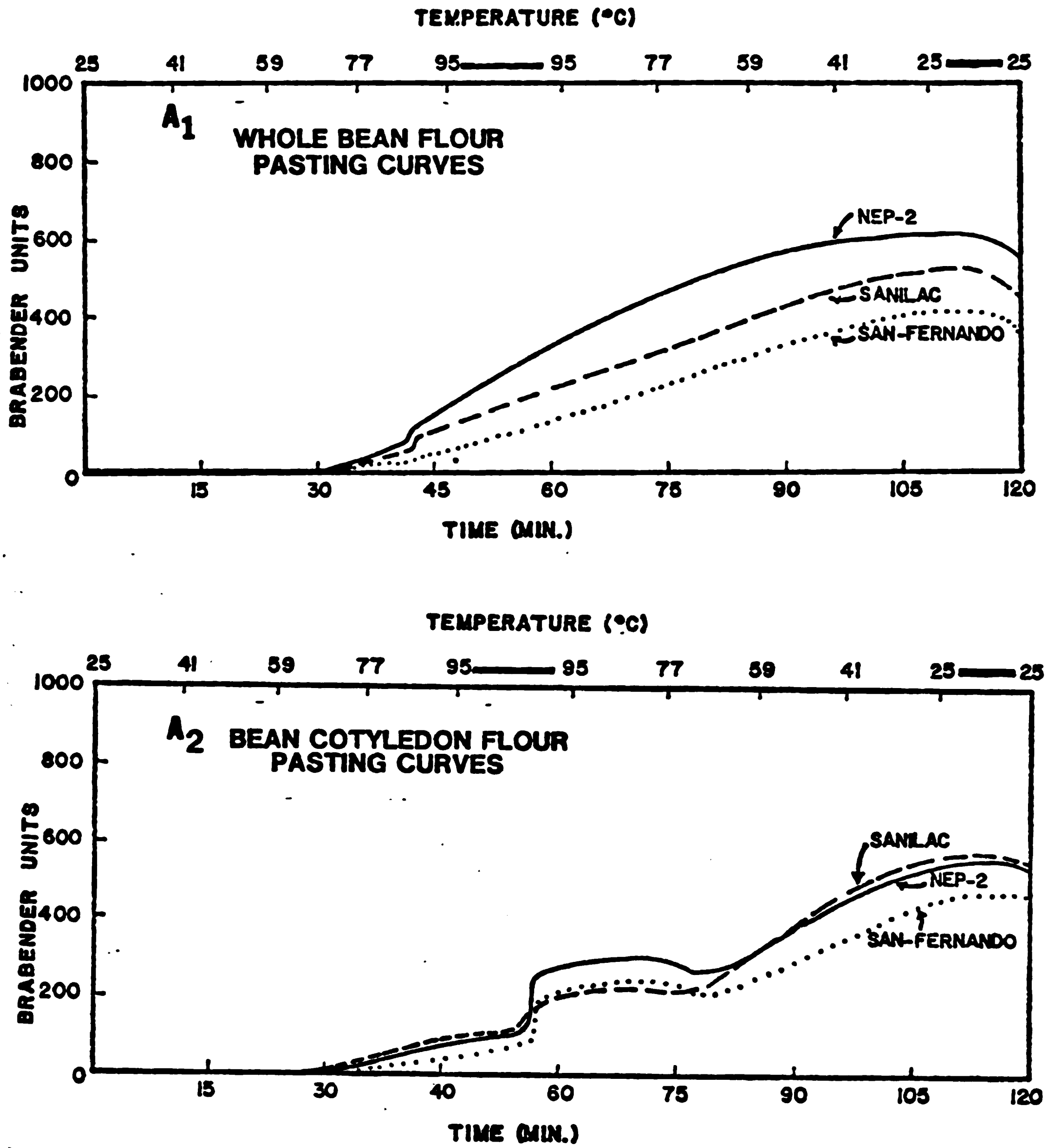

Figure B_3. Pasting curves of dry bean portions (whole bean and cotyledon) flours by cultivars (Sanilac, Nep-2 and San-Fernando) in 1 400 ml phosphate buffer solution at pH 5.30 for crop year 1980 .

Table 1_C. Hunter Lab color and color difference values of dry and processed bean cultivars (Sanilac, Nep-2 and San-Fernando) from three crop years (1978, 1979 and 1980).

Bean	Dry Beans			Processed Beans		
	L	a_L	b_L	L	a_L	b_L
Sanilac	62.7^a	0.2^c	0.2^e	50.5^g	11.1^i	16.2^j
Nep-2	58.7^a	0.1^d	11.1^e	48.8^g	11.9^i	16.7^j
San-Fernando	16.6^b	0.0^d	-0.1^f	15.7^h	5.2^j	-2.6^k

n = 24 (2 replicates/sample x 2 samples/can x 2 cans/cultivar x crop years)
Like letters denote no significant differences ($p \leq 0.05$) among cultivars

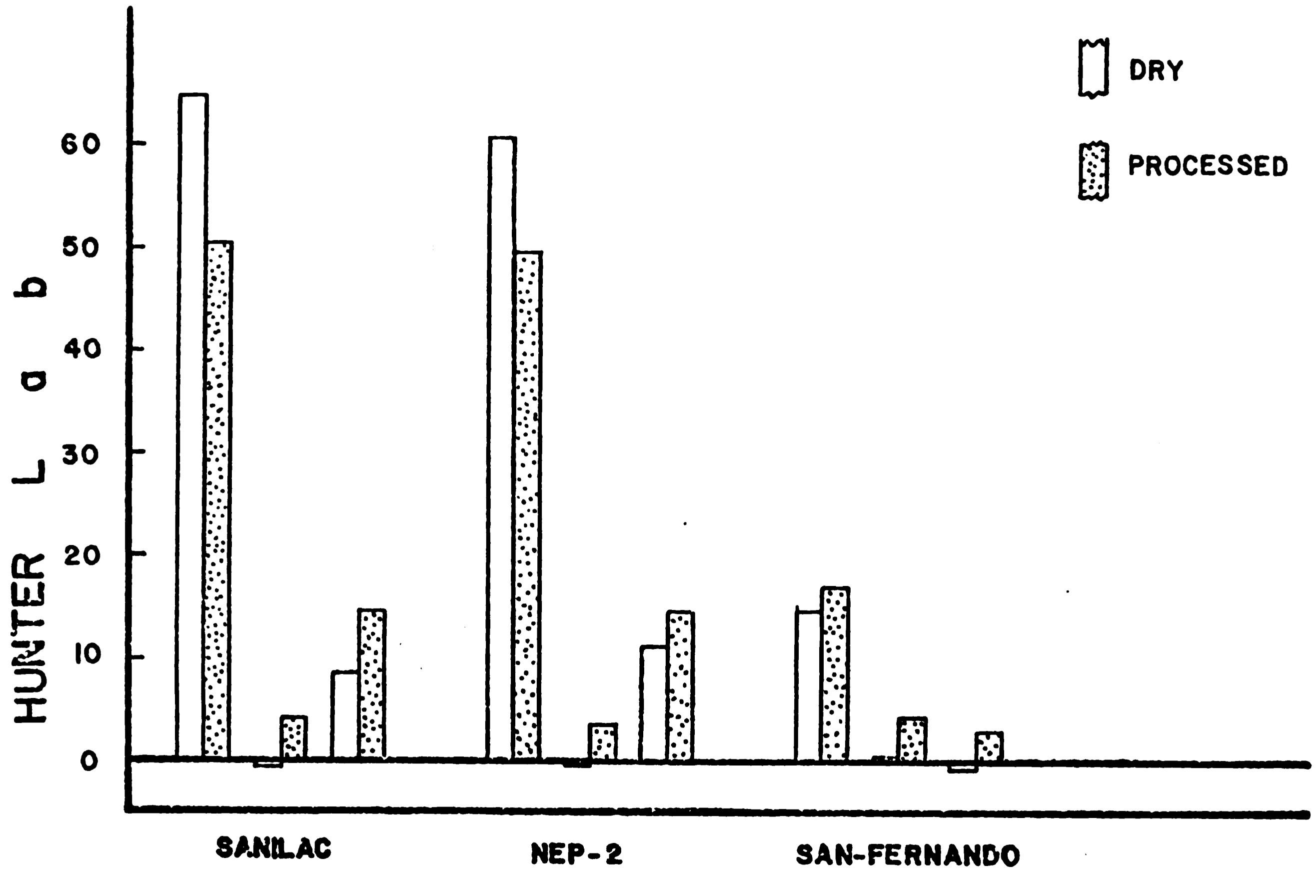

Figure C_1. Hunter color and color differences values of dry and processed bean cultivars (Sanilac, Nep-2 and San-Fernando) crop year 1978.

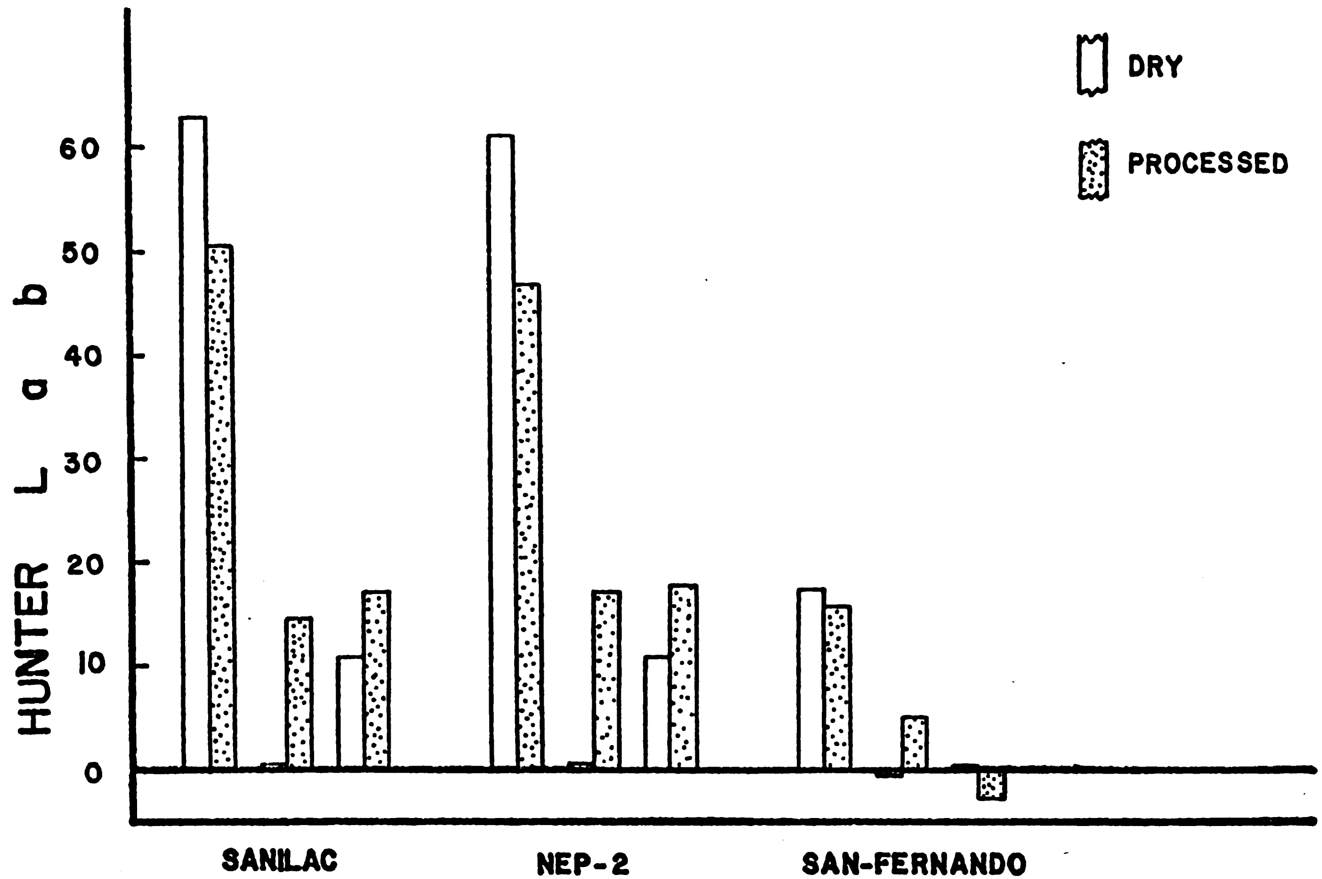

Figure C_2. Hunter color and color differences values of dry and processed bean cultivars (Sanilac, Nep-2 and San-Fernando) crop year 1979.

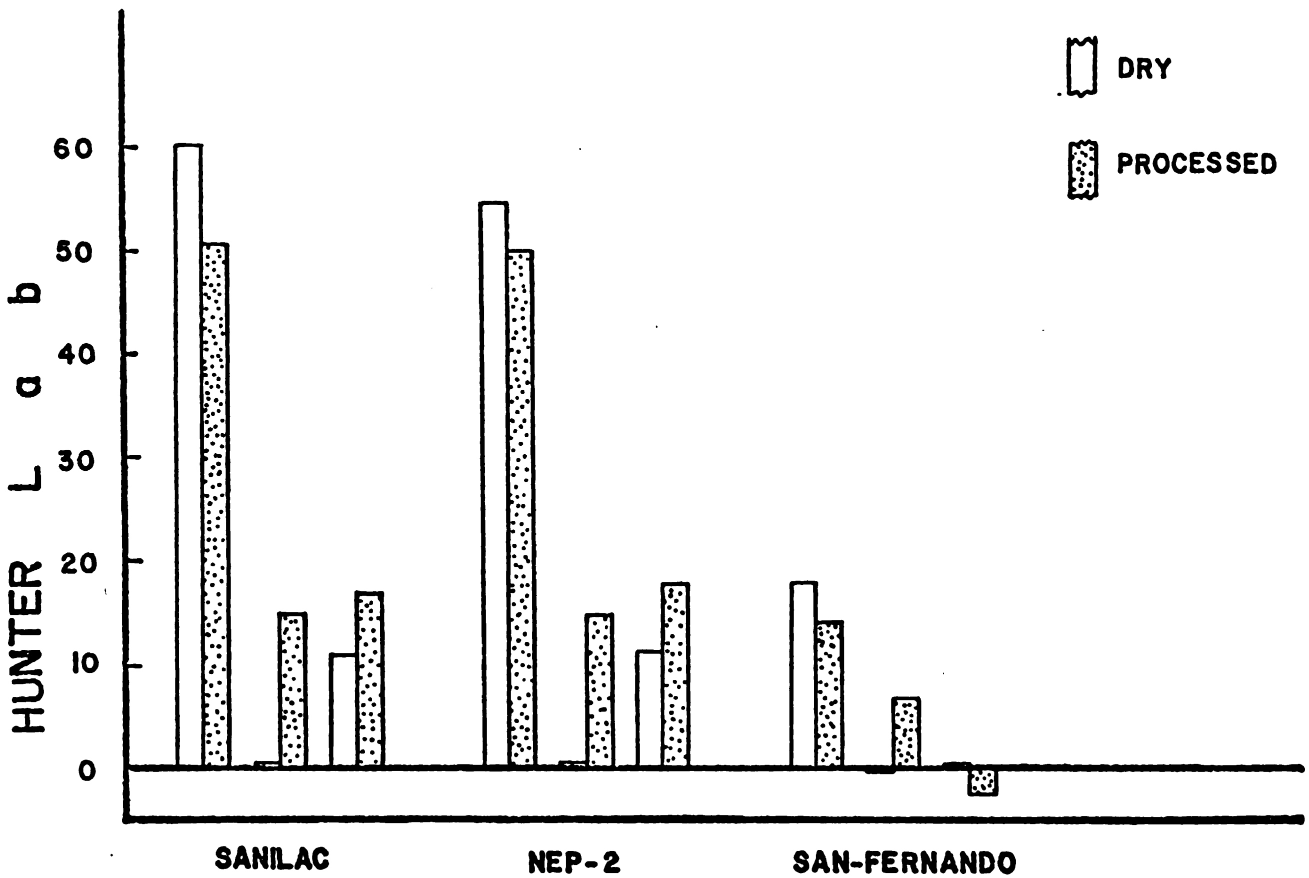

Figure C_3. Hunter color and color differences values of dry and processed bean cultivars (Sanilac, Nep-2 and San-Fernando) crop year 1980.

Table 1D. Processed bean evaluation - three crop years (1978, 1979 and 1980) bean samples.

Legumes	Quality parameters evaluation								
	Initial Moisture[1] %	Water Content After Soaking[1] %	Water Content After Processing[2] %	Drained[1] Weight g	Clump[1] Rate 1 to 3	Split[1] Rate 1 to 5	Texture[2] Peak Height (Kramer units)	Texture[2] Force Kg/100 g	Soak Ratio
Sanilac	15.0^{a}	55.3^{b}	67.2^{c}	307.9^{d}	2.4^{f}	3.0^{g}	31.9^{j}	43.4^{m}	1.9^{n}
Nep-2	16.1^{a}	53.4^{b}	69.5^{c}	305.4^{d}	1.2^{f}	2.5^{g}	36.9^{i}	50.2^{l}	1.8^{n}
SF	16.6^{a}	51.3^{b}	68.0^{c}	286.2^{e}	1.8^{f}	2.5^{g}	55.8^{h}	75.8^{k}	1.7^{n}

[1]n = 6 (1 replicate/can x 2 cans/cultivars x 3 crop years).
n = 12 (2 replicates/can x 2 cans/cultivar x 3 crop years).
Like letters denote no significant differences ($p \leq 0.05$).

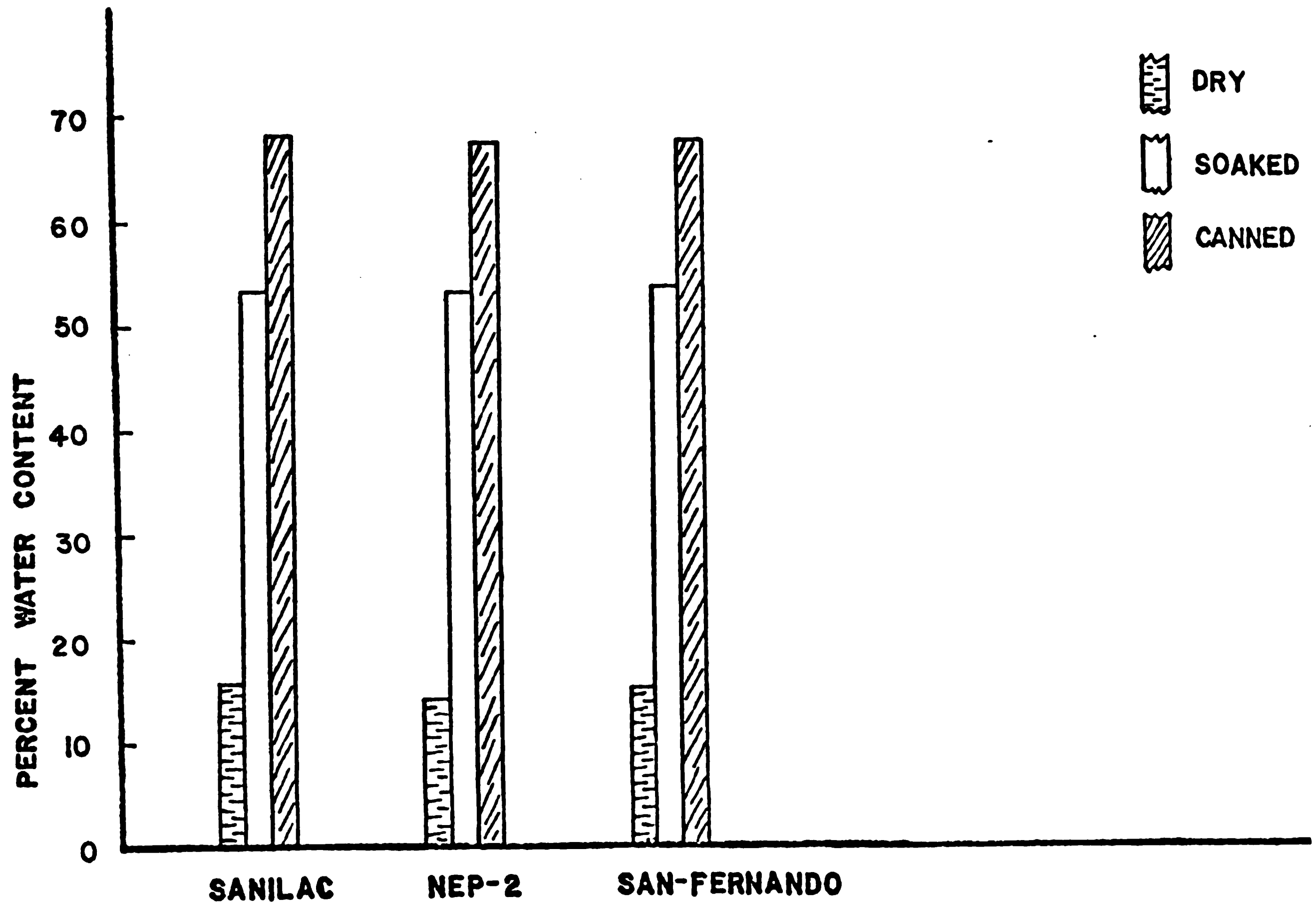

Figure D_1. Percent water content of bean cultivars (Sanilac, Nep-2 and San-Fernando) in quality parameters evaluation for crop year 1978.

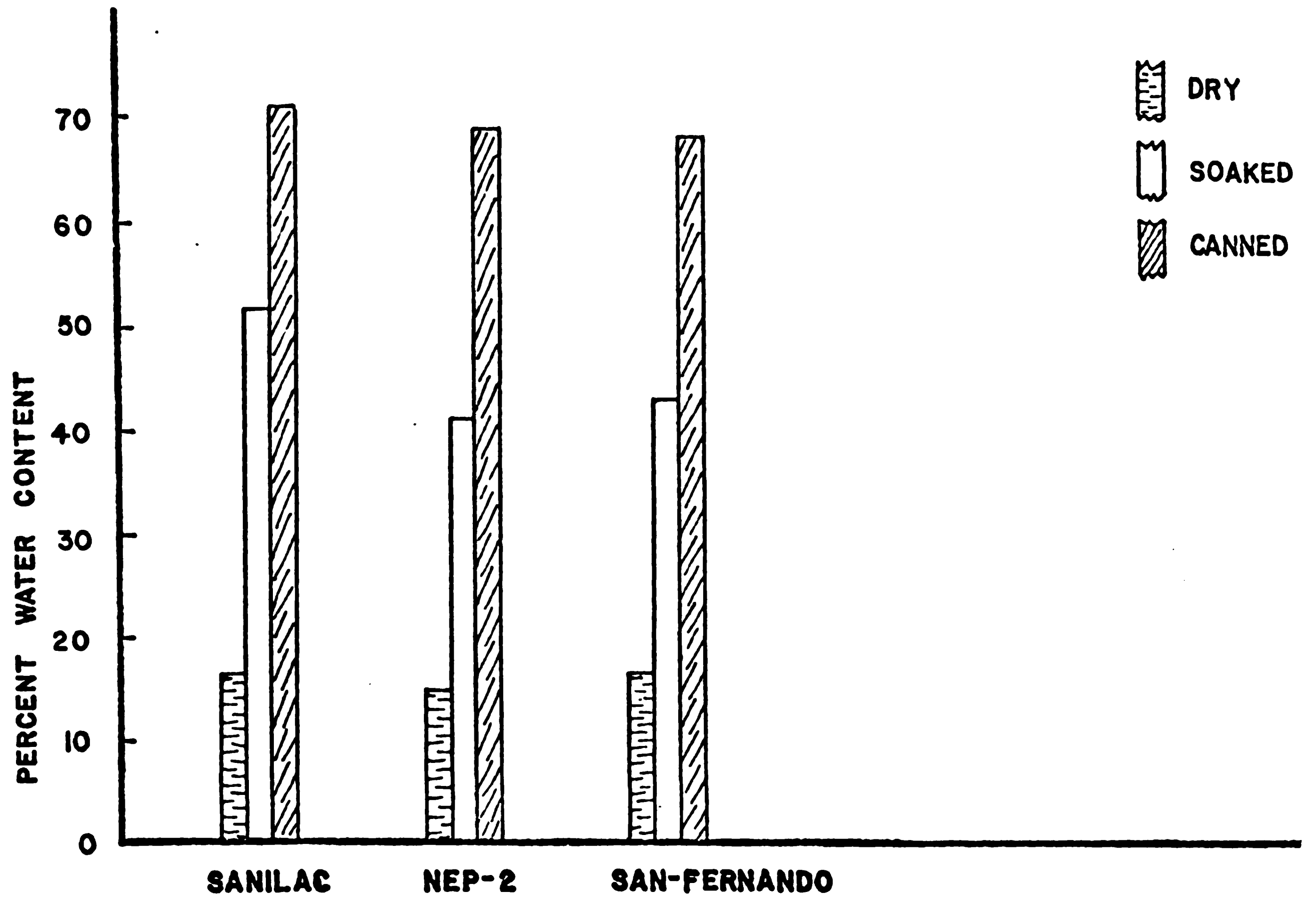

Figure D_2. Percent water content for bean cultivars (Sanilac, Nep-2 and San-Fernando) in dry, soaked and canned beans quality parameters evaluation for crop year 1979.

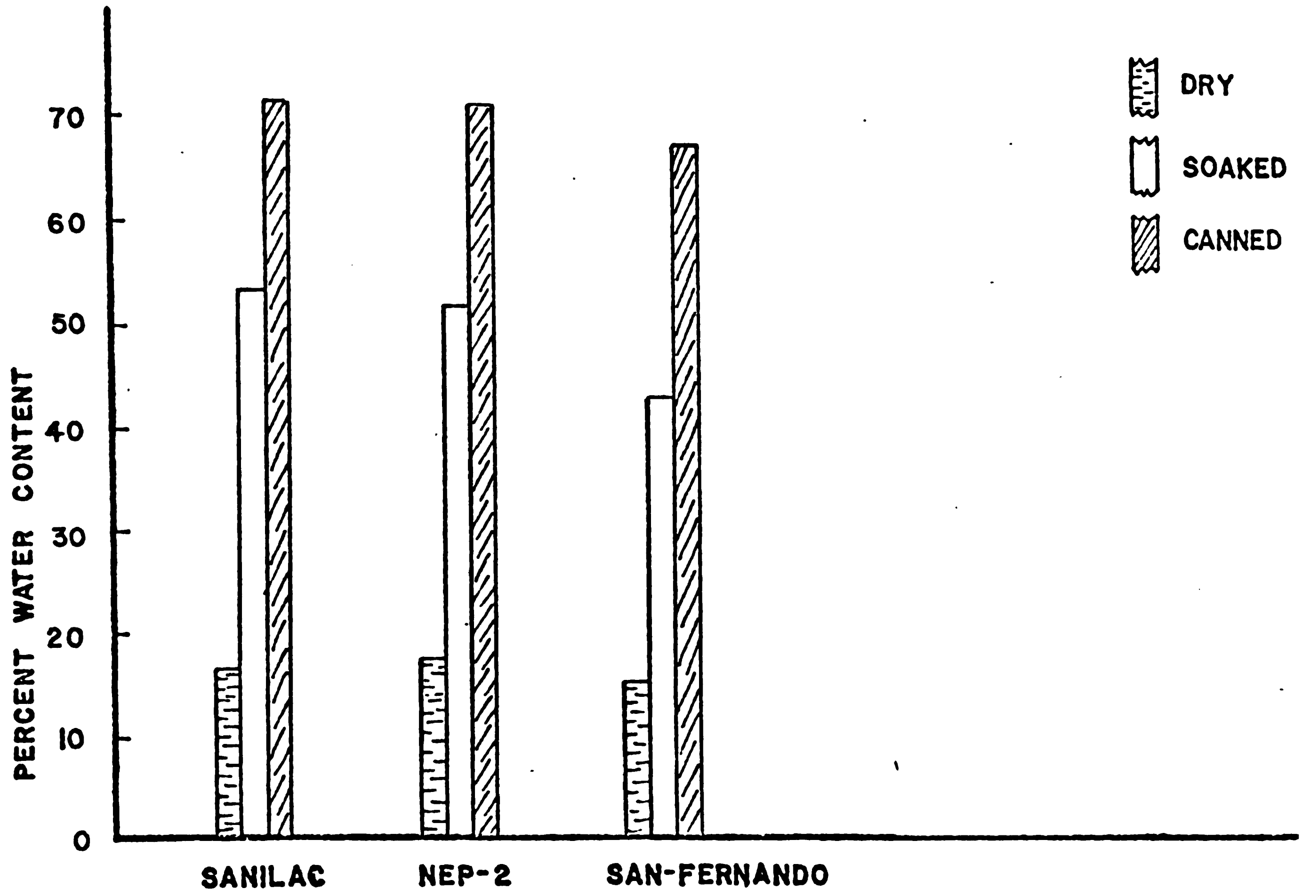

Figure D_3. Percent water content for bean cultivars (Sanilac, Nep-2 and San-Fernando) in quality parameters evaluation for crop year 1980.

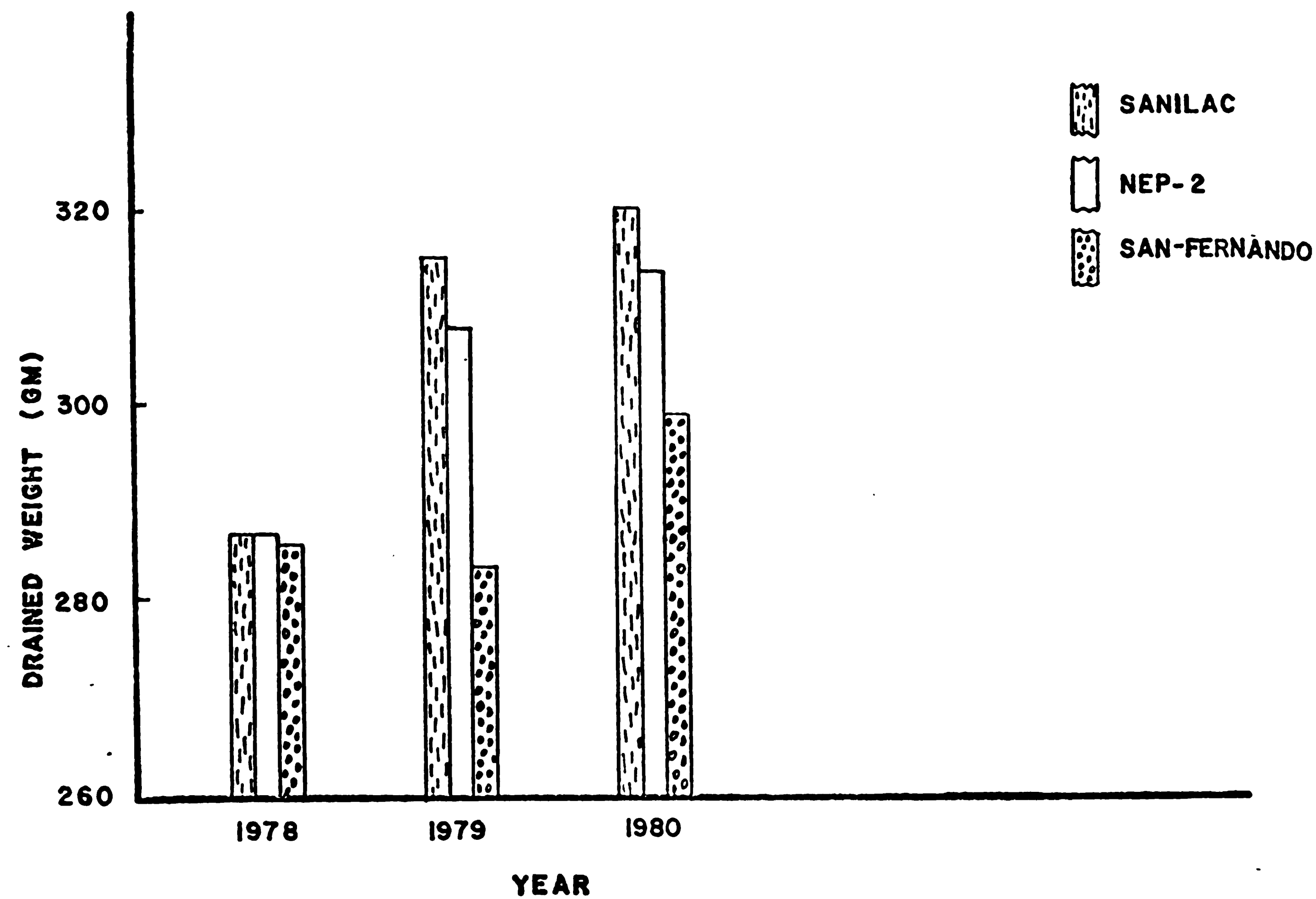

Figure E. Drained weight for bean cultivars (Sanilac, Nep-2 and San-Fernando) in canned bean quality parameters evaluation by crop years (1978, 1979 and 1980).

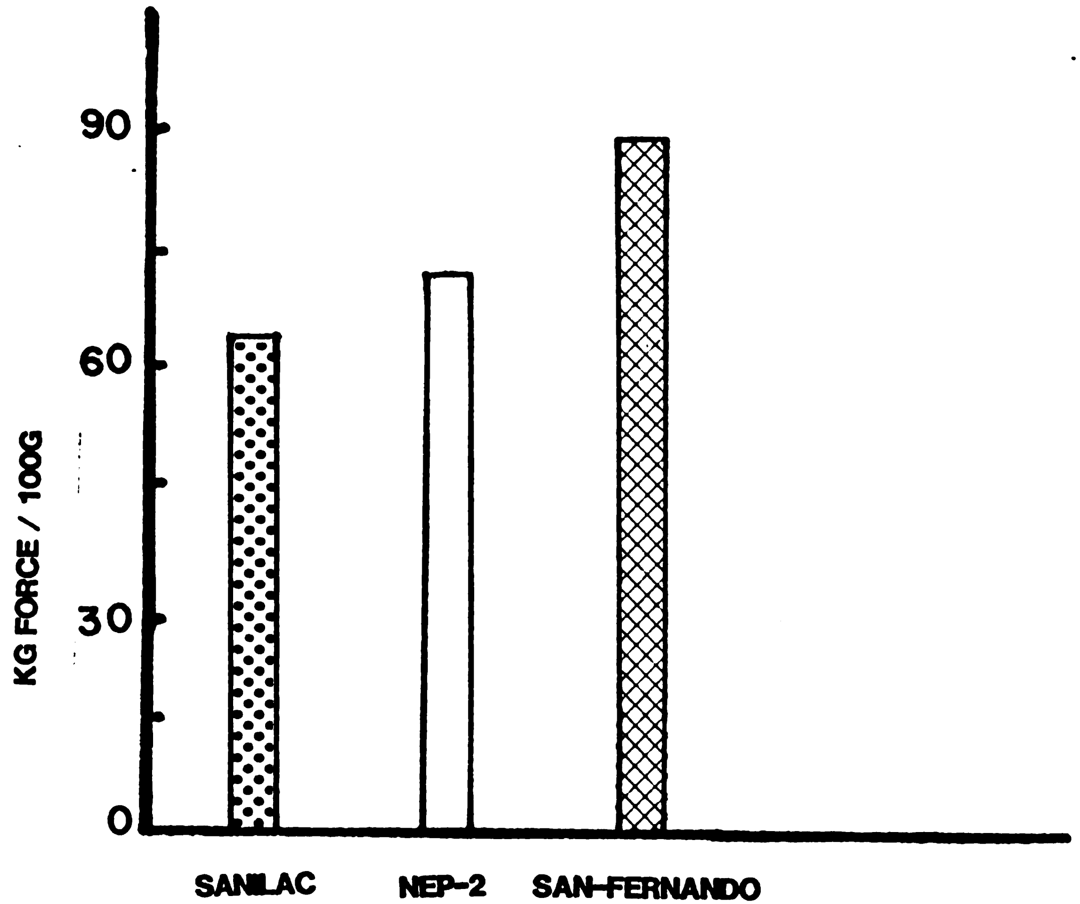

Figure F_1. Texture for bean cultivars (Sanilac, Nep-2 and San-Fernando) in canned bean quality parameters evaluation for crop year 1978.

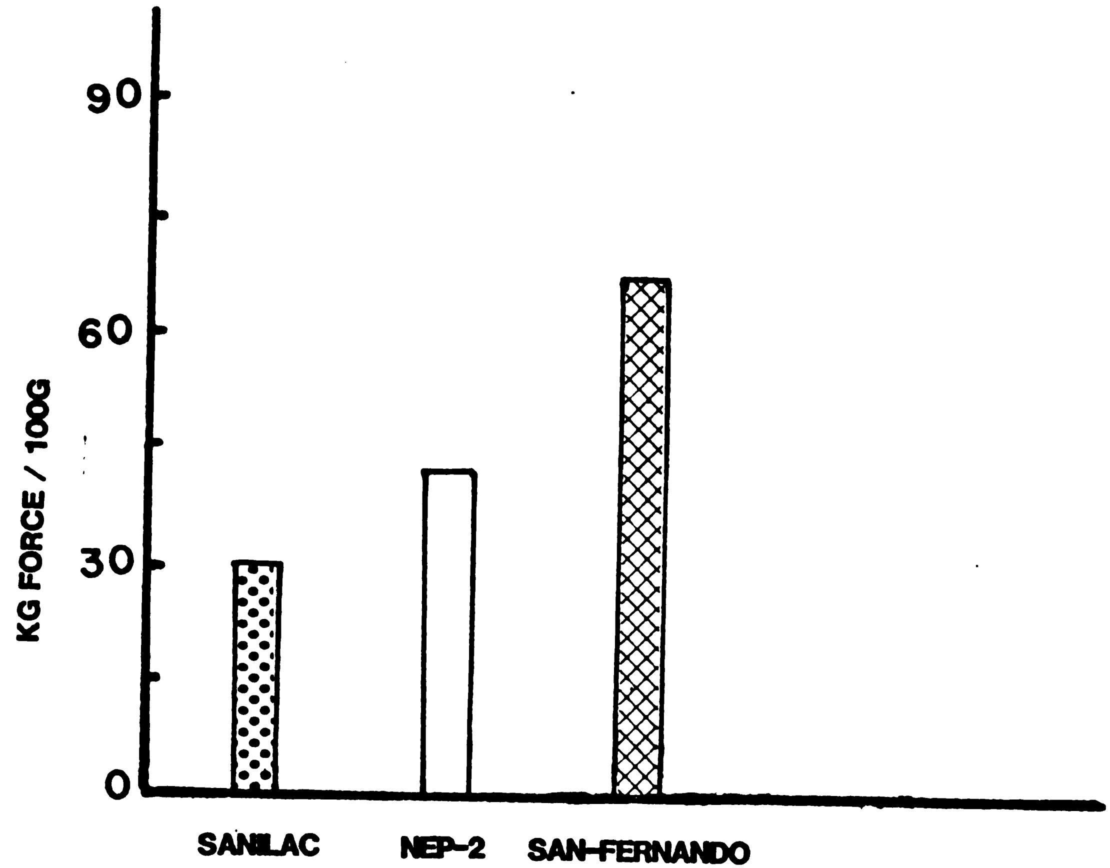

Figure F_2. Texture for bean cultivars (Sanilac, Nep-2 and San-Fernando) in canned bean quality parameters evaluation for crop year 1979.

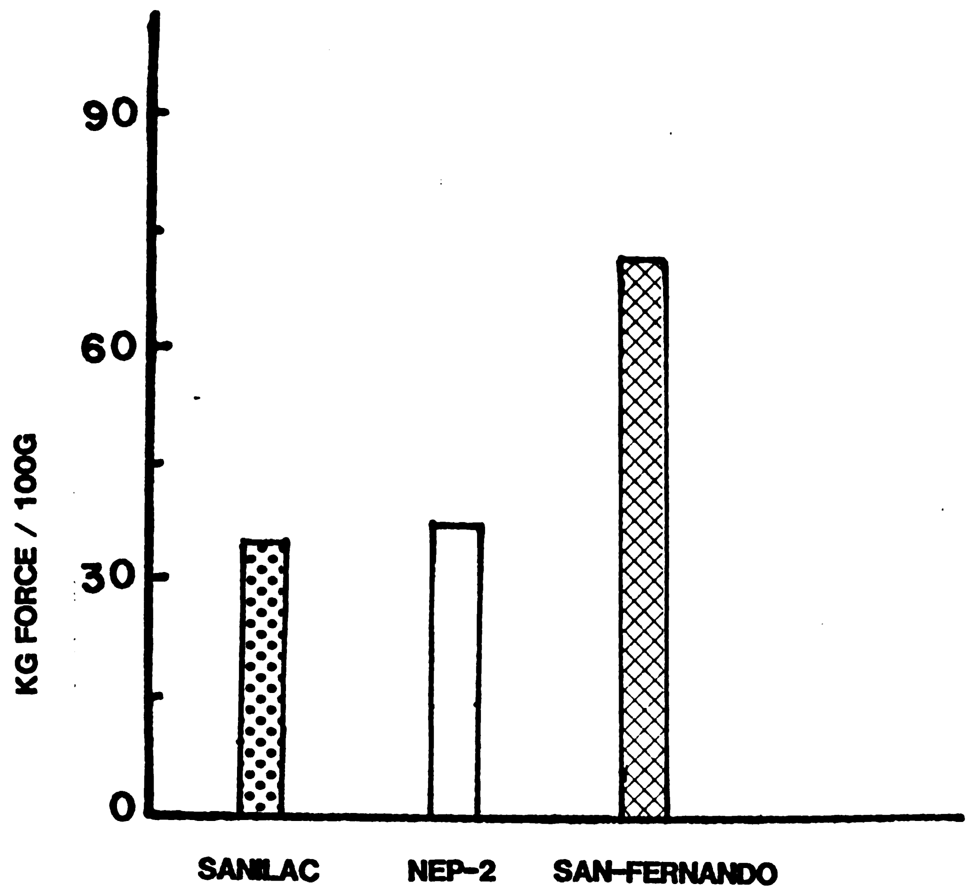

Figure F_3. Texture for bean cultivars (Sanilac, Nep-2 and San-Fernando) in canned bean quality parameters evaluation for crop year 1980.