CHANGES IN CELL WALL STRUCTURE AND STARCH DIGESTIBILITY DURING COOKING OF DRY BEAN (*Phaseolus vulgaris* L.)

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ABSTRACT

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The indigestible starch fractions of dry bean have been studied by examining the raw and cooked seeds. The effects of selected heat treatments on cell wall structural appearance and starch bioavailability were enzymatically and microscopically examined. An enzymatic procedure using β-amylase and pullulanase was used to selectively digest starch fractions. The starchy plant tissue evaluated included: navy beans, pinto bean, sweet potato and wheat.

The degree of gelatinization measured by the enzymatic method showed lower scores than that obtained by the microscopic birefringent method on differentially cooked beans. The lower values measured by the enzymatic method resulted from inaccessibility of the enzyme within gelatinized starch. Cooking whole beans for 10 min or longer caused crystallization within cell wall. The crystalline structure was observed under polarized microscope appearing a white band around cell walls. The crystallized cell walls of cooked bean was mechanically resistant and acted as a physical barrier not only to enzyme hydrolysis but also to swelling of starch granules leading to a residual of ungelatinized starch during cooking. The rigidity of the crystallized cell wall that changed during cooking was investigated by measuring available starch after reheating of freeze-

dried and milled flours. The longer the cooking time, the greater the rigidity of the crystallized cell wall of the cooked beans. The cell wall rigidity varied between bean types and among the process conditions studied. DSC thermography demonstrated that the crystalline structure formed during cooking was more stable than starch that demonstrated to be associated with resistant starch fraction. Starches of cooked sweet potato and wheat were readily available to undergo enzymatic degradation since no crystallization occurred within cell walls to limit enzyme accessibility.

The attributes of physical appearance, palatability and nutritional bioavailability are essential components associated with food quality of cooked dry beans. Dry bean cell walls undergo structural changes during cooking that limit starch digestibility. Crystallized cell walls encapsulating starch granules were observed in cooked bean exudate (brine). The amount of starch in brine was not correlated with brine viscosity however total protein and insoluble dietary fiber in the brine were positively correlated with increased brine viscosity.

Dedicated to my wife, Junghee, and parents
for their love, patience and moral support

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INTRODUCTION

Legume consumption has increased in the western world due in-part to the improved recognition of beans as a good source of dietary fiber and protein. In addition, the high quality of carbohydrate associated with controlled digestion and absorption is increasingly being recognized. Legume starch is well-known as a low glycemic index food. It has been shown that the low glycemic index diets improve metabolic variables not only in diabetes (Brand, et al., 1991) and hyperlipidemia (Jenkins, et al., 1987a) but also in healthy subjects (Jenkins, et al., 1987b). The resistance of legume starches towards hydrolytic enzymes have been of great interest to nutritionists, since they have been found to exhibit a lower glycemic index than the cereals (Jenkins, et al., 1980). However, legume starch granules are contained within thick cell walls, which is a critical factor determining the rate of digestion. The starch granules contained within intact cell walls pass the stomach and enter the small intestine. The indigestible starch also reaches colon and provide substrates for bacteria to ferment which result in flatulence.

The degree of starch gelatinization in cooked beans is of importance to their complete digestibility. Seeds of food legumes are known to contain starch fractions that do not gelatinize and therefore are resistant to digestion. *In vitro* enzymatic methods have been used for estimation of degree of starch gelatinization, these methods provide results which are closely related with true *in vivo* digestibility if appropriate enzymes and conditions are maintained. Heating of beans during processing is required for palatability.

However, the integrity of cell wall polymerized during cooking is a concern to maximum digestability.

The research reported in this dissertation was directed and conducted toward the development and assessment of accurate methodology for starch gelatinization of cooked whole beans and to attain a greater understanding nature of changes within cell wall during cooking. Both approaches are necessary for implementing technological strategies for improvement of dry bean utilization.

This research was conducted and reported in three independent Chapters. Chapter titles and their respective Null hypotheses (H_o) are presented:

Chapter 1:	Indigestible starch components in dry beans
H_o:	The presence of whole cells with crystallization in the cell wall do not prevent starch gelatinization and lower starch digestibility.
Chapter 2:	Heat-induced changes in cell wall structures reduce bioavailability of bean starch
H_o:	The crystallization of cell wall in cooked beans is not a limiting factor associated with starch gelatinization and bioavailability.
Chapter 3:	Characterization of brine obtained from canned beans possessing differential quality
H_o:	The compositional components of brine and physical bean quality after processing are not controlled by genetic background.

REVIEW OF LITERATURE

Physical and Chemical Properties of Bean Starch

Legumes are the dicotyledonous seeds of plants that belong to the family Leguminoase, which contains about 600 genera with 13,000 species. The major carbohydrate of the legume seed is starch. Within each parenchyma cell, starch granules are embedded within a protein matrix (Powrie et al., 1960; Sefa-Dedeh and Staley, 1979 and McEwen et al., 1974). Legumes contain 24% (winged beans) to 68% (cowpeas) total carbohydrate on a dry basis of which starch makes up a larger portion ranging from 24 to 56% (Reddy et al., 1984). These variations in starch contents are due to different cultivars and analytical procedures (Pritchard et al., 1973 and Cerning-Beroard et al., 1975). Starches contain two types of glucose polymers: amylose and amylopectin. The amylopectin molecules are highly branched and in general, larger than amylose molecules. The starch found in legumes has oblong granules which vary in size by species. Dry bean starch granule is resistant to swelling and rupture and generally contains high amylose content (30 - 37%) (Hoover and Sosulski, 1985). Starch granules can greatly influence the cooking characteristics of legumes. Gelatinization temperatures ranging from 60°C to over 75°C are relatively high compared to cereals and may contribute to processing variability (Hahn et al., 1977).

Granule Composition and Structure

The major energy reserve of most plant tissue is starch, which is most abundant in seeds, roots and tubers. Following the biosynthesis, starch is stored in the plant as

compact micron-sized granules that are partly crystalline. This relatively inert and water-insoluble fraction provides energy reserves which facilitate starch isolation and analytical handling (Zobel, 1984).

Granule size, shape, and microscopic appearance

The size and shape of legume starch granules are shown in Table 1. The granule size is quite variable and ranges from 4 to 85 μm depending on the starch source. Most bean starch granules are oval (greater length than width), although spherical, round, elliptical, and irregular granules are also found. This wide variation in granule size and shape is generally attributed to genetic control and seed maturity. For dry beans, however, a wide variability in shape is found in starch granules from the same source (Reddy et al., 1984).

Light microscopic studies of legumes starches clearly reveal two distinct starch granule characteristics: the presence of a hilum and the presence of lamellae. The hila have been described as furrows, grooves, cracks and stria (Naivikul and D'Appolonia, 1979), cracking dark bands (Hall and Sayre, 1971), and microfibrils (Donovan, 1979).

The hilum and lamellae observed under the light microscope are not seen under a scanning electron microscope. Instead, their surfaces appear to be smooth with some occasional "scar-like" features. These latter structures could arise from adhering cell wall materials or proteins, to both.

Under the polarized light microscope, intact granules exhibit a well-defined birefringence pattern with a dark cross. This is indicative of the highly organized nature of

Table 1. Granule dimensions and shapes of legume starches

Starch source	Range (diameter) Width (μm)	Length (μm)	Unspecified (μm)	Shape	Reference
Kidney bean	16-42	16-60		Elliptical, oval	Kawamura 1969; Hoover and Sosulski 1985
Northern bean	12-40	12-62		Oval, irregular, round	Hoover and Sosulski 1985; Sathe and Solunkhe 1981
Navy bean	12-40	12-49		Oval, round elliptical	Hoover and Sosulski 1985; Naivikul and D'Appolonia 1979
Black bean	8-34	8-55		Oval, spherical	Hoover and Sosulski 1985; Lai and Varriano-Marston 1979
Mung bean	7-20	10-32		Oval, irregular, round	Kawamura 1969; Naivikul and D'Appolonia 1979
Pinto bean	10-30	12-48		Oval, irregular, round	Hoover and Sosulski 1985; Naivikul and D'Appolonia 1979
Adzuki bean	20-55	24-70	15-45	Oval, kidney	Kawamura 1969; Tjahjadi and Breene 1984
Moth bean			6-28	Oval, round	Wankhede and Ramteke 1982
Faba bean	12-24	20-48		Oval, spherical	Naivikul and D'Appolonia 1979
Horse bean			6-31	Oval, irregular	Lineback and Ke 1975
Red bean			25-67	Oval, irregular	Lii and Chang 1981
Lablab bean			30	Oval, round, kidney	Rosenthal el al. 1971
Smooth pea			20-40	Oval, round	Vose 1977
Wrinkled pea			6-80	Round	Colonna et al. 1980
Black gram	7.5-27	7.5-28.5		Round, oval	Sathe et al. 1982
Chick pea			8-54	Oval, spherical	El Faki et al. 1983; Lineback and Ke 1975
Cow pea			4-40	Oval, spherical	El Faki et al. 1983; Tolmasquim et al. 1971
Horse gram			15-85	Oval, spherical	El Faki et al. 1983
Lentil	15-30	10-36		Oval, round, ellipsoid	Naivikul and D'Appolonia 1979; Bhatty 1988

the granule. Amylopectin may be responsible for the crytallinity since waxy starches with essentially no amylose are very birefringent (Hood, 1982).

Molecular structure

Generally, starch is defined as a polymer of D-glucose units. It is composed of two different polymers, a linear compound, amylose, and a branched component, amylopectin. Traditionally, amylose was thought to be a linear glucose polymer in which the individual monomers were connected solely by α-1,4 glucosidic linkages. It is now recognized that it has some elements of nonlinearity (Hood, 1982; Whistler and Daniel, 1984). The molecular structure is not well defined. It has a molecular weight of 150,000 - 1,000,000 depending on its biological origin. Greenwood and Thomson (1962) indicated that structural differences exist along the amylose molecule and that it is not completely linear. Amylopectin is a ramified structure containing 94-96% α-1,4 and 4-6% α-1,6 linkages. The average chain length is 20-26 glucose units. Amylopectin does not give a native starch-iodine (Amylose-iodine) blue color but rather a purple and sometimes reddish brown color depending upon its source. The molecular weight of amylopectin is on the order of 10^7- 10^8. Its molecular structure has been studied by many investigators (Haworth et al., 1937; Meyer, 1952; Wheland, 1971; French, 1972; Robin et al., 1974; Yamaguchi et al., 1979; and Manners and Matheson, 1981). Several structural models have been proposed for amylopectin molecule to account for its physicochemical properties. In 1937, Haworth et al., proposed the "laminated" structure, i.e., the ratio of A and B chains is 1:(n-2), where A chains are attached to the macromolecule by a single linkage from the potential reducing-group, and B chains are linked to two or more other

chains, where n is the total number of chains. In the same year, Staudinger and Husemann suggested the "comb" structure. All of the chains except one were designated A chains. Meyer (1952) originated the "tree type" structure in which branching from a centering region is predominant. Wheland (1971) later revised the Meyer structure such that the A:B ratio was approximately 1:1. A cluster-type structure was originally proposed by Nikuni (1969) for starch molecules; then independently, for the amylopectin component by French (1972), and later by Robin et al. (1974). More recently, an extended cluster model has been proposed by Yamaguchi et al. (1979). Manners and Matheson (1981) supported the structure of the cluster-type because it was more in accord with the physical properties, the observed structural features, and mode of biosynthesis within the starch granule than were other hypothesized models. It is becoming increasingly apparent that, except for high-amylose starch, the molecular structure of amylopectin is similar among cultivars (Hood, 1982).

The molecular structure of starch components and their arrangement are related to the crystalline properties of the granule. According to the amylopectin structure proposed by Robin et al. (1974), the α-1,6 linkage rich regions represent the amorphous area within the granule, whereas the outer short chains (DP$\leq$15) form the highly organized helical structures contribute to the crystalline properties of the granules.

Granule Crystallinity

Starch granules contain both crystalline (ordered) and amorphous (unordered) regions. This crystallinity gives rise to the birefringent property of starch granules (Elbert, 1965). X-ray diffractometry has been used to reveal the presence and characteristics of

crystalline structures of starch granules (Katz and Van Itallie, 1930; Sarko and Wu, 1978). Legume starches have been shown to exhibit a "C" type X-ray diffraction pattern (Hoover and Sosulski, 1985; Kawamura, 1969; Sarko and Wu, 1978; Colonna et al., 1980; Lai and Varriano-Marston, 1979). This is intermediate between the A (cereal) and the B type (tuber). The reasons for these differences are not properly understood. Hizukuri (1985) has suggested that slight differences in the chain length and chain profile of the constituent amylopectin molecules may be responsible for these differences in X-ray patterns. Hoover and Sosulski (1985) have shown that most legume starches are characterized by two very pronounced lines centered at 17.2 and 18.1° 2θ angles, which correspond, respectively, to interplanar spacing of 5.15 and 4.98 Å (1 Å = 0.1 nm).

Swelling Power and Solubility

Legume starches usually have restricted swelling behavior. Leach et al. (1959) indicated that legume starch swelling power is lower than that exhibited by wheat starch. Many workers have studied the swelling pattern of various legume starches; Lineback and Ke (1975), chick pea and horse bean; Lai and Varriano-Marston (1979), black bean, yellow pea, and navy bean; Lii and Chang (1981), red bean. They have observed the same single-stage pattern. Lai and Varriano-Marston (1979) indicated that bean starch had lower swelling power than wheat starch within the temperature range 60°C to 74°C; above 75°C, however, bean starch surpassed wheat starch in swelling power. The restricted swelling, plus the single-stage swelling pattern, have been interpreted to explain the crystalline and amorphous regions of starch granules and the presence of strong binding forces which relax at one temperature range. Reddy et al. (1984) suggested that the

swelling ability and solubility depend on starch source, temperature and pH. Lai and Varriano-Marston (1979), Comer and Fry (1978), and Sathe et al. (1981) reported that solubility of legume starches is less than 30%.

Gelatinization and Pasting

Starch, when heated in the presence of excess water, undergoes an order-disorder phase transition called gelatinization over a temperature range characteristic of the starch source (Donovan, 1979; Biliaderis et al., 1980). The above phase transition is associated with the diffusion of water into the granule and subsequent hydration and swelling of the starch granule. This endothermic reaction results in loss of crystallinity, decreased relaxation time of water molecules, and amylose leaching (Stevens and Elton, 1971; Lelievre and Mitchell, 1975; Donovan, 1979; Hoover and Hadziyev, 1981).

Most legume starches have gelatinization temperatures of 60°C to 90°C, with the exception of wrinkled peas which ranges over 99°C (Reddy et al., 1984). The gelatinization temperature ranges of various legume starches are presented in Table 2. The Brabender viscosity pattern of bean starches showed restricted swelling characteristics similar to those shown by chemically cross-linked starches (Schoch and Maywald, 1968; Lineback and Ke, 1975; Vose, 1977; Lai and Varriano-Marston, 1979; Naivikul and D'Appplonia, 1979; Lii and Chang, 1981; Sathe and Salunkhe, 1981). According to Schoch and Maywald (1968), these restricted swelling pastes are classified as Type C starches which show no pasting peak, but rather a very high viscosity which remains constant or increases during cooling and have relatively constant cold-paste viscosities during a holding period at 50°C. Kawamura and Fukaba (1957) classified legume starches

Table 2. Gelatinization temperature ranges reported for legume starches

Starch Source	Gelatinization Temperatures Range (°C)	Reference
Lima bean	70 to 85	Schoch & Maywald (1968)
Lentil	64 to 74	Schoch & Maywald (1968)
	58 to 61	Biliaderis et al. (1979)
Yellow pea	63 to 73.5	Schoch & Maywald (1968)
Navy bean	66 to 77	Schoch & Maywald (1968)
	68 to 74	Biliaderis et al. (1979)
Garbanzo bean	62.5 to 72	Schoch & Maywald (1968)
	65 to 71	Biliaderis et al. (1979)
Mung bean	60 to 78	Schoch & Maywald (1968)
	63 to 69	Biliaderis et al. (1979)
Wrinkled pea	69 to 83	Schoch & Maywald (1968)
	> 99	Biliaderis et al. (1979)
Black gram	71.5 to 74	Sathe et al. (1982)
Black bean	63.8 to 76	Lai & Varriano-Marston (1979)
Smooth pea	65 to 69	Biliaderis et al. (1979)
Red kidney bean	64 to 68	Biliaderis et al. (1979)
Faba bean	61 to 66	Biliaderis et al. (1979)
	61 to 69	Lorenz (1979)
Soybean (Amsoy 71)	73 to 81	Wilson et al. (1978)
Pea	54 to 66	Comer & Fry (1978)
Red bean	63 to 70	Lii & Chang (1981)
Adzuki bean	83 to 89	Biliaderis et al. (1979)

into two categories based on hot paste characteristics: 1) those which do not have a substantial rise in viscosity during heating (25°C to 92.5°C) and cooling (92.5°C to 25°C) cycles and 2) those which show a distinct rise in viscosity during heating and cooling cycles. Lai and Varriano-Marston (1979) studied the gelatinization temperature range of black bean, and reported temperature ranges of 63.8°C to 76°C, which is considerably higher than that of wheat starch, 55.6°C to 63°C. These results are similar to those reported for navy and mung bean starches according to Schoch and Maywald (1968).

Rosenthal et al. (1970) studied the pasting characteristics of jack bean and guandu bean starches and reported that there was no peak at low concentrations (up to 6%). At these low concentrations, no sensible retrogradation was observed and their viscosity curves were similar to cross-bonded starch. Vose (1977) studied the effects of acidic and basic conditions during pasting of double-milled air-classified pea flour. He found that at acidic pH values up to pH 4, there was a steady increase in viscosity during the pasting process, including a resistance to shear breakdown when 8% slurries were maintained at 96°C for 15 min; however, at pH 3 a pasting peak occurred, which was followed by a decrease in viscosity and then a set-back during cooling. Lii and Chang (1981) also observed that the different steeping solutions used during starch isolation did not affect the viscosity pattern. Sathe and Salunkhe (1981) indicated that the gelation of purified bean starch could yield a stable gel at concentrations of 7% or above (w/v).

Starch gelatinization and melting concept

Starch granules are not soluble in cold water, but can reversibly imbibe water and swell slightly. However, as the temperature is increased, the starch molecules vibrate

more vigorously, breaking the weaker intermolecular bonds (firstly in the amorphous area) and allowing their hydrogen bonding sites to engage more water molecules. This penetration of water, and the increased separation of more and longer segments of starch chains, increases randomness in the general structure and decreases the number and size of crystalline regions. Continued heating in the presence of abundant water results in a complete loss of crystallinity as judged by loss of birefringence. This process is called "Gelatinization" and the temperature at which gelatinization occurs is called the "Gelatinization Temperature". For a population of granules, the range for a gelatinization temperature is usually between 5-10°C, indicating that fractions of granules exhibit different gelatinization temperatures.

Gelatinization process also can be described as the melting of starch crystallites which should be amenable to thermodynamic analysis (Lelievre, 1973). Marchant and Blanshard (1978) postulated three constituent processes for starch gelatinization based on nonequilibrium thermodynamics: (1) diffusion of water into starch granules, (2) a hydration-facilitated helix-coil transition which is a melting process, and (3) swelling as a result of crystallite disintegration (melting). Using a light-scattering method to study melting and swelling processes during gelatinization, Blanshard (1979) reported that gelatinization may be described as a "Semicooperative process". Each granule has its own degree of crystallinity with its unique energy characteristics. The imposition of a 2°C temperature rise may result in certain granules being totally gelatinized but, in others, only some of the crystallites will have their gelatinization threshold exceeded. If the temperature increase is applied continuously, eventually the whole population of granules

will be gelatinized. Since the amorphous region hydrate initially, French (1984) has proposed that swelling of amorphous phase which occurs when starch is heated in excess water contributes to the disruption of the crystallite regions by tearing molecules from the crystallites.

Aside from swelling during gelatinization, the viscosity of the medium also increases. When the granule swells to the point that internal bonding can no longer maintain its integrity, it ruptures and the viscosity declines. During heating, amylose leaches from the granule and forms an extragranular network (Allen et al., 1977; Miller et al., 1973). Viscosity changes during the early heating stages appear to be due largely to this extragranular material. During the later stages when maximum viscosity is approached or attained the swollen granules and the extragranular material both contribute to apparent viscosity. Both the molecular and granular structures contribute to the increase in the viscosity. Based on the changes in the viscosity of starch in excess water system during and after heating, Olkku and Rha (1978) summarized the step of gelatinization as follows: (1) granules hydrate and swell to several times of their original size, (2) granules loss their birefringence, (3) clarity of the mixture increases, (4) marked, rapid increase in consistency occurs and reaches a maximum, (5) linear molecules dissolve and diffuse from ruptured granules, (6) upon cooling, uniformly dispersed matrix forms a gel or paste-like mass.

Method for determining starch gelatinization

Degree of gelatinization can be determined qualitatively and /or quantitatively by physical, chemical, and biochemical methods (Watson, 1964; Wong and Lelievre, 1982).

Birefringence End Point Method Starch granules show some degree of birefringence owing to a highly oriented nature of D-glucosyl units in amorphous and crystalline regions. Loss of birefringence is a characteristic of starch that undergoes thermal gelatinization. The measurement requires an optical microscope with crossed polarizing filters and a heating stage (Schoch and Maywald, 1956; Watson, 1964). Watson (1964) determined the degree of gelatinization by measuring the percent loss of birefrigence (2, 10, 25, 50, 75, 90, and 98%) of the granules in the field during uniform rates of heating. The 98% point is taken as the gelatinization temperature end-point. In practice, usually only initial gelatinization and 98% loss temperatures are recorded. Watson's method was modified by Berry and White (1966) to follow the progress of the gelatinization by recording the light output on a photocell as a function of hot stage temperature. This method is often used to determine the degree of gelatinization, because of its simplicity in equipment and application. It is limited, however, to dilute granule suspensions (0.1 - 0.2%).

Viscosity Method In common practice, the most widely used method for determining the degree of gelatinization is based on changes in viscosity during gelatinization. A Brabender Visco/amylo/Graph, the most commonly used instrument, provides information not only on gelatinization but also on the properties of the paste during cooling. This instrument records the torque required to balance the viscosity that develops when the starch slurry is subjected to programmed heating and cooling cycles. The temperature at which the first major rise in viscosity occurs is highly dependent on starch concentration and is generally higher than gelatinization temperatures obtained by

loss of birefringence. Viscosity is measured in arbitrary units that reflect paste consistency; accordingly, Brabender Viscoamylograph results are described in term of paste properties and pasting temperatures at given time. This methodology is highly empirical and thus, reported data obtained by this technique should include instrument model, torsion spring used, bowl speed, volume of slurry, slurry concentration (including basis), and start, hold, and final temperatures.

Freeman and Verr (1972) observed the gelatinization process and paste development of starch slurry with the simple rotational viscometer, Brookfield Synchro Lectric viscometer. However, this technique is quite tedious compared to Brabender Viscoamylograph because it lacked a temperature control system. A more sophisticated rotational viscometer with programmable temperature control system, Haake Rotovisco is becoming increasingly used for studying gelatinization process, paste development and rheology of the paste (Voisey and deMan, 1976; Lee et al., 1995).

X-Ray Diffraction Method In addition to providing information about the crystal structure of the crystallites of starch granules, x-ray diffraction can give information about the relative amounts of crystalline and amorphous phases. Therefore, it can be used as a tool to measure the extent of gelatinization (Lugay and Juliano, 1965; Varriano - Marston et al., 1980; Owus-Ansah et al., 1982).

X-rays are a form of electromagnetic radiation with a wavelength typically between 0.1 and 1.0 nm, which is comparable to the molecular spacing in a crystal. When an x-ray beam impinges on a crystal which is held in a special mount that allows the crystal to be rotated with respect to the incident beam, diffraction occurs. Diffraction is the

phenomenon that occurs whenever a wave motion interacts with an obstacles. The diffracted beams are recorded to obtain information on the structure of the crystal and the molecules within the crystals. In x-ray analysis of starch granules, satisfactory results are obtained if the specimens are properly prepared and mounted. Coarse powder gives less intense pattern. Therefore, the samples have to be less than 200 mesh inside and packed as densely as possible into a sample holder. The finished surface must be smooth and flush with the face of the sample holder. Upon recording the diffracted beams, the patterns are analyzed based on their interplanar spacings and relative intensities of the diffraction lines.

Native starches are classified according to their wide-angel X-ray diffraction patterns, as shown in Figure 1, as A-, B-, or C-type starches (Zobel, 1988; Biliaderis, 1991a). An additional diffraction pattern known as V-type crystallinity corresponds to

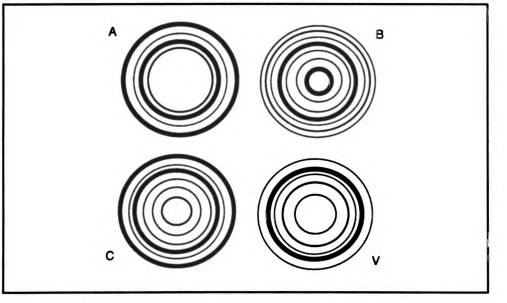


Figure 1. Schematic representation of X-ray diffraction patterns of various starches use for their classification into the A-, B-, C-, and V-type starches (Zobel, 1988)

structures of helical inclusion complexes of amylose, which is typical of amylose-lipid complexes (Biliaderis, 1991a). The A-type pattern is exhibited by cereal starches (rice, wheat, and corn); the B-type pattern is shown by tuber, fruit and high-amylose corn starches and by retrograded starch. C-type pattern starch is an intermediate between A- and B- type starches and it is typical of legume seed starches (e.g., pea and bean starch). The V-type structure has not been found in native cereal starches, but it may form due to heating of lipid-containing starches (Biliaderis, 1991b).

Amylose / Iodine Blue Value Method Amylose complexes with iodine to yield brilliant blue color and this characteristic has been used as an analytical tool to measure amylose content. A quantitative method to determine the amount of amylose present in solution base has been developed by McCready and Hassid (1963) and modified by Gilbert and Spragg (1964). The interaction of amylose with iodine generates a helical inclusion complexes in which the iodine molecules occupy the central cavity of the helical polysaccharide. The helix consisted of six glucose residues per turn, with a pitch of 8Å. The absorbance of the blue color is measured with a spectrophotometer at 600 nm. Application of amylose / iodine blue complex method to determine the degree of gelatinization has been used in many food products. Roberts et al. (1954) utilized this method to determine an index of parboiling of rice. Birch and Priestly (1973) modified their method for parboiled rice by treating with alkali solution to distinctively differentiate degree of gelatinization. Wootton et al. (1971) also used amylose / iodine method to determine degree of gelatinization in biscuits.

Differential Scanning Calorimetry (DSC) Method The term "Differential Scanning Calorimetry" was initially a source of some confusion in thermal analysis. The parent technique to DSC is "Differential Thermal Analysis (DTA)". The purpose of differential thermal systems is to record the difference between an enthalpy change which occurs in a sample and that in some inert reference material when both are heated.

The important difference between DTA and DSC is that in DSC the sample and reference are each provided with individual heaters which allows the determination to be conducted with no temperature difference between sample and reference. Running in this mode allows two important simplifications compared to DTA. First, because the sample and reference pans are maintained at the same temperature, the calibration constant for the instrument is independent of temperature. Obviously, this greatly simplifies the experimental technique since the calibration constant need only be determined for one standard material. Second, since the sample and reference pans have independent heater, the difference in heat flow to the sample and reference in order to maintain equal temperature can be measured directly. Thus, data are obtained in the form of differential heat input (dH/dt) vs temperature (or time, since constant heating rate are used). These data are readily used to obtain temperature and an enthalpy of transitions or reactions.

For optimum peak sharpness and resolution, the contact surface between pan and sample should be maximized, which is usually accomplished by having the sample as thin discs, or film, or fine granules. Frequently, in applications in foods, the sample will be dispersed or soluble in water, which obviates any problem with contact surface. Calibration of the instrument is generally carried out with a high purity metal with

accurately known enthalpy of fusion and melting point. The most commonly used calibrant is indium (ΔH = 6.8 cal/gm, mp = 156.4°C).

Stevens and Elton (1971) applied DSC to measure heat of gelatinization of starches, and since then many groups have applied DSC in the study of gelatinization (Longston and LeGrys, 1981; Ghiasi et al., 1982; Raemy and Loliger, 1982). Banks and Greenwood (1975) suggested this method was valid by considering gelatinization to be the analogue of a melting process for a crystal.

Laser Light Scattering Method The use of polarize laser-light scattering is a relatively new technique for following changes in the internal structure of starch granule during gelatinization. Since the anisotropic scattering indicates a spherulic organization (French, 1984). Many investigators (Marchant et al., 1977; Marchant and Blanshard, 1978, 1980a, 1980b) have successfully utilized this technique to observe the changes of starch granule during gelatinization.

Other Methods Other methods in addition to those previously described are available, e.g., enzymatic digestibility, nuclear magnetic resonance, light-extinction, solubility or sedimentation of swollen granules, and absorption of congo red measurement. However, these methods are less popular due to the difficulties in their procedures or degree of accuracy in measuring the degree of gelatinization.

Factors affecting gelatinization process within food systems

Time and Temperature Many investigators have studied the influence of time and temperature on the extent of starch gelatinization in order to design the efficient starch cooking systems and / or to optimize both process and product quality.

Most kinetic studies on starch gelatinization were performed under isothermal conditions. Since the temperature can then be considered as an independent variable, and the time dependence can be determined. In practice, particularly in processing of food constituents, processes to promote desirable reactions are not accomplished isothermally, but the product undergoes a real temperature change (thermally dynamic) and there is a significant lag time within the product. Lund (1984) proposed in his work and cooperated with Wirakartakusumah (1981) on the isothermal and nonisothermal kinetics of starch gelatinization:

$$\text{water + ungelatinized starch} \xrightarrow[\text{heat}]{} \text{gelatinized starch}$$

The reaction is reversible, and under isothermal conditions the form of the rate expression is:

$$r = -\frac{d[UG]}{dt} = k\,[H_2O]^m\,[UG]^n$$

where m, n = order of reaction for water and UG, respectively
k = the reaction rate constant
UG = ungelatinized starch.

If excess water is present, the reaction rate constant k and $[H_2O]^m$ can be combined into an apparent or empirical pseudo rate constant Ka.

$$\frac{d[UG]}{dt} = -Ka\,[UG]^n$$

For n = 1, a first order reaction, the integrated form is

$$\ln \frac{[UG_t]}{[UG_i]} = Ka\,t$$

where UG_i = initial amount of ungelatinized starch at time t
UG_t = final amount of ungelatinized starch at time t

The expression $[UG_t] / [UG_i]$ is unity when gelatinization reaches 100 % completion provided the time is sufficient at any given temperature. However, if the extent of starch gelatinization is temperature dependent (i.e., the starch is not complete gelatinized at a given temperature even if held an infinite time), the argument $[UG_t] / [UG_i]$ must be modified to describe the extent of reaction.

$$\ln \frac{[UG_t - UG_u]}{[UG_i - UG_u]} = Ka\, t$$

where UG_u = ultimate amount of ungelatinized starch at the given temperature

Then two procedures were used to determine the reaction rate constants. The isothermal method uses data generated by measuring the extent of reaction as a function of time at a given constant temperature. The second approach is based on the heat evolution or temperature scanning method using DSC data. The results indicate that the ultimate extent of gelatinization is directly dependent on heating temperature. Furthermore, that a significant degree of gelatinization has been accomplished at the end of lag time. However, this methodology cannot describe the rate of gelatinization over the entire range of no gelatinization to maximum extent of gelatinization. This would suggest that the process of gelatinization is complex and not readily described by pseudo first order kinetics. This observation is consistant since there is a diverse population of starch granules, each with its unique degree of crystallinity. The process can be empirically

modeled but interpretation of mechanism from the model should be done with great care. Pravisani et al. (1985) examined the kinetics of starch gelatinization in potato and confirmed the previous studies that at temperatures below certain thresholds, gelatinization was not completed even for long reaction time.

Moisture At temperature above that for complete gelatinization, several studies have shown that there is a minimum water to starch ratio (W/S) in order to achieve complete gelatinization (Wootton and Bamunuarachchi, 1979; Eliasson, 1980; Marchant and Blanshard, 1980).

Wirakartakusumah (1981) studied the influence of moisture on starch gelatinization using DSC and followed the changes in enthalpy of various W/S of rice starch suspension at temperature above the gelatinization temperature (150°C, at 10°C / min). At W/S ratio ≥ 2:1, only two endotherms are obtained. The first endotherm is observed at an onset temperature (To) of about 71°C. This single endotherm is characterized as the gelatinization phase change. In addition to the first endotherm, the second endotherm is obtained in the temperature range between 90 - 110°C. This higher temperature endotherm is associated with melting of an amylose-lipid complex. A similar type of endotherm which occurred at 100°C was also reported by Kuimiya et al. (1980) from maize and wheat starches. As the W/S ratio decrease to less than 1.5:1, the gelatinization endotherm develop a trailing shoulder with a marked decrease in the area (enthalpy). This shouldering may be due to the destabilizing effect of water and heat treatments on the amorphous and crystalline region of starch granules. The onset

temperature (To) and temperature range of gelatinization shift to higher temperature as the water content decreases.

Wirakartakusumah (1981) also reported that more than 3 moles of water / hexose unit are required to start gelatinization of rice starch and, about 13.5 moles of water / hexose unit are required to complete gelatinization. These values are in good agreement with the data reported by Donovan (1979) on potato starch. Therefore, it is concluded that a critical amount of water is necessary to initiate and to complete gelatinization.

Ingredients

Surfactants and lipids It is well established that physical characteristics of starch pastes may be radically altered by the presence of polar substances containing large hydrophobic groups. Practical instances of utilizing these effects include: (1) addition of sulfonated oils to textile starch sizes as "softeners" and antiskining agents, (2) use of soaps to modify viscosity of starch sizing in paper coatings, (3) the former application of polyoxyethylene monostearate as a bread softener, (4) use of monoglyceride to alter the paste consistency of dehydrated potato flakes, and (5) use of glycerol monostearate and other mono- and di-glycerides to prevent stickiness in cooked rice kernels. These effects are due primarily to the formation of a complex between the fatty adjunct and amylose, the linear fraction of starch polymers (Larsson, 1980: Marringat and Juliano, 1980; Ohashi et al., 1980; Eliasson et al., 1981a,b; Hoover and Hadziyev, 1981; Ghiasi et al., 1982). It is postulated that the use of pork back fat pieces in "pork and beans" has a direct impact on the starch characteristics of navy beans during canning.

Gray and Schoch (1962) performed a definitive study on the effect of nine fatty adjuncts on swelling and solubilization characteristics of starches from various sources. As a broad generalization, lower polar adjuncts containing four or five carbon atoms (e.g., butyl and emyl alcohols) markedly assist the hydration of corn starch when cooked in water, causing a substantial reduction in the gelatinization temperature and an increase in the extent of solubilization. Fatty adjuncts containing a hydrocarbon residue of about 12 carbon atoms tend to complex with the amylose below 85°C, consequently repressing swelling and solubilization. This complex breaks down at higher temperatures, and the surfactant may thereafter assist swelling. Compounds containing a hydrocarbon residue of 18 carbon atoms (stearate) generally appear to form complexes which are stable at least up to 95°C, and hence repress swelling and solubilization over the entire gelatinization temperature ranges.

The time of addition and time for diffusion of adjunct into the granule are also very important and can cause substantial differences in properties of the cooked starch or cereal grains. If the adjunct is allowed to diffuse into granule significantly, swelling and solubilization may be greatly reduced. Whereas, if the adjunct is added immediately before cooking, the amylose-lipid complex will largely form extragranularly and the adjunct will have little effect on swelling and solubilization.

Sugars, salts and other solutes Ionic and nonionic soluble constituents affect gelatinization characteristics of starches. Wootton and Bamunuarachchi (1980) presented a study on the effect of sucrose and NaCl on wheat starch gelatinization. However, the situation regarding the influence of sugar and salt on starch gelatinization is not quite

clear. Much of the previous research dealt with low starch concentration and, hence, makes correlation between the earlier studies difficult and some of these techniques are more suited to qualitative, rather than quantitative, interpretation.

Table 3 demonstrates the results of Wootton and Bamunuarachchi (1980) on ΔH_G (enthalpy of gelatinization), the extent of gelatinization and temperatures at which the endotherms began (To) reached their peak (Tp), and concluded (Tc), for wheat starch / water systems containing various levels of sucrose in the aqueous phase. This can be seen that To and Tc were not affected by increased levels of sucrose while Tp was increased. These results agreed with the findings of earlier workers, who indicated that increasing sucrose concentration caused increases in pasting temperatures corresponding with the change in Tp. From the lack of change in To and Tc, it appears that while the peak temperature of the gelatinization endotherm rises with increased sucrose concentration, the range over with the process extends is not affected by the concentration of the solutes.

Sucrose has a restrictive effect on the heat of gelatinization. This effect is widely known and several explanations for this phenomenon have been proposed including

Table 3. Effect of sucrose concentrations on the gelatinization endotherm of wheat starch

Sucrose conc. in aqueous phase (%)	ΔH_G (cal / g)	Extent of Gelatinization (%)	Endotherm temp. (°C)		
			To	Tp	Tc
0	4.7	100	50	68	86
15	3.2	68	50	70	86
30	2.8	60	50	73	86
45	2.3	49	50	75	86

From Wootton and Bamunuarachchi (1980)

competition between starch and sucrose for available water, sucrose inhibition of granular hydration, and sucrose-starch interactions. From the review paper of Lund (1984), he suggested that the actual mechanism by which sucrose restricts the gelatinization of starch is not simply lowering available water in proportion to the level of sucrose present in the system. If it is, then a linear relationship between a ΔH_G and the sucrose level in the aqueous phase may be expected however the relationship between these two parameters is not linear (Table 3).

Since the rising cost of sucrose and the increased availability of corn syrup and high fructose syrup, many investigators have studied the effects of different sugars on starch gelatinization (Koepsel, 1977; Hoseney et al., 1978; Savage and Osman, 1978). They concluded that monosaccharides delay gelatinization less than disaccharides, except maltose, which acted like a monosaccharide. By using DSC, Spies and Hoseney (1982) reported that the gelatinization-delaying characteristics of a sugar solution were related to the water activity of the solution and to the molecular size of the sugar. As the water activity of a sugar solution decreases, gelatinization temperature of starch in that solution increases. But at equal water activities, sugars with longer chain length delay gelatinization more than do shorter chain sugars.

Evans and Haisman (1982) examined starch gelatinization in the present of nonionic constituents and found that the effect of temperature and extent of gelatinization could be explained through solute effect on available water and melting point. The influence of NaCl on starch gelatinization was also studied by Wootton and Bamunuarachchi (1980). As shown in Table 4, salt has a different and more complex

effect on starch gelatinization than does sucrose. Changes in salt concentration affected all three temperatures associated with the gelatinization of the endotherm. A variation in the temperature range over which the gelatinization endotherm extends also occurs with added salt. Evans and Haisman (1982) reported that other salts such as sodium sulfate and sodium hydrogen phosphate (pH 7.0) increase gelatinization temperature, whereas $CaCl_2$ decreased and then increased gelatinization temperature with increased concentration. Oosten (1982) proposed a hypothesis to explain this phenomenon. His hypothesis is that starch acts as a weak acid ion exchanger and that cations tend to protect and to stabilize the granule structure while anions function as gelatinizing agents by rupturing hydrogen bonds.

Table 4. Effect of salt concentrations on the gelatinization endotherm of wheat starch

Salt conc. in aqueous phase (%)	ΔH_G (cal / g)	Extent of Gelatinization (%)	Endotherm temp. (°C)		
			To	Tp	Tc
0	4.7	100	50	68	86
3	2.7	57	58	71	88
6	2.5	53	64	75	88
9	2.6	55	68	78	88
12	2.7	57	65	77	88
15	2.7	57	65	77	88
21	2.8	60	61	80	90
30	3.3	70	59	79	91

From Wootton and Bamunuarachchi (1980)

Hydrocolloids Hydrocolloids have been used extensively in food products to modify texture, improve moisture retention, control water mobility, and maintain overall product quality during storage. Because starch is a basic ingredient of so many foods, the

study of changes within starch granules and pasting properties in the presence of gums were needed to extend knowledge of the function of starch in starch/gum based products.

Christianson et al. (1981) studied the gelatinization of wheat starch as modified by Xanthan gum, Guar gum and Cellulose gum. The results indicated that the gum hasten the onset of initial paste viscosity and substantially increase final peak of viscosity of wheat starch. Further investigation in hydrocolloid interaction with starches, Christianson (1984) proposed the two possible reasons why gums enhance the effect of gelatinization. First, there may be interaction between exudate from the granule (solubilize amylose and low molecular weight amylopectin) and the gums. Second, the addition of thickening gums would mean that the forces being exerted on the granules in the shear field are much larger than those encountered in starch-water suspensions of equal starch concentration. These increased forces should significantly affect granule breakdown and the amount of material exudate into medium.

Processing procedures Treatment of starch and cereal grains prior to cooking can significantly alter cooking and / or gelatinization characteristics (Gough and Pybus, 1971; Donovan et al., 1983; Lorenz and Kulp, 1979, 1981, 1982; Lund, 1984).

In processing of cereal grains, perhaps the most widely known pretreatment is parboiling of rice. Generally, parboiling consists of two processing operations: soaking and / steaming. During soaking, rice is hydrated so that sufficient water is present to allow subsequent gelatinization. During steaming, gelatinization and solubilization of starch occur which contributes to the final quality of milled rice. The gelatinization characteristics of the remaining ungelatinized starches in the intact rice kernels are altered

as a result of the parboiling process. The parboiled rice tends to gelatinize over a temperature range that is both broader and lower than the untreated rice. This indicates that the starch granules are damaged and destabilized in the previous parboiling treatment, but the crystalline structure have not been disordered completely (Lund, 1984).

Soaking and / or steeping are the indispensable steps to provide sufficient moisture in the grain for gelatinization or facilitating further processing steps. Usually moderately high water temperature (50-60°C) is used to hasten the hydration process. Gough and Pybus (1971) observed an interesting characteristic of gelatinization for wheat starch when it was steeped for 72 hr at 50°C. The treatment changed and narrowed the temperature range for gelatinization from 52 to 61°C before steeping to 65.4 to 65.8°C after steeping. However, there was no change in the x-ray pattern between the raw and treated starches. Similar studies conducted by Lorenz and Kulp (1979) for wheat starch, Wirakartakusumah (1981) for rice starch steeped at various times and temperatures confirmed the previously reported data.

The occurrence of increasing and narrowing the range of temperature for gelatinization during steeping has been thought to be similar to the annealing process of semicrystalline polymers. In synthetic polymers, exposure to an appropriate temperature and solvent environment causes a spontaneous ordering of the polymer molecules. The ordering process is known as annealing and generally occurs at a temperature near the melting point of the polymer. Since starch is a semicrystalline material consisting of amorphous and crystalline regions, holding a suspension of granules just below gelatinization temperature would be expected to give rise to more perfectly ordered

crystals with higher melting points. The increase in crystallinity level was confirmed by x-ray examination, according to Ahmed and Lelievre (1978). Another processing procedure which is known as "Heat-Moisture Treatment" also causes changes in gelatinization properties, physicochemical and functional properties of starch. This treatment consists of starch granules with limited moisture content exposed to excessive high temperature for a period of time. Lorenz and Kulp (1981) reported that the "heat-moisture treatment" causes an increase in the bread-baking potential of potato starches. Further, Lorenz and Kulp (1982) studied the effect of the "heat-moisture treatment" on tuber starches (arrowroot and cassava). They reported that the physical properties of treated tuber starches approached those of wheat starch. X-ray diffraction studies also show the change of B-pattern of tuber starches toward an A-pattern of cereal starch. Donovan et al.(1983) reported that the heat-moisture treated potato and wheat starches showed a boardening of the gelatinization temperature range and a shifting of the endothermal transition toward higher temperatures, compared to untreated starches. The interpretation of these results was also given to the effects of recrystallization and the perfection of the small crystalline regions of the granule. Changes in gelatinization properties by either annealing or heat-moisture treatments could have significant effect on cooking characteristics of starch. This area warrants further investigation.

Retrogradation

Starch granules, when heated in excess water above their gelatinization temperature, undergo irreversible swelling, resulting in amylose leaching into solution. In the presence of a high starch concentration, this suspension will form an elastic gel on

cooling. The molecular interactions that occur after cooling have been called retrogradation. These interactions are found to be both time and temperature dependent. Miles et al. (1985b) showed, with the aid of physical techniques, that amylose gelation occurs as a result of a phase separation, which produces regions that are rich and deficient in polymer and that, if the amylose concentration is sufficiently high, the region rich in polymer forms a three dimensional network.

Amylose crystallization was found to be a secondary process, occurring in the region rich in polymer. Miles et al. (1985b) and Ring et al. (1987) attributed the initial gel firmness during gelation to the formation of an amylose matrix gel and the subsequent slow increase in gel firmness to amylopectin crystallization. Heterogeneous acid hydrolysis of waxy maize amylopectin gels, followed by gel permeation chromatography studies on the residue, revealed that amylopectin crystallization occurs by association of amylopectin molecules with DP $\leq$ 15 (Ring et al., 1987). The crystallization of amylopectin was shown to be reversible at temperatures below 100°C, whereas the initial gelation and crystallization of amylose was irreversible even at 100°C (Miles et al., 1985b; Ring et al., 1987). This indicates a greater degree of molecular interaction in the latter. X-ray diffraction and shear modules studied on various starches showed that the initial rates of development and stiffness of gels were sequenced among starch sources as follows: smooth pea > maize > wheat > potato; while the long-term increase followed the order: smooth pea > potato > maize > wheat (Orford et al., 1987). The latter process was found to be more important at high starch concentrations. Differences in the fine structure of amylopectins, and also in the extent of association between starch components, may

account for the above observed retrogradation tendencies. The freeze-thaw stability of starch gels is an important characteristic of food starches. This stability is determined by gravimetric measurement of the water exuded (syneresis) from a gel after it has been frozen and thawed. This exudation occurs owing to the reassociation of linear starch molecules (retrogradation). This high retrogradation tendencies of legume starches was also shown by Hoover and Sosulski (1985) and Tjahjadi and Breene (1984) by measurement of the degree of syneresis during low temperature storage. The high degree of syneresis makes native legume starches unsuitable for use in foods requiring low temperature storage. However, modification by acetylation and hydroxy-propylation was found to significantly decrease the extent of syneresis to levels that may be acceptable to food processors (Hoover and Sosulski 1986; Hoover et al. 1988).

Nutritional Attributes of Dry Bean Starch

Dry legumes are dietary staples in many parts of the world and are important dietary sources of protein, complex carbohydrates (starch and dietary fiber), other essential nutrients, and energy. The digestion of starch in dry legumes is lower than in cereal grains and many other foodstuffs. Factors contributing to decreased digestibility include: 1) increased dietary fiber intake; 2) intact cell walls which hinder the digestive enzymes from gaining access to the starch and protein; 3) residual lectin activity; 4) limited proteolysis of certain protein sub-fractions; 5)incomplete hydration of the starch granule; and 6) amylose retrogradation. Slow digestion of starch is beneficial since it reduces hyperglycemia and hypertriglyceridemia which are risk factors in the development of cardiovascular disease. However, it is likely that slow digestion of starch and retrograded amylose are major contributors to the flatulent problem frequently associated with legume consumption. The challenge to the food processor and plant geneticist is to produce legumes that continue to provide a low glycemic index and a good source of fiber with improved protein digestibility and decreased flatulence. However, producing a low glycemic, fiber rich food with minimal flatulence may be mutually exclusive.

Factors Affecting Digestion of Starch

A factor that affects digestion of both legume proteins and starches is the physical form of the protein and starch when it enters the stomach and small intestine. Grains and root vegetables are usually ground and/or cooked prior to consumption. Grinding and cooking serve to break the cellular structures and to release the protein and starch so that proteolysis and amylolysis can occur. Legumes are generally consumed as whole seeds

that have been cooked. Cell wall structures in cooked beans are intact and surround the protein bodies and starch granules. Chewing macerates some of the cells, but most of the cells remain whole and enter the digestive tract intact. Thus, the cellular structures render legume protein and starch less available to the digestive enzymes except for the small amounts of starch that leaches from the cells (Würsch, et al., 1986; Golay, et al., 1986).

Another factor affecting digestion of both protein and starch is the amount of total dietary fiber in the diet. *Phaseolus vulgaris* beans typically contain 17.5 - 20% dietary fiber and are regarded as an excellent source of dietary fiber (Prosky, et al., 1985; Cummings, et al., 1985; Anderson and Bridges, 1988). As an individual increases dietary fiber intake, nutrient digestibility and/or absorption tends to decrease (Kies, 1981; Miles, et al., 1988). Decreased digestibility of protein and starch is presumably due to physical entrapment and/or premature release of food particles from the stomach, although direct inhibition of digestive enzymes by fiber cannot be excluded (Schneeman, 1982). Dietary fiber can encase nutrients in a fibrous matrix which impedes digestive enzymes from gaining access to protein and starch and fiber can reduce absorption of digested protein and starch by slowing or preventing diffusion of the digestive products to the mucosal cells (Southgate, 1973; Johnson and Gee, 1981; Flourie, et al., 1984; Elsenhaus, et al., 1981; Vahouny, et al., 1981). Food is normally held in the stomach until it is reduced to particles less than 1 mm (Meyer, et al., 1979; Meyer, et al., 1981); but fiber can cause the premature release of large particles from the stomach (Meyer and Doty, 1988). Large particles represent another type of physical entrapment which limits digestion (Williams, et al., 1984; Doty and Meyer, 1988). The many possible effects of fiber on nutrient

bioavailability have been thoroughly reviewed (Vahouny and Kritchevsky, 1982; Trowell, et al., 1985; Vahouny and Kritchevsky, 1986).

A potential factor which could limit protein and starch digestion is the presence of naturally occurring protease and amylase inhibitors. During processing and cooking, most of these inhibitors are inactivated; however, residual levels of active inhibitors may remain, particularly in improperly cooked beans. It is reported that for individuals accustomed to consuming beans with residual levels of inhibitors, the pancreas enlarges and secretes more digestive enzymes to compensate for the enzymes which have been inactivated by the inhibitors (Schneeman and Lyman, 1975; Madar, et al., 1976). As a result of pancreatic hyperplasia and hypertrophy, adequate amounts of digestive enzymes are secreted and digestion is not limited. The best evidence to support this conclusion is based on the experience with exogenous amylase inhibitors ("starch blockers"). Several groups demonstrated that chronic consumption of amylase inhibitors was ineffective in reducing starch digestion and caloric utilization (Savaiano, et al., 1977; Granum and Eskeland, 1981; Bo-Linn, et al., 1982; Carlson, et al., 1983; Garrow, et al.,1983). Essentially the same results are found with continued consumption of protease inhibitors (Madar, et al., 1976).

Legumes contain lectins which can potentially reduce nutrient digestion and absorption. Cooking beans will inactivate much of the lectin activity in raw beans; however, fully cooked beans can still contain a significant amount of the original lectin activity (Thompson, et al., 1983; Rea, et al., 1985). The deleterious effects of raw beans or comparable levels of isolated lectins are well documented (Sgarbieri and Whitaker,

1982; Liener, 1986). But, it is difficult to measure the effect of residual lectins on digestion and absorption. Data provided by Donatucci et al. (1987) strongly suggest that small quantities of lectins are sufficient to reduce absorption of glucose and presumably amino acids and small peptides. There maybe sufficient residual lectin activity in cooked beans to cause at least a small decrease in protein and starch digestibility.

Digestibility of Legume Starch

Legume starch is digested more slowly than starch from most other sources. The rate of *in vivo* starch digestion is illustrated by the rise in blood glucose and insulin following the consumption of starch. The "glycemic index" has been developed to aid dietary treatment of diabetics (Jenkins, et al., 1982) and is defined as:

$$\frac{\text{glucose response curve for a food}}{\text{glucose response curve for the equivalent amount of glucose}} \times 100$$

Canned beans have a low glycemic index compared to other food groups. Thus, bean starch is digested more slowly than starch from most other foods. The importance of the glycemic index is that elevated blood glucose promotes triglyceride and cholesterol synthesis by the liver which results in more very low density lipoproteins (triglycerides and cholesterol) being secreted into the blood (Anderson, et al., 1984; Jenkins, et al., 1985; Thompson, 1988). Also, elevated blood glucose promotes glycosylation of proteins in the vascular system (Jenkins, et al., 1988). The net effect of elevated blood lipids and unwanted protein glycosylation is an increased risk of cardiovascular disease. Thus, consumption of legume starch reduces the risk of premature cardiovascular disease

compared to consuming starch with a high glycemic index. While slow starch digestion (a low glycemic index) is desirable, it is undesirable for the starch to be digested too slowly since there will be incomplete digestion in the small intestine. Undigested starch will pass to the colon where rapid fermentation will take place and flatulence will result.

Two major factors affect the rate and perhaps the extent of starch digestion. The first factor is gastric emptying time and the second factor is accessibility of the glycosidic bonds in starch to pancreatic amylase and other digestive enzymes. If the rate of starch digestion is constant, starch which is released from the stomach slowly and over an extended time period will be digested and absorbed more slowly than when gastric emptying is rapid and over a short time period. There is a strong negative correlation between postprandial changes in blood glucose and gastric emptying (Mourot, et al., 1988). Likewise, if gastric emptying is constant, starch digestion rate determines how high the blood glucose rises in response to starch ingestion. The digestion rate of starch strongly influences the glycemic response to different foods (Thompson, 1988; Jenkins, et al., 1982). The glycemic index obviously reflects both gastric emptying and starch digestion rate.

Legume starch digestion rate is affected by conditions which slow or prevent digestive enzymes from gaining access to the glycosidic bonds of starch. Much of the legume starch reaches the stomach and small intestine within intact cell walls. Thus, the fiber matrix of cell walls is the first parameter to hinder starch digestion since amylase must penetrate the cell wall before amylolysis can proceed. Another parameter affecting digestion rate is the hydration state of the starch; starch must be hydrated to be digested.

Many types of starch, including legume starch, must be gelatinized to hydrate the starch and the extent of gelatinization often determines the rate of starch digestion (Holm, et al., 1988; Daniel and Whistler, 1985). When beans are cooked, the starch is not fully hydrated (Golay, et al., 1986) and there are data which suggest that some of the starch still retains its bifringence (Lai and Varriano-Marston, 1979; Hahn, et al., 1977; Varriano-Marston and DeOmana, 1979). It appears that more energy is required to break the bonds between starch chains in legume starch than in most other types of starch (Biliaderis, et al., 1981; Hoover and Sosulski, 1985a, 1985b).

Another factor which limits starch digestibility is the tendency for some starches to retrograde. When gelatinized starch cools slowly, some of the water between the starch chains is squeezed out and hydrogen bonds within or between starch chains reform. Amylose molecules with a degree of polymerization (DP) between 200 and 1200 glucose units, have a high tendency to retrograde to crystalline "beta sheet" type configurations. The average DP for legume amylose is DP 1000 - 1400 (Biliaderis, et al., 1981a), which is highly conducive to formation of retrograded crystalline starch structures. Retrograded amylose is indigestible within the small intestine (Englyst and Cummings, 1987). Legume starch has a higher percentage of amylose than most starches which increases the potential for formation of indigestible retrograded starch. During cooking some of the amylose leaches from the starch granule. Since the cell structure in cooked beans remain intact, much of the leached amylose remains within the cell and fills spaces between protein bodies and starch granules. If amylose retrogradation occurs, another indigestible matrix

(similar to fiber in cell walls) in and around the protein and starch granules is formed. This retrograded amylose matrix is another factor which will slow starch digestion.

Indigestible Starch

It is feasible that normally digestible carbohydrates (such as starch) may not be completely digested if the organism lacks sufficient digestive capacity relative to the amount or type of carbohydrate ingested. This may be due to consumption of carbohydrates which are less accessible or resistant to hydrolysis, a deficiency in the hydrolyzing capacity of the individual (inherited or temporary), the result of insufficient reaction time to digest and absorb the carbohydrate properly, or the result of a too rapid food transit (Hellendoorn, 1978).

Stephen (1983), using starch mixtures from navy beans, rice and potatoes, directly measured the passage of carbohydrates by inserting an aspirator at the human ileocecal junction. It was observed that after serving meals of different proportions to subjects, that the percent starch could be recovered at ranged from 2.3 to 20.1% (mean of 9.3%) for smaller meals, and 2.2 to 10.9% (mean of 6.0%) for the larger meals. The conclusion drawn from this work was that 2 to 20% of the dietary starch escaped absorption in the small intestine.

Starch digestibility may also be dependent on cooking time, preparation of dry beans or the type of starch itself. Accordingly, 5 to 15% of bean starch can remain indigestible even after prolonged cooking (Hellendoorn, 1969). Faki and Bhavanishangar (1983), using *in vitro* and *in vivo* studies, demonstrated that apart from oligosaccharides, the starch and hemicelluloses of chickpea, cowpea and horse gram contributed

substantially to the total flatulent effect. Roasting or boiling were ineffective in reducing flatulence, but removal of oligosaccharides and hemicelluloses by preliminary water soaking and sieving followed by precipitation of protein resulted in a product significantly non-flatulent as well as non-nutritive.

All starch resisting digestion in the small intestine is subjected to bacterial fermentation in the large intestine with the subsequent production of volatile fatty acids. Most of the starch which reaches the colon is not totally resistant to pancreatic amylase, but its hydrolysis is retarded so that it is not completely digested during its passage through the small intestine . The reasons for this incomplete digestion are separated into intrinsic (physical inaccessibility, resistant starch granules and retrograded starch) and extrinsic factors.

Physical inaccessibility occurs when starch is contained within undisrupted plant structures such as whole or partly milled grains and seeds. The cell walls may entrap starch and prevent its complete swelling and dispersion(Würsch, et al., 1986) thus delaying or preventing its hydrolysis with pancreatic amylase in the small intestine. It has also been observed that after a meal of sweetcorn, peas and beans, up to 20% of fecal solids may be starch contained in recognizable, undigested food (Englyst, 1985).

The actual crystalline structure of the starch granule is suggested to depend on the chain length of amylopectin. A-type starch is the normal pattern for cereal starch granules, B-type is typical of potato, amylomaize and retrograded, and C-type (combination of A and B patterns) is characteristic of certain pea and bean starches. In general, starch granules showing X-ray diffraction patterns B or C tend to be the most

resistant to pancreatic amylase, though the degree of resistance is dependent on the plant source (Fuwa et al., 1980; Englyst and Cummings, 1990). Cooking disrupts the granules and facilitates the hydrolysis of the starch contained within them. Crystallization at higher temperature and lower water content will favor the formation of A pattern, and lower temperature and high water content, the B pattern. On cooling, gelatinized starchy foods will retrograde, solubility of the starch molecule decreases and so does its susceptibility to hydrolysis by acid and enzymes.

The extent of crystalline bonding in amylopectin is limited by the branch length. Therefore amylopectin retrogrades to a lesser extent than amylose, the retrograded amylopectin being not so firmly bound as retrograded amylose. Pure amylose crystallized with the B pattern can be solubilized only by autoclaving. The resistance to dissolution is due to the extensive network of intra- and interhelical hydrogen bonds that stabilize the double helical structure of crystalline amylose. Retrograded starch may be separated into that redispersed at 100°C (mainly retrograded amylopectin) and that resistant to dispersion in boiling water (mainly retrograded amylose). Human studies suggest that the mainly retrograded amylose fraction resists virtually complete digestion in the small intestine (Englyst & Cummings, 1985; 1987).

Socorro et al. (1989) reported that dietary fiber decreased digestibility of black bean, corn, rice and wheat starches by porcine enzyme, especially when whole grain fiber was used. However, black bean and rice starch digestibility by human pancreatic α-amylase was not affected by fiber, while corn and wheat starch was slightly inhibited. The use of the human enzyme is therefore recommended for *in vitro* amylolysis assays,

although the difficulties of extrapolating results obtained with animal enzymes to humans should be carefully considered .

Studies by Fernandez and Berry (1989) reported that germination sharply increased the susceptibility of chickpea starch to digestibility by α-amylase, but no change in the appearance of SEM could be attributed to germination. *In vivo* and *in vitro* methods with experimental rats and commercial digestive enzymes, respectively, were used by Nnanna and Phillips (1990) in assessing the protein and starch digestibility and flatulence potential of germinated cowpeas. Germination reduced the flatulence potential of seeds . *In vivo* digestibility of starch and protein was also significantly increased by germination. Germination did not affect *in vitro* protein digestibility, but reduced *in vitro* digestibility of freeze-dried and 70°C-dried starch. Cooking in boiling water significantly increased *in vivo* protein and starch digestibility of both ungerminated and germinated seed.

Tovar et. al. (1990a) found the starch content of a raw red kidney bean (*Phaseolus vulgaris*) flour (RBE) was higher than that of a cooked and blended (CBB) and of a cooked, freeze-dried, and milled (CBF) preparation. However, wet homogenization as well as pepsin pretreatment of CBF increased the starch yield, indicating that starch in the cooked samples is not completely available to enzymatic degradation unless cell wall entrapped granules are released by mechanical or enzymatic disruption of the fibrous walls. This could partially explain a number of inconsistencies in digestibility values reported in the literature (Fleming and Vose, 1979; Jenkins et. al., 1982; Würsch et. al., 1986; Socorro et. al., 1989 and O'Dea and Wong, 1983). Influence of encapsulated and

resistant starch fractions on dietary fiber values was also noticed. CBF showed remarkably low values of *in vitro* amylolysis rate and starch digestibility index in a digestion/dialysis system, features that seemed to depend also on the integrity of cell walls.

Large differences exist in the degree to which different starch containing foods affect the blood glucose levels of both normal volunteers and diabetics. These differences appear to relate to the digestibility of the starch and the factors determining this, including: the interaction of starch with fiber, antinutrients (e.g., phytate) and protein in the food, together with the nature of the starch itself and its physical form (e.g., raw or cooked, ground or whole). In this respect legumes exemplify a class of foods, high in fiber, protein and antinutrients, with a starch which is digested slowly in vitro. They also produce relatively small blood glucose rises after consumption by both normals and diabetics and in the long term result in improved diabetic control. Identification of foods which yield the response and further understanding of factors determining starch digestibility will allow greater therapeutic use of diet in the management of diabetics and disorders of carbohydrate metabolism.

Thompson, et al. (1987) determined digestion of wheat starch (WS) and red kidney bean (RKB) starch by pancreatic (PA) and salivary (SA) amylose in the presence or absence of lectins. Compared with WS, digestion of RKB starch by PA and SA was 70.0% and 66.6% lower, respectively. RKB lectin added to WS at the hemagglutinin activity level in RKB starch resulted in significantly decreased digestion with PA (63.9%) and SA (43.8%) as did heated RKB lectin with insignificant hemagglutinin activity (41.1% with PA, 35.8% with SA). Jack bean lectin (concanavalin A) also resulted in reduced rate

of starch digestibility. Kinetic analyses revealed noncompetitive inhibition by RKB lectins on both amylases. Results confirmed the role of lectins in reducing the rate of starch digestion and its possible health benefit.

Würsch, et al. (1986) studied the factors responsible for the slow digestibility of starch in leguminous seeds by examining microscopically the cooked seeds after various treatments and by measuring starch digestion *in vitro*. Starch in leguminous seeds is entrapped in parenchyma cells and swells only partially during cooking. The α-amylase cannot easily penetrate within the gelatinized starch granules due to steric hindrance and the physical nature of the leguminous starch. Disruption of the cells, especially before cooking increases the susceptibility of starch to α-amylase digestion.

The objective of a comprehensive study recently conducted was to determine whether legumes in a physical form which is rapidly digested *in vitro* give rise to proportionately greater metabolic responses *in vivo* than legumes which are slowly digested *in vitro* (O'Dea and Wong, 1983). Samples of cooked whole and ground lentils were incubated in vitro with pancreatic amylase for 30 min and the percentage starch hydrolysis determined. Grinding the lentils before cooking resulted in a 5-fold increase in the rate of starch hydrolysis (whole lentils 12.1%, ground lentils 60.9%). For the *in vivo* studies six healthy, young lean subjects consumed two test meals containing 50 g starch: whole lentils and lentils that had been ground finely before cooking. Postprandial glucose and insulin responses were measured over 4 hrs. Peak glucose and insulin responses occurred 60 min postprandially for the whole lentils and 30 min postprandially for ground lentils. Although the increase in plasma glucose after ground lentils (1.6mM) was

significantly higher than that after whole lentils (0.09mM), there was no difference in the magnitude of the insulin responses. These results indicate that, unlike for cereals, the rate of intestinal starch hydrolysis is not the major factor determining the metabolic responses to legumes. By virtue of their low post prandial glucose and insulin responses, irrespective of their physical form and digestibility, legumes would appear to be ideal for inclusion in the diet of diabetics.

Cell Wall Components and Chemistry in Plant Seeds

Definition of Dietary Fiber

Dietary Fiber (DF) was defined by Trowell (1974) as that part of plant material in our diet which is resistant to digestion by secretions of the human digestive tract. As this definition did not include polysaccharides present in some food additives (such as plant gums, algal polysaccharides, pectins, modified celluloses, and modified starches) Trowell (1976) extended the definition to include all the polysaccharides and lignin that are not digested by endogenous secretions of the human digestive tract. Accordingly, the term DF now refers mainly to nonstarchy polysaccharides and lignin in the diet (Southgate, 1976).

Plant cell walls are the main source of DF, and most of our DF intake comes from the cell walls in foods such as fruits, vegetables, and cereal products. The principal components of DF are complex polysaccharides and some of which are associated with lignin and protein.

Components of Dietary Fiber

The main components which make up DF are summarized in Table 5. The parenchymatous tissues are particularly important in connection with DF, because the walls of these tissues comprise the bulk of the DF from fruits and vegetables, and the endosperm of cereals. The parenchymatous tissues have mainly thin primary cell walls, whereas the lignified tissues have cell walls that have ceased to grow and have undergone secondary thickening. The lignified tissues are of greater importance with some cereal product, e.g., wheat bran and bran based product.

Table 5. Components of dietary fiber

Category of foods	Tissue types	Main constituent groups of DF polymers
Fruits and vegetables	Mainly parenchymatous with some lignified and cutinised tissues	Cellulose. hemicelluloses (eg, xyloglucans), pectic substances, and some glycoproteins Cellulose. hemicelluloses (eg, glucuronoxylans), lignin and some glycoproteins Cutin and waxes
Cereals	Parenchymatous and lignified tissues	Hemicelluloses (eg, arabinoxylans and β-D-glucans), cellulose, proteins, and phenolic esters Hemicelluloses (eg, glucuronoarabinoxylans), cellulose, lignin and phenolic esters and proteins
Seed other than cereals (eg, leguminous seeds)	Parenchymatous (pea cotyledons) and cells with thickened endosperm walls (guar endosperm)	Cellulose, hemicelluloses (eg, xyloglucans), pectic substances, and glycoproteins Hemicelluloses (mainly galactomannans), and some cellulose, pectic substances, and (glyco)proteins
Food additives		Gums--gum arabic, alginates, carrageenan, guar gum, carboxymethylcellulose, modified starches, etc

The cellulose microfibrils, the main structural elements, contain highly ordered crystalline regions, in which linear chains of cellulose molecules are highly packed, and less ordered amorphous regions in which the cellulose chains are less closely packed and in which other polysaccharides may be found. The microfibrils are closely associated with hemicelluloses, pectic substances, and glycoproteins deposited as an amorphous matrix of macromolecules.

Organization of Cell Wall Structure

Dicotyledonous plants

The seeds of dicotyledonous plants can be classified into those which are free of an endosperm, referred to as nonendospermic (e.g.. bean, pea, etc.) and those which have an endosperm, referred to as endospermic (as in certain leguminous species, e.g., guar, locust bean, etc.). The nonendospermic seeds usually have starch as the main storage polysaccharide, and their cell wall material is derived mainly from the tissues of the cotyledons and there is some contribution from the testa (which are usually lignified). The cell wall polysaccharides of the cotyledons are similar to those of parenchymatous tissues. The main cell wall polymers of parenchymatous tissues of dicotyledons are pectic substances, hemicelluloses (e.g., xyloglucans), and cellulose, whereas those of lignified tissues are lignin, hemicelluloses (glucuronoxylans), and cellulose; usually different types of hemicellulosic polysaccharides occur in the cell walls of the two types of tissues.

By contrast to nonendospermic seeds, all the endospermic leguminous seeds contain galactomannans, which are located in the endosperm cell walls (Meier and Reid, 1982). The galactomannans are essentially linear molecules but are highly substituted on

C-6 of the β-(1-4)-linked-D-Man p residues with single Gal p residues, which confers on them properties which are quite different from those of unbranched, cellulose-like, water-insoluble mannans and glucomannans. The galactomannans are hydrophilic and are usually obtained from the crushed seeds (or endosperms) by hot water extraction. Based on the interactions of galactomannan with water and other polysaccharides, uses of galactomannan form guar and locust bean seeds appears to slow glucose absorption in the small intestine by interacting with intestinal mucosa (Johnson and Gee, 1981).

Monocotyledonous plants

The cell walls of certain organs of monocotyledonous plants, particularly those of cereal grains, are an important source of DF. All cereals have endospermous seeds; the endosperm of wheat, for example, represents about 80 to 85% of the grain, and is the source of white flour. The cell wall polysaccharides of parenchymatous tissues of cereals are mainly arabinoxylans and β-D-glucans, but they all contain small but significant amounts of cellulose, usually associated with glucomannan.

The most notable differences between the cell walls of the endosperm (and aleurone layer) of cereals and those of parenchymatous tissues of dicotyledons are that unlike the latter, the former are virtually free of pectins and pectic substances, and the amount of cellulose is very low (Mares and Stone, 1973; Fincher, 1975; Bacic and Stone, 1981). Despite the relatively low levels of cellulose, the endosperm cell walls consist of a microfibrillar phase embedded in an amorphous matrix (Mares and Stone, 1973); in this respect they are similar to most primary plant cell walls. The primary cell walls of most cereal grains have cellulose microfibrils, which are closely associated with glucomannan,

and these fibrillar structures are embedded in an amorphous matrix of hemicelluloses, which consist mainly of arabinoxylans and/or β-D-glucans, some of which are cross-linked by phenolic esters and /or proteins.

Constituents of Cell Walls

All the wall layers consisted of two phases, a microfibrillar phase and a matrix phase. The microfibrillar phase is distinguishable from the matrix phase by its high degree of crystallinity and its relatively homogeneous chemical composition.

Fibrillar polysaccharides

The fiber fractions termed fibrillar polysaccharides include the basic structural units of microfibrils which have extremely long, thin structures and are made up mainly of cellulose molecules, which are aligned parallel to the long axis of the microfibril. Cellulose is an unbranched β 1,4-glucan. The cellulose chains are held in a crystalline lattice within the microfibril. The lattice is stabilized by both intramolecular and intermolecular hydrogen bonds.

It is clear from X-ray diffraction and chemical studies that the bulk of the microfibril is made up of crystalline β 1,4-glucan. However, there is evidence that some degree of structural heterogeneity may exist within the microfibril. First, α-cellulose fraction of cell walls almost always contains a small amount of some sugars other than glucose, usually mannose and xylose. Secondly, there is less crystalline region examined in the electron microscope with negative stains, in which the non-glucose residue would be expected to be found in the less crystalline region of the microfibril.

Matrix polysaccharides

Matrix polysaccharides are made up of linearly orientated polymers, which are present at all stages of the development of the wall, and also of highly branched polysaccharides that are deposited at particular stages of growth. These polysaccharides may, at the surface of the microfibril, be incorporated into its structure. There are two major fractions in the matrix polysaccharides.

Pectic substances

By definition, pectic substances are polysaccharides that are solubilized from the cell wall by aqueous solutions of chelating agents such as ethylenediaminetetra-acetate or ammonium oxalate. The solvent action of the chelating agents depends on their ability to combine with Ca^{++} and Mg^{++}. The soluble pectic substances comprise the methyl ester, pectin, the deesterified pectic acid and its salts, pectates and certain neutral polysaccharides. The pectic polysaccharides are made up of a group of a polysaccharides rich in galacturonic acid, rhamnose, arabinose and galactose. They are characteristic of the middle lamella and primary wall of dicotyledonous plants, and to a lesser extent of monocotyledonous plants. They may also be linked covalently to phenols, cellulose and protein. The most abundant component of the pectin polysaccharides are polyuronic acids. The polyuronic acids composed mainly of two different polysaccharide: rhamnogalacturonan and homogalacturonan (Albersheim, 1974).

Rhamnogalacturonan (RG) This polysaccharide is a major component of the middle lamella and primary cell wall of dicotyledonous plants, with the greatest concentration in the middle lamella. It contains a backbone of α1,4-linked galacturonic

acid and α1,2-linked rhamnose. Many of the galacturonic acid residues of RG are methyl esterified, and some may contain acetyl group esterified to their hydroxyl groups. The backbone is long and the degree of polymerization is around 2000. RG contains a number of different side-chains, attached to the C4-position of rhamnose. These side-chains are composed principally of arabinose and galactose.

Homogalacturonan Homogalacturonans are made up of α1,4-linked chains of galacturonic acid, which may be partly methyl esterified. Other sugar residues are absent or present only in low quantities. They are found in considerable quantities in some fruits, and are also present in the primary walls of suspension-cultured dicotyledonous cells. Homogalacturonon with a low degree of methyl esterification may be referred to as 'pectic acid', while more highly methylated molecules may be referred to as 'pectinic acid', or simply as 'pectin'. This terminology can also apply to molecules containing covalently-linked homogalacturonan and rhamnogalacturonan.

Arabinan This is a highly-branched molecule containing a backbone of α1,5-linked arabinose and side-chains of single arabinose residues linked by α(1-2) or α(1-3) bonds to the main chain.

Galactan Galactans are largely unbranched chains of β1,4-linked D-galactopyranose residues, with little or no additional sugar material present in the molecule. In some cases a few of the galactose residues appear to be 1,6-linked.

Hemicelluloses

Hemicelluloses are the polysaccharides solubilized by alkali from the depectinated (and delignified) cell walls. Selvendran and O'Neill (1982) suggested that some of the

alkali-soluble polymers are polysaccharide-protein-polyphenol complexes. In addition to polysaccharides, small amount of glycoproteins, both hydroxyproline-rich and hydroxyproline-poor, are also present, and the associated sugars are mainly arabinose and galactose. The hemicellulose is connected with cellulose microfibrils by strong hydrogen bonding. In contrast to the pectins, the hemicelluloses vary greatly in different cell types and in different species.

Xylans These polysaccharides have a backbone of β1,4-linked xylose residues. The backbone is substituted by α-linked 4-o-methylglucuronic acid on C2 of some xylose residues, by α-linked arabinose on C2 or C3 and by acetyl esters on C2 or C3. The primary cell wall of monocotyledonous plants include, as a major hemicellulose, an arabinoxylan in which arabinose is the dominant side-chain. Secondary walls of monocotyledonous plants contain an arabinoxylan with rather more glucuronic acid. The primary walls of dicotyledonous plants have small amounts of glucuronoarabinoxylan, containing both glucuronic acid and arabinose side-chains. The secondary walls of dicotyledonous plants contain glucuronoxylan, with only a low proportion of arabinose, as their major hemicellulose.

The xylans are capable of crystallization under certain conditions, though they are not thought to be crystalline in the cell wall. They may crystallize with either a two-fold or a three-fold screw axis, depending on the degree of acetylation.

Mannans and Galactomannans Mannans and galactomannans have been found in the cell walls of some seed endosperms, where they function as a food reserve. In some cases, they also have a role in imbibing and retaining water. They contain a β1,4-linked

mannose chain, and where galactose is present it is linked by an α(1-6) bond to mannose. The mannans are able to form very hard, crystalline structures.

Galactans The galactan subgroup of the hemicelluloses is mainly composed of arabinogalactans. Their molecules consist of a main chain of β-galactopyranose units linked to each other by β1,3-linkages.

Xyloglucan Xyloglucan is the principal hemicellulose of the primary walls of dicotyledonous plants. It consists of a backbone of β(1-4)-linked glucose residues, to the majority of which xylose residues are attached by α(1-6) bonds. Xyloglucans are also found as storage polysaccharides in some seed endosperm cell walls. Primary walls of monocotyledonous plants contain small amounts of xyloglucan, with a lower xylose: glucose ratio than in dicot primary walls.

CHAPTER 1 INDIGESTIBLE STARCH COMPONENTS IN DRY BEANS

Introduction

Procedures to determine the degree of gelatinization of starch within a food matrix or purified starch have been developed by several researchers. It has been recognized that selective use of appropriate enzymes and proper handling are important to consistently determine the degree of gelatinization of bean (*Phaseolus vulgaris* L.) starch in-situ. The enzymatic methods are based on the hydrolysis of gelatinized starch by glucoamylase to form glucose (Chiang and Johnson, 1977). These methods are useful to differentiate between native (raw) starch and gelatinized (cooked) starch. However, these methods are not suitable to distinguish between gelatinized starch and retrograded starch due to the limited selectivity of glucoamylase. According to Kainuma et al. (1981), the combination of β-amylase (E.C.3.2.1.2) and pullulanase (E.C.3.2.1.41) (BAP) provides a highly sensitive approach for the detection of structural changes in raw, gelatinized and retrograded starch. β-amylase is unable to act on raw starch and pullulanase (debranching enzyme) requires thermal disruption of the three dimensional structure of amylopectin molecules for active hydrolysis. For instance, corn starch paste induced to the retrograded state through frozen storage (13 days at -20°C), resulted in 21% of the retrograded starch being over estimated by glucoamylase compared with data obtained using the BAP. Another example is that 35% of the rice starch paste hydrolyzed by glucoamylase was not detected by the β-amylase and pullulanase enzyme complex. It has been recognized that proper sample handling is necessary for accurate assessment of total starch content and degree of gelatinization of raw and processed bean starch, because the starch is

encapsulated within cell walls. This structural partitioning and the appearance of resistant starch are among the unique characteristics of processed beans compared to cereal grain (Tovar et al., 1990a).

The degree of starch gelatinization in cooked beans is of importance to their complete digestibility. Most studies associated with indigestible starch fractions of leguminous seeds have used the terminology "resistant starch" (RS), which is defined as dietary starch that does not digest in the small intestine (Englyst and Cummings, 1985). It has been recognized that the portion of RS in cooked beans consists of not only resistant starch granules but also ungelatinized starch. Previous research has shown that some portion of the bean starch retains its crystalline structure after prolonged cooking (Lai, and Varriano-Marston, 1979; Varrano-Marston and DeOmana, 1979). Loss of birefringence under polarized light microscopy is commonly used as an indicator of starch gelatinization (Schoch and Maywald, 1956; Watson, 1964). The appearance of birefringency due to crystalline structures may be a valuable phenomenon to observe crystallization within cell walls. It has been suggested that a rigid cell wall is a physical barrier to digestive enzymes and is an important determinant limiting the relatively low bioavailability of starch in cooked beans (Würsch et al., 1986; Flemming et al., 1988). The cell walls in cooked beans may also inhibit starch gelatinization due to limited swelling potential of the starch granule and restricted or limited water availability necessary for complete gelatinization (Thorne et al., 1983; Würsch et al., 1986).

The purpose of this study was to investigate the degree of starch gelatinization during cooking of whole beans using enzymatic and microscopic techniques. Enzymatic

digestion of the starch fraction of cooked beans was conducted with β-amylase and pullulanase to measure the degree of starch gelatinization during differential heating times.

Experimental Plan

Whole dry navy beans (variety Seafarer) were soaked in water for 12 hrs and thermally processed with differential time at 115.5°C. Samples are prepared as fresh cooked and lyophilized. Degree of gelatinization was measured by an enzymatic method and a visual microscopic method. Reheating was conducted on lyophilized sample to investigate the rigidity of crystallized cell walls.

The null hypothesis tested in this research is stated as follows:

H_o: The presence of whole cells with crystallization in the cell wall do not prevent starch gelatinization and lower starch digestibility.

The experimental protocol used in this study is outlined in Figure 2.

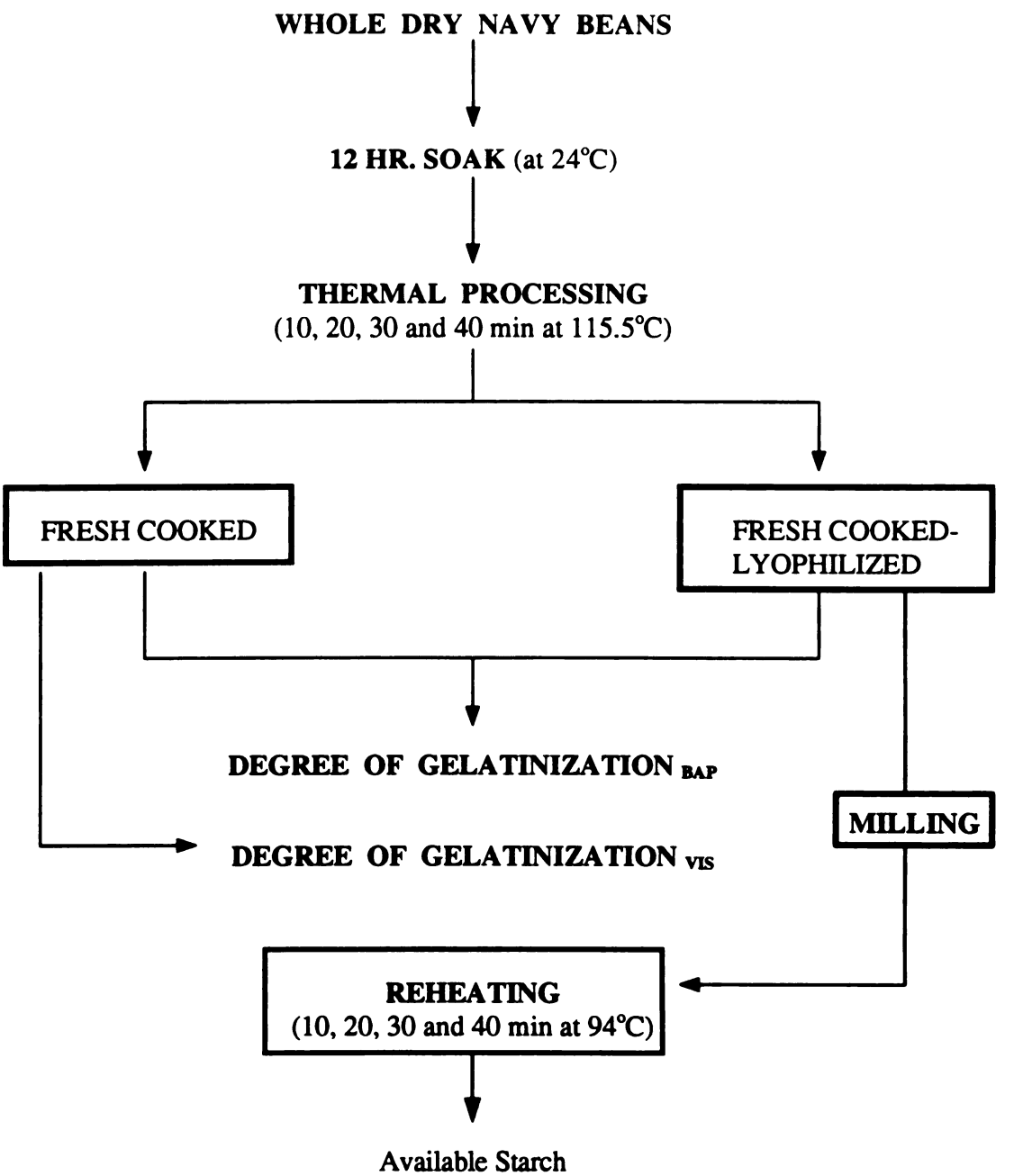


Figure 2. Experimental protocol used to assess the degree of starch gelatinization in differentially cooked and prepared navy beans. (Degree of gelatinization BAP: β-amylase and pullulanase enzymatic method; VIS: microscopic method)

Materials and Methods

Materials

Navy beans (*Phaseolus vulgaris*, var. Seafarar) were obtained from the Michigan Foundation Seed Association, Okemos, Michigan and stored at 4°C until used. The seed was produced in the 1993 growing season. Bean samples were randomly selected and used directly as whole bean seeds for cooking and preparation procedures.

Thermal Processing

Dry bean samples were soaked in prepared soaked water (distilled water containing 100 ppm Ca^{++}) for 12 hrs at room temperature prior to processing. The soaked beans were thermally processed in 4 oz cans (204×214) at 115.5°C in a still retort. Beans were cooked for 10, 20, 30 and 40 min. The experiment was replicated twice for each cooking time. Distilled water containing 100 ppm Ca^{++} was used as brine. The hermetic seal of all cans were punctured immediately after the cooling cycle due to inadequate heating to achieve commercial sterility.

Sample Preparation

Cooked (fresh) sample

Cooked whole beans (canned) were referred to as "fresh cooked". Seed coats were removed from the fresh cooked sample and the cotyledons were macerated using a mortar and pestle for immediate microscopic observation and enzymatic assay.

Cooked-Lyophilized sample

Cooked samples including brine were frozen, freeze-dried and milled by passing through a Udy Cyclone Mill (Udy Co., Fort Collins, CO) equipped with a 20 mesh screen.

All samples were held in tightly capped glass bottles in a desiccator to minimize physical and chemical changes prior to analyses.

Cooked-Lyophilized + Reheated sample

Additional heating of the cooked-lyophilized sample was conducted by suspending 80 mg meal in distilled water (8 ml) in a covered culture tube (50 ml) and reheating for 10, 20, 30, and 40 min at 94°C in a water bath with occasional shaking.

Light Microscopy

The samples for microscopic examination were prepared by macerating cotyledons from fresh cooked or lyophilized beans with a mortar and pestle. A portion of the resultant bean paste was suspended in distilled water and mounted on a glass slide and observed under light microscopy. Fully cooked samples were prepared by hand cutting the whole beans with a razor blade and then suspending and mounting cross sections on a glass slide. A laser scanning confocal microscope (Zeiss 210, West Germany) was used with a red laser (Helium-neon emitting at 633 nm) and a blue laser (Argon-ion emitting at 488 nm). Photomicrographs of representative specimens were obtained after viewing numerous fields and used as the basis to draw conclusions.

Determination of Starch Gelatinization

Enzymatic hydrolysis

The degree of gelatinization (DG) of bean starch was estimated by the method of Kainuma et al.(1981) using a combination of β-amylase and pullulanase (BAP). One ml of the enzyme solution contained 3.4 IU of pullulanase (E.C. 3.2.1.41, Sigma No. P5420) and 0.8 IU of β-amylase (E.C. 3.2.1.2, Boehringer Mannheim No. 102822).

Samples (80 mg, db) were placed in a glass homogenizer (30 mL) with 8 mL of distilled water and dispersed with up and down movement of a polyester piston (10-20 times) by hand. Two samples of 2.0 mL were transferred to 25-mL volumetric flasks. One flask was filled with acetate buffer (0.8M, pH 6.0) and used for the determination of DG of the sample (DISPERSED SAMPLE). To the other flask, 0.2 mL of 10M sodium hydroxide solution was added. The flask was then heated 3-5 min at 50°C to gelatinize the starch completely; 1.0 mL of 2M acetic acid is then added to neutralize to pH 6.0. This flask is then filled with 0.8M acetate buffer, pH 6.0. It is used as the reference of 100% degree of gelatinization (ALKALINE GELATINIZED SAMPLE). Each sample solution (4.0 mL) from both dispersed and alkaline gelatinized sample was mixed with enzyme solution (1.0 mL) and the mixture was incubated 30 min at 40°C with shaking. After the reaction, the enzyme was inactivated by boiling for 5 min. Then 1.0 mL of the reaction mixture was diluted five times and 1.0 mL and 0.5 mL of the diluted reaction mixture were analyzed by the Somogyi-Nelson procedure (Robyt and Whelan, 1968) for reducing sugar and the phenol-sulfuric acid assay (Dubois et al., 1956) for total carbohydrate, respectively. The procedure and flow diagram for the BAP method are shown in Figure 3.

Degree of gelatinization (DG) of a starch sample was expressed as the percentage of the degree of hydrolysis compared to the completely gelatinized starch sample under alkaline conditions.

$$\text{Degree of gelatinization}_{BAP}\,(\%) = \frac{A / B}{A' / B'} \times 100$$

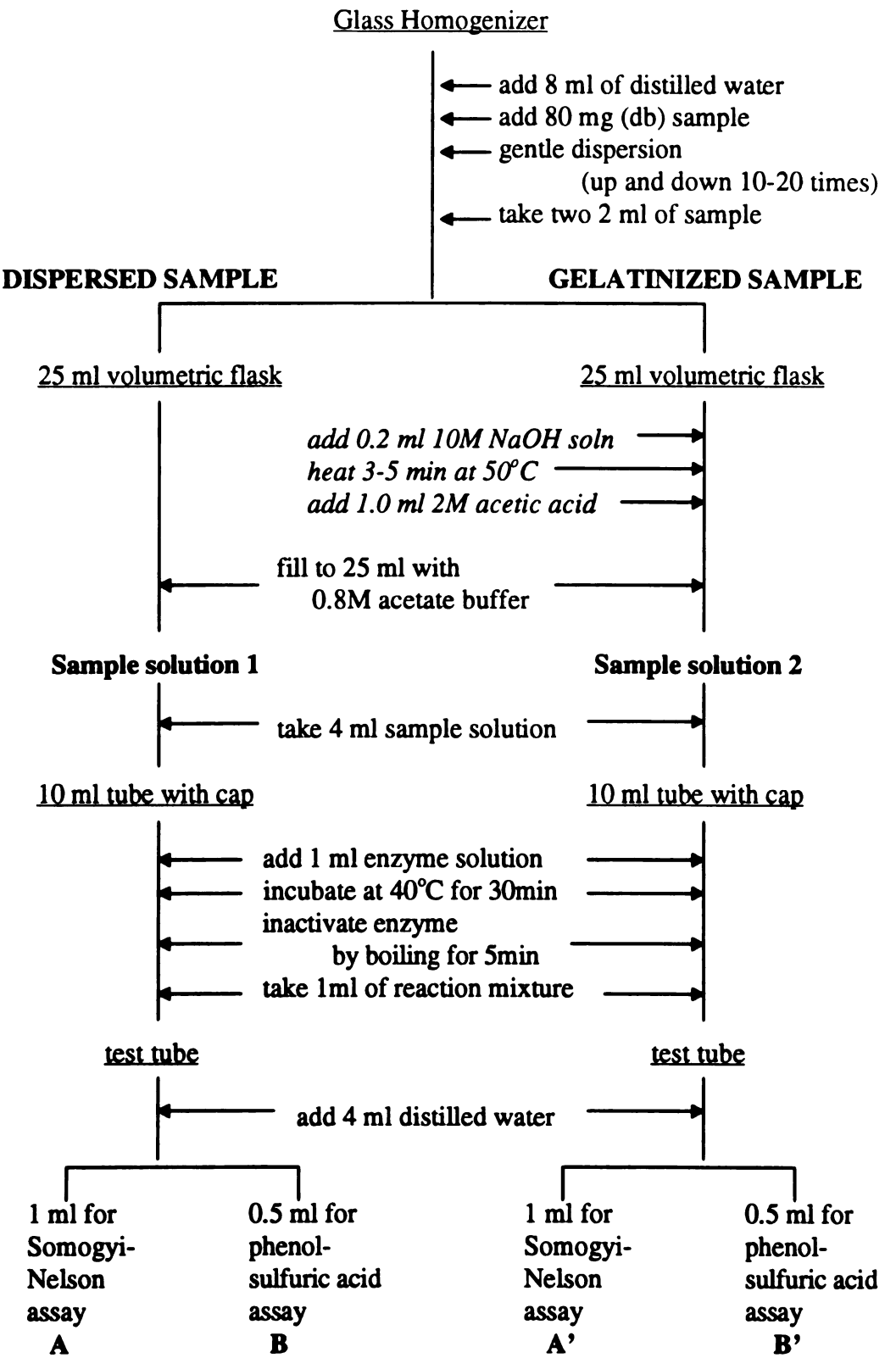


Figure 3. Flow sequence to determine degree of gelatinization (DG): DG (%) = [(A / B) + (A' / B')] × 100; (Modified: BAP method of Kainuma et al., 1981)

where, A = amount of reducing sugar in dispersed sample
B = amount of total carbohydrate in dispersed sample
A' = amount of reducing sugar in alkaline gelatinized sample
B' = amount of total carbohydrate in alkaline gelatinized sample

Direct polarized microscopy

The degree of gelatinization was quantitated by counting the number of cells which consisted of gelatinized, partially gelatinized, and ungelatinized starch granules. Classification was made based on visual rating of cellular brightness using a 5 point scale: 1 = ungelatinized, bright-white / numerous occlusions; 3 = partially gelatinized, light-white / few occlusions; 5 = gelatinized, clear / no occlusions. Three representative photomicrographs under polarized light were taken on fresh cooked samples and the degree of gelatinization estimated by visual microscopy method is defined as "degree of gelatinization$_{VIS}$" and calculated as follows:

$$\text{Degree of gelatinization}_{VIS}\ (\%) = \frac{X + 3Y + 5Z}{5\,(X + Y + Z)} \times 100$$

where, X = number of ungelatinized cells
Y = number of partially gelatinized cells
Z = number of gelatinized cells

Statistical Analysis

Degree of gelatinization measured by enzymatic methods was performed in duplicate and each sample was analyzed in triplicate. All other determinations were made in triplicate. The mean, standard errors, mean square errors, one factor ANOVA were conducted using Super ANOVA software (version 1.11, 1991, Abacus Concepts, Inc.,

Berkeley, CA). Mean separations were performed using LSD with the mean square error term at the 95 % confidence level.

Results and Discussion

General

Table 6 shows total soluble solids and carbohydrate relationships during cooking of beans in brine. The total solids of beans decreased with increased cooking time while brine solids increased. These results demonstrated that the cooking process caused the leaching of solids into the brine in response to bean tissue breakdown. The amount of total carbohydrate leached into the brine after 10 min cooking was 18.5 % and was significantly lower than that of extended cooking times. The prolonged cooking over 10 min did not affect the amount of total carbohydrate leached into brine. The amount of available starch in brine, which is measured by β-amylase and pullulanase, increased with cooking time up to 30 min and dropped at 40 min of cooking time.

Table 7 shows the degree of gelatinization of beans for the different preparation conditions used and measured by the BAP method. The degree of gelatinization increased linearly up to 15% after 30 min cooking of the fresh cooked sample. However, further cooking resulted in a reduced hydrolytic response. It was observed that the pattern of enzyme hydrolysis on starch in brine (Table 6) was similar to that of the degree of gelatinization in freshly cooked beans (Table 7). This relationship may be attributed to release of encapsulated starches from the bean cotyledon tissues during cooking. A previous study by Tovar (1995) supports this explanation. He presented his work on hydrolysis of cooked brown beans and cooking water. Beans were boiled in water for 2 hrs and beans and the cooking water were freeze-dried separately, powdered and fractions observed. Both fractions contained cell wall-surrounded starch in an encapsulated form.

Table 6. Total solids and carbohydrate contents of whole beans and brine after cooking[1]

Cooking Time (min)	Total Solids (%)		% of Total Solids in Brine	
	Bean	Brine	Total CHO	Available Starch[2]
10	36.2 ± 0.8a	2.3 ± 0.6a	18.5 ± 1.2a	0.1 ± 0.1a
20	33.4 ± 1.1a	2.3 ± 0.6a	27.2 ± 2.0b	1.6 ± 0.2b
30	33.0 ± 3.2a	2.5 ± 0.4a	28.5 ± 0.6b	2.2 ± 0.4c
40	31.6 ± 2.2a	2.7 ± 0.3a	29.0 ± 1.8b	0.4 ± 0.1a

[1] Values are means of duplicate. Means in a column with different letters were significantly different ($p < 0.05$).

[2] Available by the BAP enzyme method (β-amylase and pullulanase)

Table 7. Degree of gelatinization of beans held in different conditions measured by the enzymatic method[1]

Conditions	Degree of Gelatinization$_{BAP}$ (%)
Soaked	5.3 ± 0.6ab
Cooked (Fresh)	
10 min	6.2 ± 0.4ab
20 min	10.1 ± 1.1b
30 min	15.4 ± 3.3c
40 min	7.2 ± 0.8ab
Cooked - Lyophilized	
10 min	2.1 ± 0.7a
20 min	2.5 ± 1.3a
30 min	5.4 ± 4.6ab
40 min	2.4 ± 2.0a
Soaked - Lyophilized + Reheated (30 min / 94°C; Homogenate)	116.7 ± 12.3d
Corn Starch (30 min / 94°C)	100.4 ± 5.2d

[1] Treatments were done in duplicate and each sample was analyzed in triplicate. Means in a column with different letters were significantly different ($p < 0.05$).

He noticed that the cooking process caused a breakdown of cotyledons and starches released from the seeds were encapsulated within intact cell walls. This similar pattern suggests that susceptibility of the enzymes to starch has been changed during cooking due to conformational change of starch by gelatinization and the integrity of cell wall due to crystallization.

Heating generally ruptures cell walls and frees starch granules from their cellular compartmentalization. It also has been observed that some carbohydrates leach from intact beans into the brine during cooking. However, this study showed that crystallization within the cell walls of beans during heating results in individual cells which resist rupture and starch release. The heating of beans caused cell walls to crystallize which prevented the release of starch, even though prolonged heat caused more gelatinization of starch within cells as shown by microscopic observations.

The degree of gelatinization of the lyophilized samples throughout different cooking times was significantly less than that of the fresh samples. Retrogradation of starch occurred during preparation by freeze-drying. Over 50% of gelatinized starch in the fresh cooked samples was retrograded during the freeze-drying process. Bean starches from the soaked, lyophilized and reheated homogenate treatments were completely hydrolyzed by the enzyme, β-amylase and pullulanase, indicating that the cell wall was readily ruptured and starches were available for digestion.

The extent of starches hydrolyzed by β-amylase and pullulanase upon different cooking and reheating treatments is shown in Table 8. Freeze-dried flours prepared with different cooking time without subsequent reheating showed no significant differences on starch bioavailablity as measured by the enzyme digestion. It is proposed that low

Table 8. Starch available by β-amylase and pullulanase after different cooking and reheating treatment[1]

Reheating[2] (min)	Available starch (g / 100g sample)				
	Soaked	10 min cook	20 min cook	30 min cook	40 min cook
0	0.3 ± 0.1a	0.5 ± 0.4a	0.5 ± 0.3a	0.7 ± 0.3a	0.4 ± 0.3a
10	19.1 ± 1.9f	7.7 ± 2.5e	4.2 ± 1.9bcd	0.8 ± 0.6a	1.1 ± 0.3a
20	16.8 ± 1.0f	5.1 ± 2.8de	3.8 ± 0.9abcd	1.4 ± 0.6ab	1.5 ± 0.5ab
30	15.9 ± 0.3f	4.8 ± 3.0cde	2.3 ± 1.1abcd	2.1 ± 1.3abcd	2.1 ± 1.2abc
40	17.7 ± 2.2f	4.2 ± 4.8bcd	2.4 ± 2.3abcd	2.2 ± 0.2abcd	2.2 ± 1.7abcd

[1] All samples were freeze-dried and milled after soaking and/or cooking. Values are means of triplicate samples. Means with different letters were significantly different ($p < 0.05$).

[2] Reheating was undertaken in a water bath with occasional shaking at 94°C.

digestibility values obtained for the soaked and uncooked sample was due to ungelatinized starch granules while those obtained for any cooked samples was caused by crystallization of cell walls. Many studies have revealed that starch granules of the cooked bean are encapsulated within thick-walled cell (Würsch et al., 1986; Tovar et al., 1991). Tovar reported that the thick and mechanically resistant cell walls acted as a physical barrier to swelling and enzymatic breakdown of starch granules *in vitro*. In this study, we found that the rigidity of the cell walls crystallized during the cooking process varied with cooking time (Table 8). Reheating of the 10 min-cooking and freeze-dried flour showed higher values for available starch than those cooked for 20, 30, and 40 min. Approximately 32% of the total starch measured as free starches of soaked and freeze-dried flour, was liberated by the reheating process in the 10 min cooked sample while only 10% of the total starch was available in the 40 min-cooking and freeze-dried sample. The amount of starch released after reheating showed no significant difference among 20 min, 30 min, and 40 min cooked samples. Tovar et al. (1990b) observed that milling of the freeze-dried bean samples cooked in boiling water for 60-70 min resulted in a flour that retained cell structure. We confirmed their observations and additionally demonstrated that the crystallized cell walls induced by initial heating (just 10 min) was resistant to enzymatic attack and milling. However, the milling process of the soaked and freeze-dried sample liberated starch granules and rendered them available through heat induced gelatinization. There were no significant differences among reheated samples of the soaked-freeze-dried and milled bean flour heated for differential times (Table 8). These data confirm previous

studies that milling of the samples before cooking increased starch availability (Golay et al., 1986; Fleming et al., 1988).

A mechanism of crystallization of a thick cell wall during cooking based on the above observations could be proposed as follows. The cell wall of raw bean was readily broken by milling. However, once heat was applied to the soaked bean, crystallization of cell walls inhibited the mechanical breakdown and release of starch granules. Thus, most starch granules are entrapped within thick cell walls after cooking. Crystallized cell walls become barriers to enzyme hydrolysis and continuation of starch granule swelling. During the soaking process, most starch granules absorb water and swell which increases potential to be gelatinized during heating. However, some starch granules remain intact after soaking and are not able to be gelatinized even with prolonged heating because the crystallized cell walls inhibit expansion of starch granules after cooking. These data are conclusive that during cooking of whole beans some portion of starch granules remain intact. The structural characteristics of these intact starch granules result in reduced bioavailability.

Microscopy

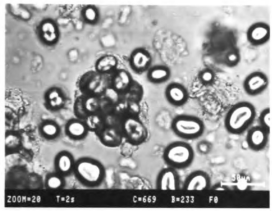
Plate 1 shows the appearance of aqueous suspensions of cold soaked (12 hrs) and lyophilized bean meal viewed under transmitted (a,c) and polarized light (b,d). These duplicate fields demonstrate the diversity and typical appearance of cellular structures within the sample and illustrate the free starch granules (a,b) and starch granules bound within the cell (c,d). These samples received no heat treatment and the granules are clearly ungelatinized. These liberated starch granules (Plate 1b) showed characteristic and

clear birefringence light scattering patterns while starch granules packed within parenchyma cells (Plate 1d) appeared white. The appearance of the cell wall of the soaked beans viewed under the polarized microscope did not show any evidence of crystalline structure. One of the typical characteristics associated with gelatinized starch is a loss of birefringence patterns within the starch granule when viewed under polarized light. Cross sections of soaked bean and cooked bean, which is viewed under polarized microscope, were shown in plate 2.

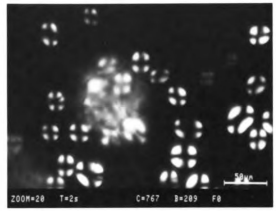
Two distinctive features were observed in cooked whole beans (Plate 3a-d): 1) a decreased percentage of ocular illumination (used as an indicator of the degree of gelatinization) resulted with increased cooking times (10 min, 33.9%; 20 min, 38.6%; 30 min, 39.6% and 40 min, 43.5%) and 2) crystallization of the cell wall (as evidenced by a white band) resulted during the initial heating period of 10 minutes. Cell walls had a highly dense crystalline appearance after this minimal heat treatment. The white ring was produced by crystallization within the cell wall. There was no crystallization in cell walls of soaked beans (Plate 1d). However, cooking for 10 min or longer caused crystallization within cell wall. The energy provided during this 10 minute cook was not sufficient to soften cotyledonary tissue and render beans palatable. However, it did transform the cell walls into firm and stable structures which resisted breakdown during subsequent cooking of up to 40 minutes duration (Plate 3b-c-d).

Plate 4 shows that ungelatinized starch can occur in beans cooked for 2 hrs. Prolonged cooking in an open kettle causes disruption of the testa (seed coat / hull) and liberation of most of the solids into brine. The samples in Plate 4 were taken from whole

beans which remained intact after cooking for 2 hrs. The observation of prolonged cooking without the expected complete gelatinization of starch suggests that there is a physical barrier which limited the hydration or granular swelling typically observed for gelatinized starch. It is suggested that the heat-induced crystalline structure observed in the cell wall limits complete gelatinization of intact starch granules.



a



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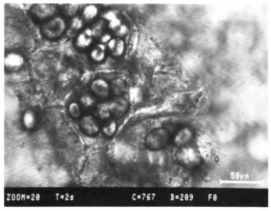
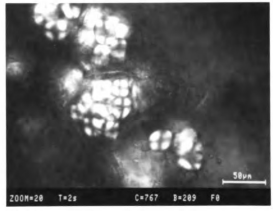
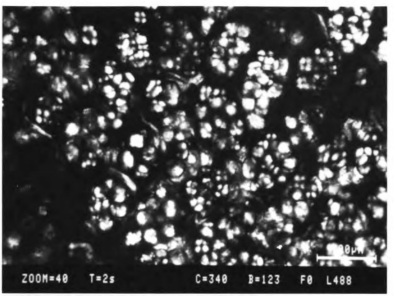


c

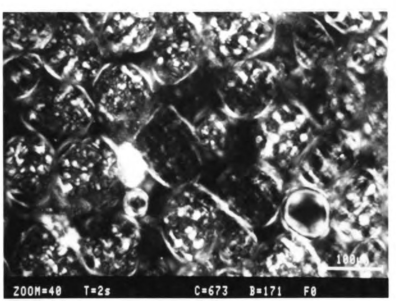


d

Plate 1. Light microscopy images of soaked beans presented as representative fields a, c) transmitted and b, d) polarized



a



b

Plate 2. Polarized light microscopy images of the cross sections for differentially prepared beans
a) soaked b) cooked for 40 min

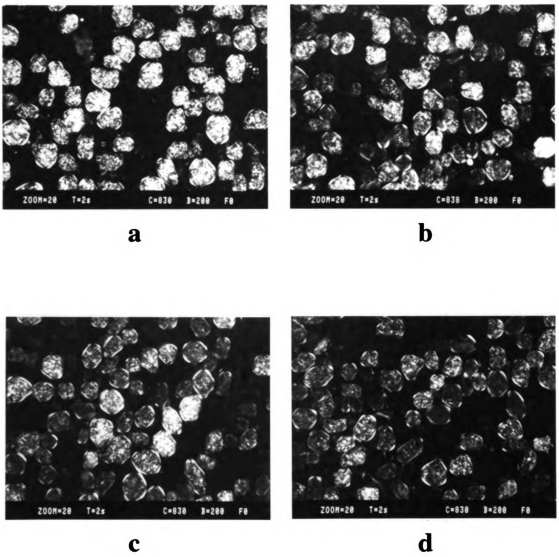
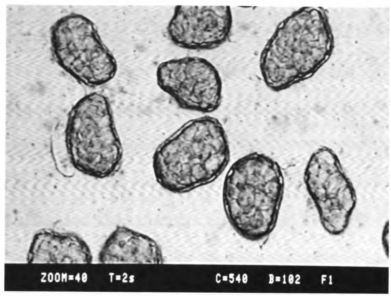
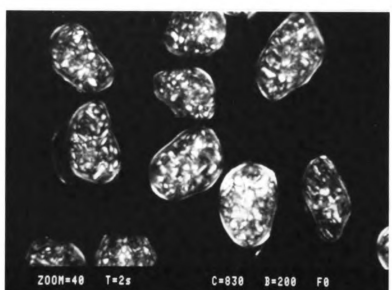


Plate 3. Polarized light microscopy images of the fresh beans cooked for different times
a) 10 min, b) 20 min, c) 30 min and d) 40 min



a



b

Plate 4. Light microscopy images of intact cooked beans heated for 2 hr at 98°C
a) transmitted b) polarized

Conclusions

Heating of whole beans in a liquid medium (brine) caused crystallization of constituents within cell walls which prevented cellular breakdown and release of starch to the extra-cellular milieu. These starches can not be readily digested and, thus, are not biologically available as nutrients. The heat induced crystallization observed in cell walls after heating were resistant to disruption using mechanical homogenization. The degree of gelatinization of the fresh cooked beans measured enzymatically was lower (about 60% when compared at 30 min cook) than that measured microscopically due to limited enzymatic access to the crystallized cell wall. The rigidity of cell walls cooked for 10 min was sufficiently firm to hinder enzyme access yet sufficiently weak to be broken by reheating. The prolonged cooking (over 10 min) increased the rigidity of crystallized cell wall resulting in firmer structure that limited starch availability on reheating of the freeze-dried flour. The freeze-drying process caused retrogradation of the gelatinized starches in fresh cooked beans and it lowered available starches over 50% as determined by BAP method.

The results obtained from this study demonstrate "ungelatinizable starch" in fully cooked edible beans. These data have direct implications on the maximum nutritional bioavailability and digestibility of bean products. Thus, there is a negative nutritional impact using beans as a dietary staple within subsistent populations dependent upon beans as an energy and protein resource and an economic impact depressing total bean consumption within cultures sensitive to intestinal gas formation and flatulence. Improving the gelatinization potential of bean starch granules could increase bean

hydration capacity (cooked bean yield), increase available caloric value, reduce flatulence and perhaps decrease cooking time required to render a palatable product.

H_0: The presence of whole cells with crystallization in the cell walls do not prevent starch gelatinization and lower starch digestibility.

Reject the H_0 as stated and conclude that the presence of whole cells with crystallization in cell walls prevent starch gelatinization which lowers starch digestibility.

CHAPTER 2 HEAT-INDUCED CHANGES IN CELL WALL STRUCTURES REDUCE BIOAVAILABILITY OF BEAN STARCH

Introduction

Legume consumption has increased in the western world due to their recognition as a good source of dietary fiber and protein. In addition, the high quality of carbohydrate related with digestion and absorption is increasingly being recognized. Legume starch is well-known as a low glycemic index food. Low glycemic index diets have been shown to improve metabolic variables not only in diabetics (Brand, et al., 1991) and hyperlipidemia (Jenkins, et al., 1987a) but also in healthy subjects (Jenkins, et al., 1987b). The resistance of legume starches towards hydrolytic enzymes has been of great interest to nutritionists, since they have been found to exhibit a lower glycemic index than the cereals (Jenkins, et al., 1980).

Recently, there has been much interest in characterizing resistant starch (RS), since it functions as a component of dietary fiber, in escaping digestion in the small intestine, but being readily fermented in the colon by microorganisms (Asp et al., 1986; Björck et al., 1986, 1987; Englyst and Cummings, 1985; Englyst and MacFarlane, 1986; Siljestrom and Asp, 1985; Wyatt and Horn, 1988). Most of the starch which reaches the colon is not totally resistant to pancreatic amylase, however its hydrolysis was retarded so that it is not completely digested during its passage through the small intestine. Many factors are involved in formation of resistant starch in leguminous seeds during cooking. One of the important and perhaps unique characteristics is thermally induced crystallization of the cell wall. The crystallized cell walls entrap starch granules and prevent their complete swelling

and dispersion. This results in granules being physically inaccessible for its hydrolysis with pancreatic amylase (Würsch et al., 1986). As a result of encapsulation, some of the bean starch remain intact and ungelatinized (retains crystalline structure) during even a prolonged cooking. In my previous work, it has been found that a minimal heating (10 min at 115.5°C) caused crystallization of cell wall which resisted breakage from milling and homogenization processes.

The purpose of this study is to investigate the changes in cell wall structure of dry beans, sweet potato, and wheat, all starchy foods during cooking and the effect of reheating on the integrity of crystallized cell walls. Starch bioavailability was examined *in vitro* using an enzyme complex, β-amylase and pullulanase.

Experimental Plan

Previous research conducted by USDA (G.L. Hosfield, Research Genetist), Michigan State University (J.D. Kelly, Professor) and The Michigan Dry Edible Bean Research Advisory Board agronomist (Gregory V. Varner, Director) has shown significant variability among bean cultivars and breeding lines for canning quality. One navy bean (experimental line, N 84004) and one pinto bean (variety, Carioca) were selected to compare starch bioavailability with other sources of starchy foods including: 1) sweet potato and 2) wheat. The experimental line, N 84004, has been consistently characterized as possessing excessively soft texture (extensive tissue breakdown and cellular disruption) upon canning and is thus unacceptable for commercial use. Carioca

has previously been shown to possess low starch digestibility among a screening selection of pinto beans (Mridvika et al., 1994).

The null hypothesis tested in this research is stated as follows:

H_o: The crystallization of cell wall in cooked beans is not a limiting factor associated with starch gelatinization and bioavailability.

The experimental protocol is outlined in Figure 4.

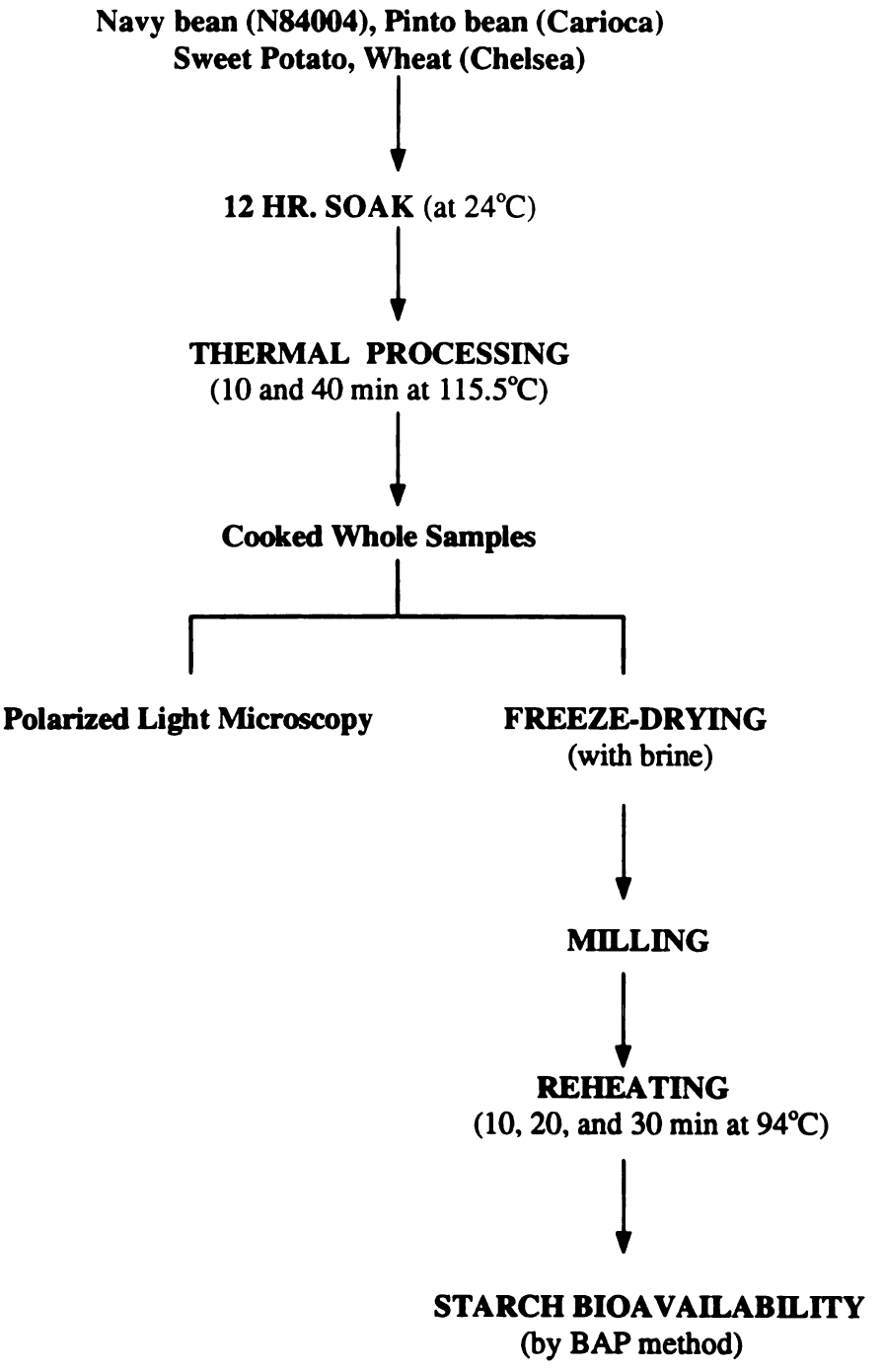


Figure 4. Experimental protocol used to assess starch availability and cellular appearance of foods prepared under selected preparation procedures

Materials and Methods

Materials

Navy beans (experiment line, N 84004) were obtained from the Cooperative Elevator Company during 1992 crop year. Pinto beans (cultivar, Carioca) were obtained from the USDA, ARS: Sugar Beet and Bean Research Center in East Lansing, MI. Bean samples were field-dried, harvested, sorted, packaged, and stored in a cooler maintained at 4°C prior to processing. Sweet potatoes (*Ipomoea batatas Lam.*) were obtained from local market and wheat (*Triticum aestivum L.*, cultivar, Chelsea) samples were obtained from Department of Crop and Soil Science at Michigan State University, East Lansing.

Thermal Processing

Each sample was soaked for 12 hrs at room temperature in distilled water with 100 ppm Ca^{++} prior to processing. A simmering brine containing only 100 ppm Ca^{++} was added before sealing. The soaked samples were thermally processed in 4 oz cans (204 × 214) at 115.5°C for 10 and 40 min. The experiment was replicated twice for each cooking time. All cans were punctured to disrupt hermetic seal immediately after the cooling cycle due to inadequate heating to assure commercial sterility.

Sample preparation

Seed coats of cooked whole beans were removed for microscopic observations. Cooked whole seeds including brine were frozen at -20°C and freeze-dried for two days. The lyophilized samples were ground with a Udy Cyclone Mill to a particle size that would pass through a 20 mesh screen to produce a milled flour suitable for analyses. Sweet potatoes were cut into cubes [maximum ½ inch (12.7mm), minimum ¼

inch(6.35mm)] prior to soaking and the same procedure used for beans was applied. Chelsea wheat was cooked and the cooked whole wheat including kernels was freeze-dried and milled to have wheat flour. All samples were held in tightly capped glass bottles in a desiccator to minimize physical and chemical changes prior to analyses. Additional heating treatment to flour samples was conducted by suspending 80 mg meal in distilled water (8 ml) in a covered culture tube (50 ml) and reheating for 10, 20 and 30 min at 94°C in a water bath with occasional shaking.

Light Microscopy

The samples for microscopic examination were prepared by macerating cotyledons from fresh cooked samples with a mortar and pestle. A portion of the resultant sample paste was suspended in distilled water and mounted on a glass slide and observed under a light microscope. Additionally, some samples were prepared by hand cutting the whole samples with a razor blade and then suspending and mounting cross sections on a glass slide. A laser scanning confocal microscope (Zeiss 210, West Germany) was used with a red laser (Helium-neon emitting at 633 nm) and a blue laser (Argon-ion emitting at 488 nm). Photomicrographs of representative specimens were obtained after viewing numerous fields and used to draw conclusions.

Available Starch

Available starch of each treatment was measured with some modification of enzymatic method using β-amylase and pullulanase (Kainuma et al., 1981). The procedure of estimating degree of gelatinization was applied to measure available starch only on the fraction termed DISPERSED SAMPLE. Reaction mixture hydrolyzed by the

enzymes are analyzed by the Somogyi-Nelson procedure (Robyt and Whelan, 1968) for reducing sugar and maltose was used to make standard solutions. The available starch content was obtained by multiplying the μg of maltose in each sample by a factor of 0.95 to account for the weight of the water gained during the hydrolysis of starch to maltose and by dividing a dilution factor of 128.

$$\text{g starch / 100 g sample (db)} = \frac{\text{maltose (}\mu\text{g / ml of reaction mixture)} \times 0.95}{128} \times 100$$

Differential Scanning Calorimetry (DSC)

DSC measurements were performed with a Du Pont Model 2920 Modulated DSC instrument equipped with a 2200 Thermal Analysis data station. Samples of approximately 10 mg were weighed accurately into aluminum sample pans and the pans were hermetically sealed. An empty sealed pan was used as a reference. The DSC was run from 20 to 250°C at the 10°C/min heating rate and the analysis was performed in a nitrogen atmosphere (50cc/min). Transition enthalpy (ΔH) computed as cal/g were calculated from the area under the curve described by recorded trace and a straight baseline joining T_i (initial transition temperature) and T_c (completion transition temperature).

Statistical Analysis

Available starch measured by enzymatic methods was performed in duplicate and each sample was analyzed in triplicate. The mean, standard errors, mean square errors, one factor ANOVA were done using Super ANOVA software (version 1.11, 1991,

Abacus Concepts, Inc, Bekeley, CA). Mean separations were performed using LSD with the mean square error term at the 95% confidence level.

Results and Discussion

Starch Availability

Available starch obtained for all starchy food differentially prepared in this study is shown in Table 9. For navy beans (experimental line, N 84004), reheating of the soaked, freeze-dried and milled flour (SFM) caused release of starch granules from cells and thus, was available to the hydrolytic enzymes. However, not all cells were broken down by the milling process. The evidence presented in the microscopic image (Plate 5a) of the SFM revealed that intact cells remained. The BAP assays indicated that entrapped starch granules did not undergo hydrolytic degradation with the enzymes. An average of 8.5g of starch per 100g navy bean (dry basis) was available upon reheating of the SFMs. The available starch of cooked (10 min and 40 min), freeze-dried and milled flour (CFM) was significantly lower than that of SFM even when additional heat treatment was applied. This observation confirms previous work by Tovar et al. (1990b) that the difference in susceptibility to enzymatic attack observed between precooked flours and the corresponding raw flours were mainly due to the release of starch granules during milling of uncooked seeds, whereas starch-containing cotyledon cells remained in the precooked red kidney bean flours.

After reheating the CFMs, there was an increase in the amount of available starch. A 10 min additional heating of the cooked flour (CFM_{10}) showed a 7-fold increase in available starch while the treatment of 40 min cooked flour (CFM_{40}) sample increased only 3-fold when compared to the cooked flour which had no reheating treatment. Starch

Table 9. Available starch of flour samples prepared differentially on reheating[1]

	Available starch (g starch / 100 g sample)		
	Soaked	Cooked	
Reheating (min)		10 min	40 min
Navy bean (N84004)			
0	0.30 ± 0.14a	0.95 ± 0.35a	1.10 ± 0.14a
10	8.95 ± 0.21e	6.60 ± 1.98c	3.55 ± 0.07b
20	8.15 ± 0.21de	6.80 ± 0.57c	2.85 ± 0.50b
30	8.75 ± 0.50de	7.70 ± 0.14cd	3.45 ± 0.07b
Pinto bean (Carioca)			
0	0.35 ± 0.07a	1.10 ± 0.42á	0.70 ± 0.71a
10	11.80 ± 2.55d	12.85 ± 0.78de	7.00 ± 0.28bc
20	13.30 ± 4.12de	12.45 ± 1.63d	5.20 ± 2.55b
30	16.60 ± 1.98e	11.10 ± 3.54cd	7.40 ± 0.28bc
Sweet Potato			
0	27.15 ± 1.49a	57.95 ± 3.75b	58.75 ± 0.07b
10	64.00 ± 3.68b	60.10 ± 4.10b	59.85 ± 2.33b
20	63.20 ± 1.98b	58.55 ± 6.29b	61.15 ± 5.59b
30	62.50 ± 0.71b	61.40 ± 2.26b	59.75 ± 0.35b
Wheat			
0	0.20 ± 0.01a	18.25 ± 6.44b	27.40 ± 0.40bc
10	33.95 ± 2.33c	26.50 ± 7.07bc	30.00 ± 1.56bc
20	29.70 ± 4.53bc	27.60 ± 7.21bc	36.10 ± 4.53c
30	32.35 ± 3.18c	29.70 ± 1.13bc	36.10 ± 3.11c

[1] Means within a specific product (columns and rows) followed by different letters are significantly different ($P<0.05$), n = 2

leaching as a consequence of thermal cell wall disruption was observed with polarized light microscope (Plate 5b, c). Tovar (1991) showed a starch release and a melted appearance of particulate structures by scanning electron microscope (SEM) with additional heat treatment. However, some encapsulated starch granules remained intact within a crystallized cell wall. In our study, the rigidity of the cell wall was varied with cooking time. There was a significant difference in available starch between CFM_{10} and CFM_{40} navy bean samples after additional heat treatment (Table 9). Relative starch hydrolysis of navy bean (experimental line N84004) prepared on different cooking time after reheating treatment was shown Figure 5. Reheating of CFM_{10} enabled 80% of starch to be hydrolyzed by the enzymes while reheating of CFM_{40} allowed only 40%. The rigidity of crystallized cell walls in beans cooked for 10 min was significantly less than that of 40 min cooked samples.

Starch availability by reheating of SFM and CFM of pinto beans showed similar trends observed with navy bean flours. Again, the cell wall of cooked pinto beans encapsulating starch granules is a factor lowering the enzymatic availability of starch. However, starches available to the enzymes after reheating of SFM in pinto beans were relatively higher than that of navy beans. Available starch on pinto bean CFM_{10} and CFM_{40} with an additional heating was 11-fold and 6.5-fold increased, respectively when compared to the flour without an additional heating. The incremental increase in available starch when an additional heat treatment was applied to pinto beans (cultivar Carioca) was twice that obtained for navy beans (experimental line, N84004). The results from cooked beans indicate that the longer the cooking time, the harder the rigidity of cell wall of the

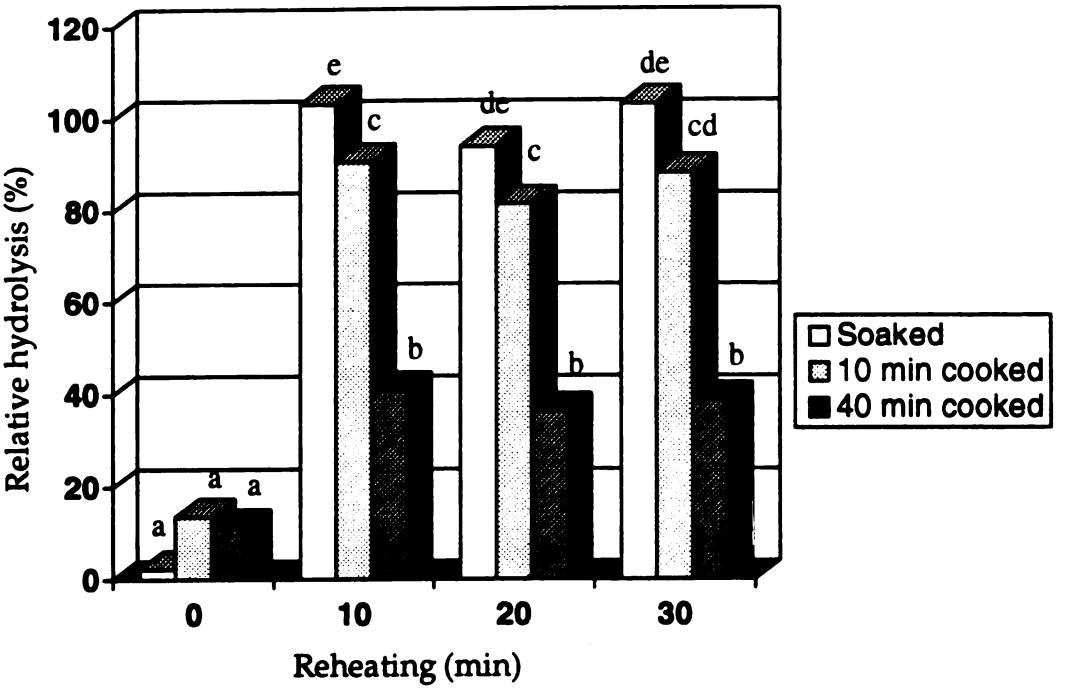


Figure 5. Available starches of navy bean (experimental line, N84004) prepared with different cooking times

cooked beans. Figure 6 demonstrated that relative starch hydrolysis upon reheating. Reheating of CFM_{10} enabled 90% of starch available by the enzymes while reheating of CFM_{40} allowed only about 40%. It is concluded that the rigidity of crystallized cell wall after cooking varies among varieties and process conditions. The factors affecting the rigidity of crystallized cell walls could be due not only to cooking time but also other factors such as age of seeds, storage conditions, cell wall components and the genotype.

Results also demonstrated available starch of SFM and CFM of sweet potato with reheating. About 40% of starch in soaked sweet potato was hydrolyzed by the enzymes even without gelatinization. Reheating of SFM enabled most starches to be available for enzymes due to complete disruption of cellular structure or complete gelatinization. Reheating of CFM showed no significant difference in available starch of both sweet potatoes cooked for 10 min and 40 min. There were no significant differences in the amount of starch hydrolysis, which is maximum, among all flour samples reheated for different times (Figure 7). Cooking caused sweet potato starches to gelatinize and to be retained inside the cells. In contrast to bean flours, starch granules were entrapped within the cells, however, there was no evidence of cell wall crystallization in cooked sweet potato. Most starches were readily available to the hydrolytic enzymes (Plate 7)

Cooking whole wheat increased available starch gradually and dramatically with increased cooking time (Table 9). Sixty percent and eighty-six percent of wheat starch were hydrolyzed by the cooking for 10 min and 40 min, respectively. In raw wheat, the starch is embedded in a protein matrix and this restricts the availability of starch for amylolysis (Holm et al., 1985). The disruption of the protein matrix during cooking

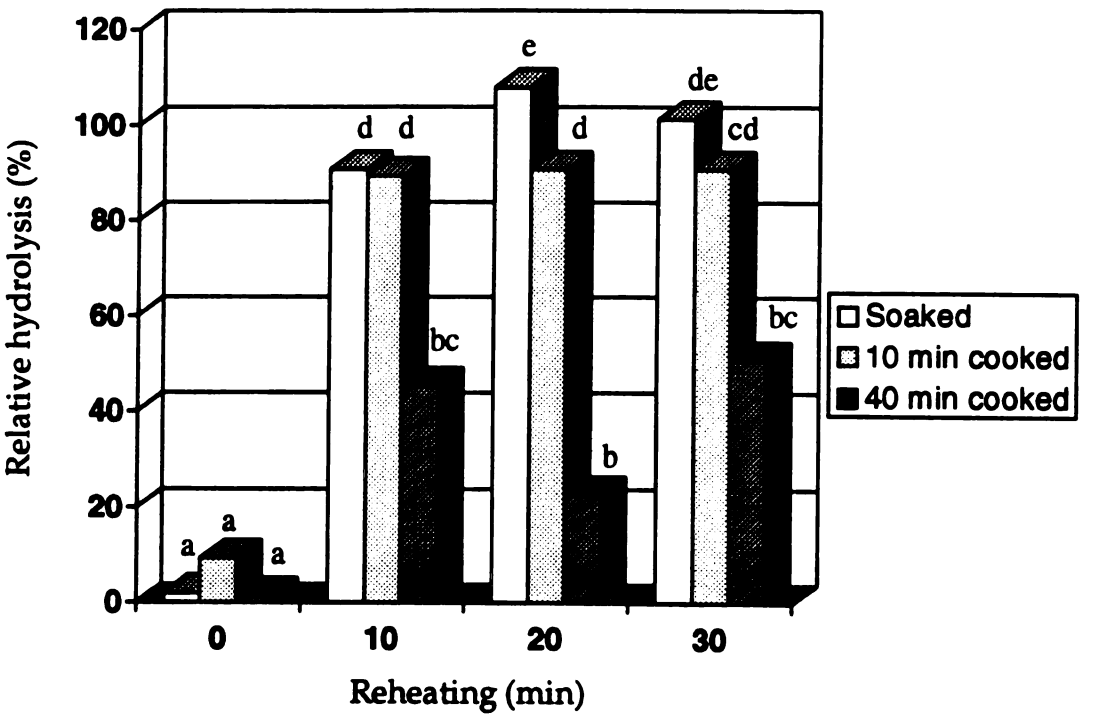


Figure 6. Available starches of pinto bean (var. Carioca) prepared with different cooking times

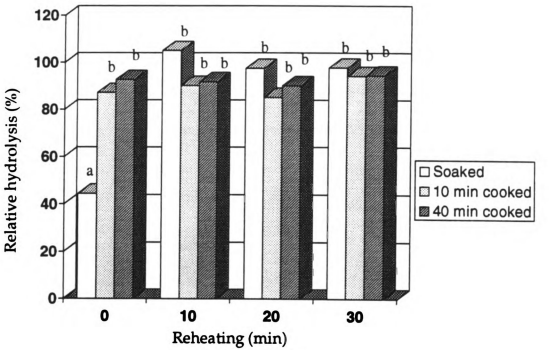


Figure 7. Available starches of sweet potato prepared with different cooking times

(reheating) contributed to the increased starch hydrolysis. No hydrolysis was detected in raw ground wheat. However, the release of starch granules from the wheat endosperm by grinding soaked and freeze-dried sample made almost all starch available to the enzymatic hydrolysis (Figure 8). Reheating of the SFM wheat flour led to the gelatinization of all the starches and thus approached the maximum attainable availability. Holm and Björck (1988) reported that the availability to α-amylase of starch in the drum-dried flours was restricted substantially by the protein structure. In our study the reheating of the cooked-milled wheat samples increased the availability of starch by eliminating the obstacle to the enzymatic hydrolysis. Reheating the wheat flour showed the same level of starch availability regardless of reheating time. Once the highly organized structures within starch granules are destroyed, the availability is not increased further by disintegration of the granule.

Polarized light microscopy

Plate 5 shows polarized light microscopy image of reheated (10 min) bean flour. All free starches were gelatinized but the cells unbroken by milling retained adhesion in the soaked, freeze-dried and milled flour(SFM) sample. Most cell walls were broken by reheating but some encapsulated starch granules remained intact in the CFM_{10} sample while more intact cells remained after reheating in the CFM_{40} sample due to rigidity of the cell walls. A view of cooked navy beans under increased magnification focused directly on the cell wall is shown in Plate 6. A thick cell wall was evident (likely due to crystallization) on both cooked beans (10 min and 40 min) however there was no apparent difference on thickness due to the heating period. Polarized light microscopy image of

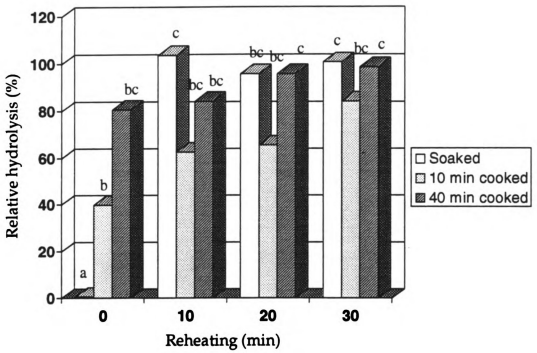


Figure 8. Available starches of wheat prepared with different cooking times

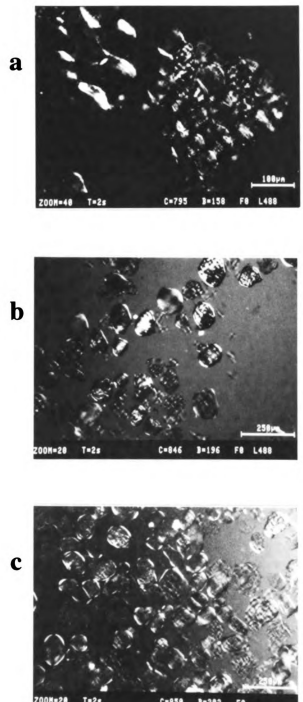
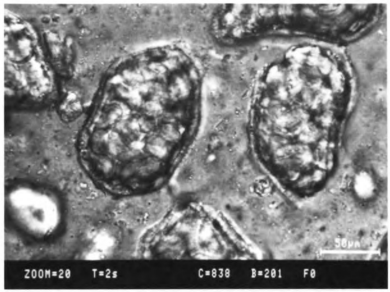
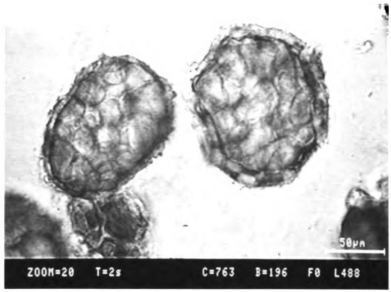


Plate 5. Polarized light microscopy image of reheated (10 min) bean flour prepared after differential cooking treatments
a) soaked, b) 10 min cooked and c) 40 min cooked



a



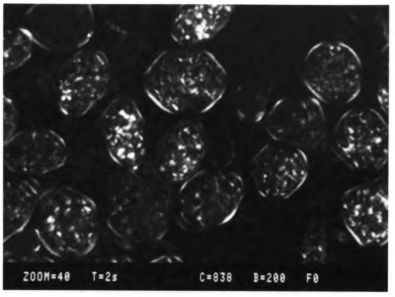
b

Plate 6. Light microscopy images illustrating cell wall structure for differentially cooked navy beans
a) 10 min cooked b) 40 min cooked

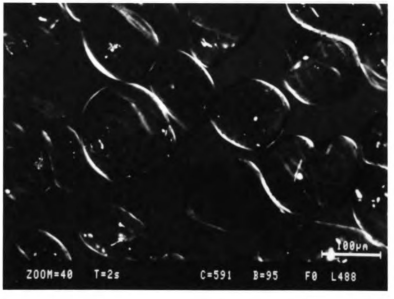
cooked (40 min) bean and sweet potato were shown in Plate 7. It is quite clear that starch granules were entrapped within cell walls in both cooked samples. Crystallization occurred within cell walls of beans and ungelatinized starch remained within the cells (Plate 7a). However, no evidence of crystallization occurred within cell walls of cooked sweet potato and it is clear that most starches were gelatinized since birefringence is lost within the cells (Plate 7b).

Differential scanning calorimetry (DSC)

DSC was used to characterize the endothermic heat flow of flour samples. DSC thermograms obtained are illustrated in Figure 9. The DSC scans constitute measurements of pinto bean flours, soaked flour (SFM), 10 min and 40 min cooked flour (CFM_{10} and CFM_{40}). All flour samples exhibited two endothermic transitions. One is over a temperature range 95 to 180°C, which may be due to dissociation of a crystalline structure of bean flour. The broad range of the first peak obtained from bean flours (solid state and mixture of food components) includes the melting temperature of retrograded starch and resistant starch, which was estimated at ~ 160°C. These results are consistent with those reported by Sievert and Pomeranz (1990 and 1989) and Ring et al. (1987). The other endothermic transition occurred in the range from 190 to 230°C. The peak temperature was ~210°C. Transition enthalpies ΔH (cal/g) measured on SFM, CFM_{10} and CFM_{40} of pinto bean were 0.59, 1.51 and 2.09, respectively. The findings from thermoanalytic measurements indicated that a crystalline structure which is more stable than resistant starch was formed during cooking processes. Further research is needed on separation and characterization of the crystallized cell wall.



a



b

Plate 7. Polarized light microscopy images of bean and sweet potato tissue cooked for 40 min

a) whole bean b) whole sweet potato

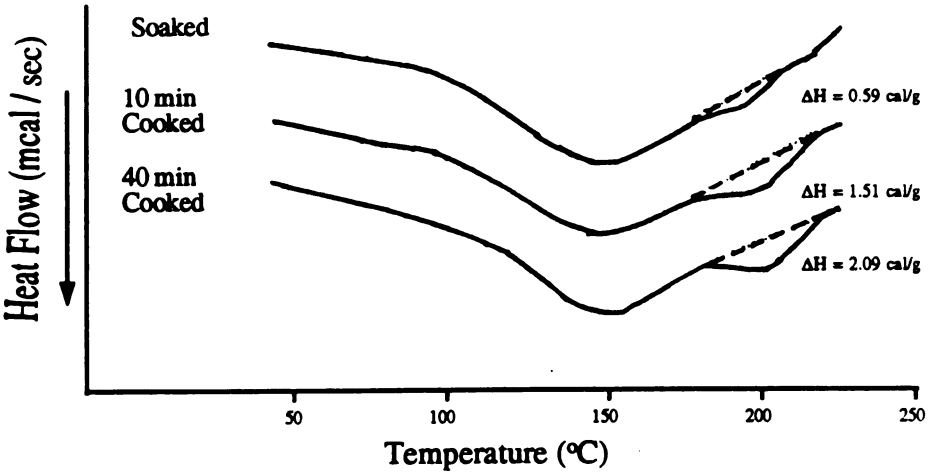


Figure 9. DSC thermograms of pinto bean flour prepared after differential cooking treatments

Conclusions

The rigidity of cell wall crystallized during cooking beans varied with cooking time. The longer the cooking time, the greater the rigidity of cell wall of the cooked beans. The cell wall integrity varied between bean type and among process conditions. Starch granules of cooked beans encapsulated within crystallized cell wall are restricted from enzymatic hydrolysis while starches of cooked sweet potato are readily available since no crystallization occurs within cell wall. Based on DSC thermograms, the crystalline structure formed during cooking processes is more stable than resistant starch. These findings are consistent with physiological responses (low glycemic index, flatulence), commonly observed with bean consumption.

H_o: The crystallization of cell wall in cooked beans is not a limiting factor associated with starch gelatinization and bioavailability.

Reject the H_o as stated and conclude that the crystallization of cell wall is a critical limiting factor for starch gelatinization and bioavailability.

CHAPTER 3 CHARACTERIZATION OF BRINE OBTAINED FROM CANNED BEANS POSSESSING DIFFERENTIAL QUALITY

Introduction

During thermal processing of the beans differential amounts of nutrients are leached into the cooking brine which lowers consumer acceptability because of the formation of cloudy, viscous, or grainy brine. Moreover, the nutritional value of beans is lowered if the brine is discarded. Much research has been conducted on nutrient loss and on approaches to minimize nutrient loss through processing as well as genetic improvement (Augustin and Klein, 1989). A better understanding of the relationship between canned bean quality and brine composition would provide insight to appropriate processing and selection of breeding lines. The purpose of this study is to investigate plant materials selected for their differential canned bean and brine characteristics and to characterize the leached components present in the brine of these materials.

Experimental Plan

Much effort has been conducted to improve canning quality of dry edible bean genetically and technologically by USDA (G.L. Hosfield, Research Genetist) and Michigan State University (M.A. Uebersax and J.D. Kelly, Professors). Navy beans (*Phaseolus vulgaris*) grown at Michigan State University Agricultural Experimental Station in 1993 crop year have shown diversities on characteristics of brine and integrity of cooked bean. Eight cultivars (and/or experimental lines) of navy beans were selected based on visual canning quality of beans and brines. The quality characteristics were categorized into two intersecting groups: 1) bean integrity (intact or split), 2) brine

viscosity (thick / viscous / cloudy or thin / fluid / clear). Relationships of the categorized bean and brine with physical characteristics of canned beans and chemical composition of brine were investigated.

The null hypothesis tested in this research is stated as follows:

H_o: the compositional components of brine and physical bean quality after processing are not controlled by genetic background.

Materials and Methods

Thermal Processing Procedure

Moisture content of dry bean samples was determined using a Burrows Digital Moisture computer 700 (Burrows equipment Co., Evanston, IL). Dry bean samples (equivalent to 100 g dry solids) were placed in nylon mesh bags and soaked in water for 30 minutes at room temperature (21°C). Immediately after this soaking, beans were transferred to a 88°C water bath for an additional 30 min. All soaking was performed in water containing 100 ppm calcium ion prepared using distilled water and reagent grade $CaCl_2$. After hot soaking, beans were momentarily cooled under cold tap water, completely drained and weighed. After weighing, beans were filled into 303x406 cans and covered with boiling brine (1.249% NaCl, 1.561% sucrose and 100 ppm calcium). Cans were sealed and processed in a still retort for 45 minutes at 116°C. After thermal processing, cans were uniformly cooled to 38°C under cold tap water and stored for two weeks at room temperature before quality evaluation. The storage period after processing permits canned beans to completely equilibrate with the canning medium.

Canning Quality Evaluation

After the cans were opened, the free flow brine was decanted and retained for observation and analyses. The washed-drained weight of processed beans was determined by decanting the can contents on a number 8 mesh sieve, rinsing them in cold tap water to remove adhering brine, and draining for 2 min on the sieve positioned at a 15° angle. One hundred grams of washed processed beans was weighed and transferred to a sample dish for color measurement. Texture was determined by using a Texture Test System (Model TSM-90) equipped with the FT 3000 transducer and a Standard Multiblade Shear Compression Cell (Model CS-1, Food Technology Corp., Reston, VA). The water content of canned beans (final moisture percentage) was determined from the 100g texture samples. These were oven dried at 80°C until the weight remained constant. The identification and descriptions of navy bean samples selected for use in this study are shown in Table 10.

Brine Characteristics

Representative samples of brines were obtained for chemical analysis through stirring and pipeting the brine suspensions. Caution was exercised to assure a homogeneous distribution.

Total solid content

Approximately 3 g of brine sample were weighed in a pre-tarred aluminum tray and dried in a vacuum oven at 75°C with 27"Hg overnight.

% total solid = dried weight of sample (g) / initial weight of sample (g) × 100

Table 10. Visual characteristics of canned navy beans and brine

	Quality Class Description	
Cultivar / Breeding line	Bean integrity	Brine appearance
Fleetwood	Intact	Thick / Viscous / Cloudy
Crestwood	Intact	Thick / Viscous / Cloudy
Midland	Split	Thick / Viscous / Cloudy
I 92912	Split	Thick / Viscous / Cloudy
Mayflower	Split	Thin / Fluid / Clear
Seafarer	Split	Thin / Fluid / Clear
N 90563	Intact	Thin / Fluid / Clear
N 90598	Split	Thin / Fluid / Clear

Alcoholic insoluble solids

About 3 g of brine sample were taken in a pre-tarred aluminum tray and four volumes of 85% ethanol were added. They were stirred with a glass rod and incubated for an hour. The alcohol supernatants were decanted and precipitates were dried at 40°C in convection oven overnight.

$$\% \text{ alcoholic insoluble solids} = \frac{\text{dried weight of alcohol precipitates (g)}}{\text{initial weight of samples (g)}} \times 100$$

Protein content (Kjeldahl nitrogen analysis)

The protein content of the brine samples was determined using AACC method 46-12, 1983. Five milliliters of concentrated sulfuric acid and one catalyst tablet were added into each digestion tube containing pre-weighed sample (ca 5 g of brine). This tube was then slowly heated to 400°C until the digestion was completed (approximately 5 hr). The protein content was determined on a dry basis using a nitrogen conversion factor of 6.25. The following equation was used to calculate the % protein of sample.

$$\% \text{ protein of sample} = \frac{\text{(ml of HCl titrated) (Nomality of HCl) (1.4007) (6.25)}}{\text{Sample weight (g)}}$$

Total starch (Kainuma et al., 1981)

Dried powders prepared from alcohol precipitation were used to measure the starch content. The starch content was quantitatively determined using an enzymatic method. This method is based on enzymatic hydrolyses using β-amylase and pullulanase

and thus termed "BAP" method. The enzymes, β-amylase and pullulanase, were used to hydrolyze the alkali gelatinized starch sample and Phenol-sulfuric acid assay was used to measure maltose units in the digested sample. The total starch content in each sample was obtained by multiplying the mg of maltose in each sample by a factor of 0.95 to account for the weight of the water gained during the hydrolysis of starch to maltose.

Dietary fiber content (Lee et al., 1992)

Sample Preparation Canned bean brine contains relatively high amounts of sucrose (1.56% by weight, formula preparation). The desugaring procedure by extracting with 85% EtOH was employed prior to determining dietary fiber. Five hundred ml of the 85% ethanol were added into the centrifuge tube containing about 50g of homogenized brine sample. After stirring and incubation for an hour, the samples were centrifuged at 4000 rpm for 10 min, the supernatant decanted and the residues dried overnight at 40°C. The dried sample was used to measure dietary fiber, pectin content, and total starch. The enzymatic method of Lee et al. (1992) for determining insoluble and soluble dietary fiber is outlined in Figure 10.

Digestion Approximately 1g of dried alcohol precipitate powder was accurately weighed into 600mL beaker and 40mL of MES/TRIS buffer (0.05M, pH 8.2) was added. After stirring on magnetic stirrer until sample is completely dispersed, 50μL of heat stable α-amylase solution (No.A-0164) was added and incubated for 35 min at 95°C with continuous agitation. After this starch hydrolysis, the treated solution was cooled to 60°C and any precipitate resuspended by dispersion with a spatula and rinsed with 10 mL H_2O. Protein hydrolysis was carried out by adding 100μL protease (No.P-3910) solution and

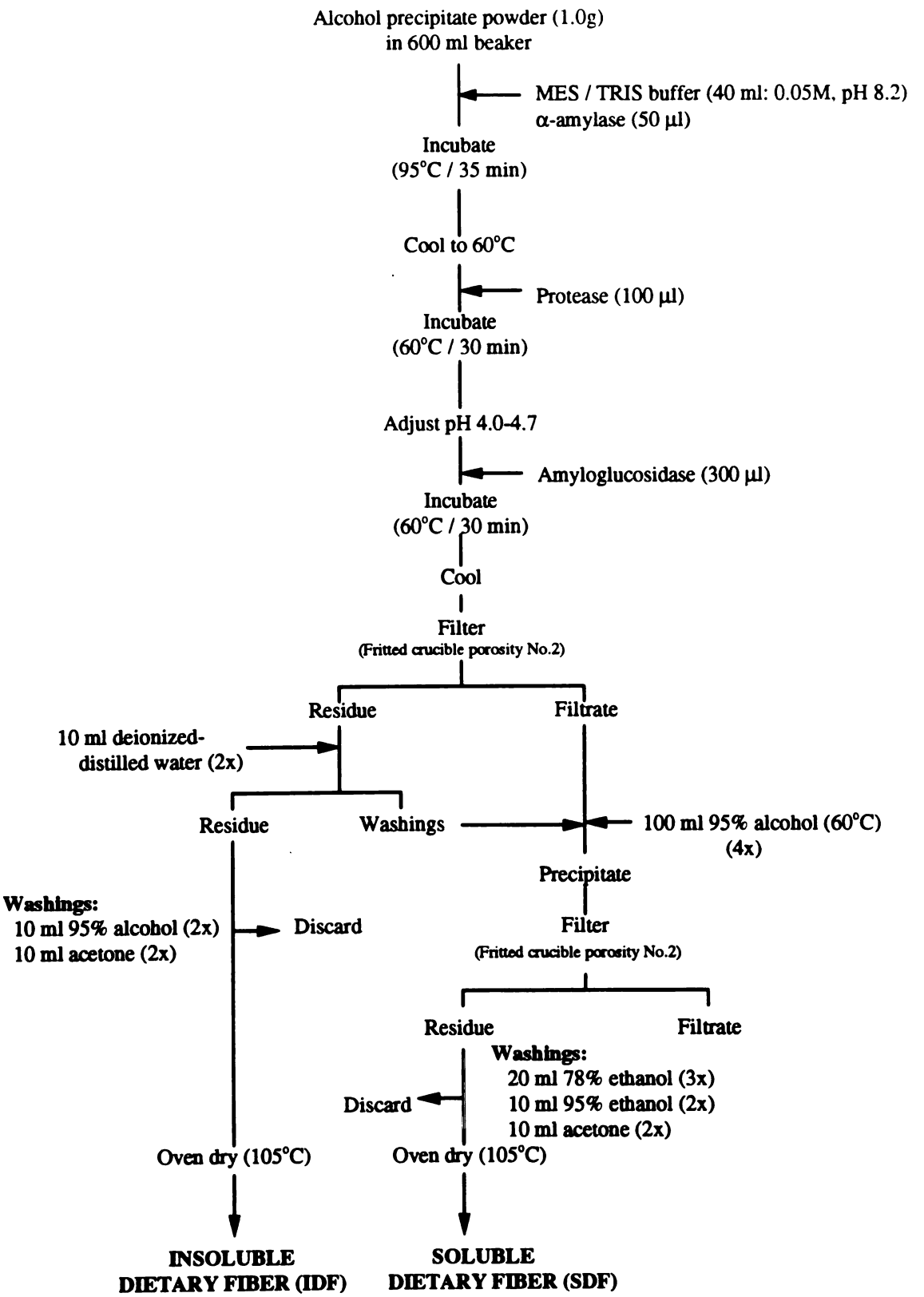


Figure 10. Flowchart of the enzymatic method to determine the insoluble and soluble dietary fiber of alcoholic precipitated flour (Lee et al., 1992)

incubation at 60°C for 30 min with continuous agitation. Following protein hydrolysis, 5mL of 0.561N HCI was dispensed into beaker and pH was adjusted to 4.0-4.7 at 60°C using 1N HCI or 1N NaOH solution. Starch hydrolysis was catalyzed using 300µL of amyloglucosidase (No. A-9913) solution maintained at 60°C for 30 min with constant agitation.

Insoluble Dietary Fiber The solution was cooled and filtered through a previously ashed and weighed fritted glass crucible (No.2) containing celite, using the Fibertec System E 023 Filtration Module (Tecator, England). The residue was washed with two 10 ml portions of deionized-distilled water. The filtrate and water washings were set aside for the determination of soluble fiber. The residue was washed with two 10 ml portions of 95% ethanol and then with two 10 ml portions of acetone. The crucible containing the residue was dried overnight in a 105°C (221°F) air oven. Residue from one set of duplicates was analyzed for protein, while the duplicate was incinerated overnight at 525°C (977°F) to analyze for ash content. Blank determinations were maintained throughout the procedure. Percent Insoluble Dietary Fiber (IDF) was calculated using the following formula:

$$\text{IDF (\%)} = \text{mg insoluble residue} - \frac{[\,(\%\ \text{protein in residue} + \%\ \text{ash in residue}) \times \text{mg residue}\,] \times 100}{\text{sample weight (mg)}}$$

Soluble Dietary Fiber The weight of combined filtrate and water washings was adjusted to 100 g with water. Four 100 ml portions of 95% ethanol preheated to 60°C (140°F) was added and allowed to precipitate at room temperature for at least an hour. The enzyme digest was filtered through previously ashed and weighed fritted crucibles

containing Celite. The residue was sequentially washed with three 20 ml portions of 78% ethanol, two 10 ml portions of 95% ethanol and finally two 10 ml portions of acetone. The residues were dried and analyzed for protein and ash following the same protocol for the insoluble fiber. Percent Soluble Dietary Fiber (SDF) was calculated as follows:

$$SDF(\%) = \text{mg soluble residue} - \frac{[\,(\%\ \text{protein in residue} + \%\ \text{ash in residue}) \times \text{mg residue}\,] \times 100}{\text{sample weight (mg)}}$$

Uronic acid

Estimates of pectin content were made based on total uronic acid. Preparation and digestion steps were the same as for the dietary fiber. Zero point three (0.3) mL of the hydrolysate were mixed with 0.3mL of a solution containing 2g of sodium chloride and 3g of boric acid / 100ml in a 50-mL tube. Five mL of concentrated sulfuric acid was added and vortex-mixed. The tube was placed in heating block at 70°C for 40 min and cooled to room temperature.

When cool, 0.2 mL of dimethylphenol solution (0.19 in 100 mL of glacial acetic acid) was added and vortex-mixed immediately. Between 10 and 15 min later, the absorbance at 400nm and 450nm were read against a water reference. The difference obtained by subtracting the reading at 400nm from that at 450nm was used to calculate sample concentration with glucuronic acid standards over the range 0.025 to 0.125 mg/mL.

Results and Discussion

The description of visual characteristics of the canned beans and brine used in this study are presented in Table 10. The integrity of cooked beans was divided into two groups: intact and split beans. Brine characteristics was grouped by viscosity and appearance: thick / viscous / cloudy and thin / fluid / clear. Brine characteristics was not related with integrity of cooked beans. Cooked beans whose brine appearance was viscous and cloudy showed either intact or split. Similar observation was made with the cooked beans in which brine appearance was fluid and clear.

Under identical process conditions, the eight navy bean cultivars selected for study exhibited canning quality differences (Table 11). Soaking dry beans before canning is considered as a necessary step to decrease cooking time, increase drained weight and ensure uniform bean expansion in the can during processing (Nordstorm and Sistrunk, 1977; Quast and da Silva, 1977). Soaked weight indicates the seed hydration ability during soaking treatment. The hydration ability during soaking did not influence the potential for bean breakage, i.e., there is no apparent relationship between bean breakage and soaked weight. However, soaked weights of beans in which brine appeared thick and viscous was slightly less than that obtained for beans in which brine appeared thin and fluid. Seed coat characteristics were suggested to be major factors in controlling seed hydration during soaking (Smith and Nash, 1961; Quast and da Silva, 1977) and may be associated with soluble solid leaching.

The processed yield of canned beans was determined by its washed drained weight. A high water holding capacity of beans with an intact seed coat is one of the most desired

Table 11. Canning characteristics of eight selected navy beans[1]

Cultivar / Breeding line	Appearance Bean	Appearance Brine	Soaked Wt (g)	Drained Wt (g)	Texture (N)	Total Solids (%,db)
Fleetwood	Intact	Thick / Cloudy	206.9	301.0	657.7	30.5
Crestwood	Intact	Thick / Cloudy	212.4	284.4	842.2	32.8
Midland	Split	Thick / Cloudy	218.4	292.8	906.4	31.4
I 92910	Split	Thick / Cloudy	207.8	287.2	818.2	31.4
May Flower	Split	Thin / Clear	213.0	283.3	882.3	32.2
Seafarer	Split	Thin / Clear	224.0	278.4	826.2	31.1
N 90563	Intact	Thin / Clear	220.2	277.0	954.5	33.0
N 90598	Split	Thin / Clear	216.4	284.4	633.7	30.7

[1] N = 1

Table 12. Average value of canning characteristics of eight selected navy beans grouped by visual appearance[1]

Category of appearance	Soaked Wt (g)	Drained Wt (g)	Texture (N)	Total Solids (%,db)
Bean				
Intact[2]	213.2 ± 6.7a	287.5 ± 12.3a	818.1 ± 149.9a	31.4 ± 0.9a
Split[3]	215.9 ± 6.0a	285.2 ± 5.3a	813.4 ± 107.1a	31.8 ± 1.0a
Brine				
Viscous / cloudy[4]	211.4 ± 5.3a	291.4 ± 7.3b	806.1 ± 105.7a	31.5 ± 1.0a
Fluid / clear[5]	218.4 ± 4.8a	280.8 ± 3.6a	824.2 ± 137.4a	31.8 ± 1.0a

[1] Means within a category of appearance (columns and rows) followed by different letters are significantly different ($P<0.05$).
[2] N=3, [3] N=5, [4] N=4 and [5] N=4.

overall product quality characteristics in canned bean products. Differences in drained weight occurred among the cultivars studied (Table 11). The Fleetwood cultivar possessed highest drained weight (301.0 g) with intact bean appearance. Drained weight of cooked beans in which brine appearance was thick and viscous was significantly higher than that obtained for cooked beans in which brine appearance was thin and fluid (Table 12).

Bean texture is also a primary canning quality character because texture affects the perceived stimuli for chewing and , hence, influences to a large degree a consumer's acceptance of a food product. Beans may be unacceptable if they are perceived as either "too firm" or "too soft" after cooking. The experimental line N 90563, in which the bean is intact and brine is fluid and thin, showed highest texture value (954.5 N). The alternate experimental line N 90598 showed lowest texture value (633.7 N), in which the bean appearance was split and brine character was fluid and thin (Table 11). Bean integrity appeared to have an associated relationship with texture value. Hosfield and Uebersax (1985) presented results showing that a firming effect of associated with addition of $CaCl_2$ during soaking and processing was helpful in decreasing seed coat splitting during canning. In this study however, Midland, in which bean appearance is split showed relatively high texture value (906.4 N) while Fleetwood, in which bean appearance is intact showed relatively low texture value (657.7 N). Further research designed to elicit control factors determining texture value of cooked beans and cultivar quality is warranted.

The amount of bean solids (%, w/w) is another quality term used for determining water holding capacity of canned beans. Theoretically, percent (%) bean solids and drained weight of canned beans demonstrate an inverse relationship. However, Fleetwood showed lowest total solids with highest drained weight and N 90563 possessed lowest water holding capacity (highest total solids) resulting in lowest drained weight (Table 11). Average value of total solids in each group showed no significant differences among treatments (Table 12).

Table 13 presents proximate composition of the brine of the canned navy beans. Average percentage of brine composition grouped by visual appearance is shown in Table 14. Solids in viscous brine were higher than those in fluid brine. The amount of protein in viscous brine was usually higher than that in thin brine. Most of the solids consisted of soluble dietary fiber (SDF) and starch ranging from 61.2% to 41.6%. From Tables 13 & 14, it is evident that starch leakage from the bean was not related with bean breakage. Fleetwood was mostly intact but had higher starch content in the brine whereas N 90598 had severely split bean but had less starch in brine. In general, viscous brine contained more SDF and protein than did the fluid brine. The amount of insoluble dietary fiber (IDF) appeared to have some effect on viscosity of the brine. In summary, there was no one specific component identified which adequately served as a single determinant of bean brine viscosity. Combinations of the components such as protein, soluble dietary fiber and starch and their interactions are suspected to be the determining factors. Further studies on brine composition are required to assess the effects of each component for their individual contributions toward viscosity changes in brine.

Table 13. Percentage of proximate composition in brine[1]

Cultivar /	Appearance		Solid		Protein		IDF[2]		SDF[3]		Pectin		Starch	
Bleeding line	Bean	Brine	Total	E-ppt[4]	Total	E-ppt	Total	E-ppt	Total	E-ppt	Total	E-ppt	Total	E-ppt
Fleetwood	Intact	Thick	9.2	7.6	9.5	11.4	1.6	1.9	36.9	44.4	7.1	8.6	24.3	29.3
Crestwood	Intact	Thick	9.1	7.0	13.3	17.1	1.3	1.7	40.0	51.6	7.3	9.5	17.9	23.1
Midland	Split	Thick	8.0	6.3	13.6	17.4	1.9	2.4	38.6	49.1	7.1	9.0	21.4	27.3
I 92910	Split	Thick	8.7	6.4	12.9	17.5	1.1	1.5	24.9	33.8	4.7	6.4	24.1	32.7
Mayflower	Split	Thin	6.8	4.9	8.4	11.7	0.5	0.8	19.0	26.3	6.2	8.6	22.6	31.2
Seafarer	Split	Thin	7.8	5.9	8.4	11.2	0.3	0.5	31.0	41.3	5.9	7.9	23.0	30.7
N 90563	Intact	Thin	7.3	4.4	10.5	17.5	0.1	0.1	26.4	43.8	5.2	8.6	15.5	25.7
N 90598	Split	Thin	8.2	6.3	7.7	9.9	1.8	2.3	34.7	44.6	6.5	8.4	21.0	27.0

[1]Calculated as dry base, N=1
[2]Insoluble dietary fiber
[3]Soluble dietary fiber
[4]Ethanol - precipitate

Table 14. Average percentage of brine composition of eight selected navy beans grouped by visual appearance[1]

Category of appearance	Solid	Protein	IDF	SDF	Pectin	Starch
Bean						
Intact[2]	8.5 ± 1.1a	11.1 ± 2.0a	1.0 ± 0.8a	34.4 ± 7.1a	6.5 ± 1.2a	19.2 ± 4.5a
Split[3]	7.9 ± 0.7a	10.2 ± 2.8a	1.1 ± 0.7a	29.6 ± 7.8a	6.1 ± 0.9a	22.4 ± 1.2a
Brine						
Viscous / cloudy[4]	8.8 ± 0.5b	12.3 ± 1.9b	1.5 ± 0.4a	35.1 ± 6.9a	6.6 ± 1.2a	21.9 ± 3.0a
Fluid / clear[5]	7.5 ± 0.6a	8.8 ± 1.2a	0.7 ± 0.8a	27.8 ± 6.8a	6.0 ± 0.6a	20.5 ± 3.5a

[1] Means within a category of appearance (columns and rows) followed by different letters are significantly different ($P<0.05$).
[2] N=3, [3] N=5, [4] N=4 and [5] N=4.

Statistical analysis (Correlation matrix)

Several studies have examined the relationships among and within physico-chemical characteristics and canning quality of beans (Uebersax, 1985;; Srisuma, 1989; Ruengsakulrach, 1990; Hsia, 1994). In this study, an effort was made to investigate possible correlations among the various parameters.

The relationships among visual appearance of cooked bean and brine, bean canning quality and brine composition are shown in a correlation matrices presented in Table 15. Lower left side of the matrices was obtained from the relationships with brine composition based on total solids while upper right side of the matrices was the relationship based on alcohol insoluble solids. The correlation coefficients between visual appearance and bean integrity (canning quality) in lower left side of the matrices demonstrate similar levels of significance to those in upper right side because the values are not dependent on either total solids or alcohol insoluble solids of the brine.

Relationships between bean integrity and canning quality characteristics

Correlation analysis was conducted by grouping and designating the bean integrity as "intact = 1" and "split = 2". No correlation was found between cooked bean integrity and texture (r = - 0.022). As described above, texture of cooked bean could not be estimated only by degree of visual bean integrity, either intact or split, among bean samples selected in this study. The correlation between bean integrity and drained weight is also very low (r = - 0.149). Total solids of cooked beans exhibited a negative

Table 15. Correlation matrix indicating relationships among visual appearance, canning quality and brine composition

		Correlation Coefficient (r)											
		Visual Appearance		Bean Canning Quality				Brine Composition					
		Bean Integ.	Brine Char.	Soaked Wt	Drained Wt	Texture	Total Solid	% Solid	Protein	Starch	SDF	IDF	Pectin
Visual	Bean Integ.		-.258	.239	-.149	-.022	-.410	-.185	-.271	.609	-.478	.163	-.472
Appearance	Brine Char.	-.258		-.629	.726*	-.085	-.129	.744*	.511	-.092	.373	.600	.000
Bean	Soaked Wt	.239	-.629		-.660	.405	.177	-.594	-.060	-.219	.223	-.411	.210
Canning	Drained Wt	-.149	.726*	-.660		-.485	-.571	.749*	-.076	.122	.193	.708*	.088
Quality	Texture	-.022	-.085	.405	-.485		.779*	-.660	.682	-.111	-.119	-.553	.113
	Total Solid	-.410	-.129	.177	-.571	.779*		-.566	.615	.479	.005	-.533	.339
	% solid	-.388	.775*	-.573	.603	-.555	-.355		-.113	-.080	.461	.735*	.049
Brine	Protein	-.193	.791*	-.243	.232	.450	.358	.413		-.315	.269	-.047	-.018
Composi-	Starch	.534	.242	-.413	.540	-.485	-.786*	.219	-.209		-.797*	-.175	-.782*
tion	SDF	-.333	.526	.014	.459	-.359	-.269	.649	.341	-.118		.419	.552
	IDF	.089	.612	-.414	.742*	-.567	-.537	.621	.299	.287	.695		.165
	Pectin	-.248	.340	-.094	.522	-.307	-.176	.359	.072	.004	.746*	.617	

* statistically significant at 0.05 porbability level

correlation with bean integrity (r = - 0.410). Intact beans showed high value of total solids while split beans possessed low value of total solids.

Relationships between brine characteristics and canning quality characteristics

Correlation analysis was conducted by grouping and designating the brine characteristics as "thin / clear = 1" and "thick / cloudy = 2". Drained weight and the thickness of brine showed fairly strong correlation (r = 0.726), which is statistically significant at 95 % confidence level ($P<0.05$). As the viscosity in brine increases, the drained weight of cooked beans increased. High viscous brine was associated with low soaked weight of beans (r = - 0.629). Texture of cooked bean showed no correlation with brine viscosity and clarity (r = - 0.085). A non-significant negative correlation was obtained between total solids and brine appearance.

Relationships between bean integrity and brine composition

The amount of starch in brine showed close relationship with visual integrity of cooked bean (r = 0.609, AIS; r = 0.534, TS). Thermal processing broke down cellular structure and released cellular components into the brine matrix. The greater the percent breakdown of bean resulted increased amount of starch in the brine. However, negative correlations were obtained between bean integrity for either the amount of total protein or percent total solids in brine.

Relationships between brine characteristics and brine composition

As can be seen, strong positive correlations were obtained between brine characteristics and percent total solids in the brine. Thick and cloudy brines contain high percent solids estimated by both total solids and alcohol soluble solids. The amount of

protein in brine is strongly and positively correlated with brine viscosity and clarity (r = 0.791, TS; r = 0.511, AIS). The more protein present in the brine, the greater the brine viscosity. Another factor affecting brine viscosity is the amount of insoluble dietary fiber (IDF). An important physical property of IDF is its capacity to bind water. A high correlation between amount of the insoluble dietary fiber in the brine and brine viscosity was observed (r = 0.612). No significant correlations were found between the amount of starch in brine and brine viscosity.

Statistical analysis (T-test)

Two tailed T-test was conducted for variables grouped both by brine characteristics and bean integrity based on total solids and alcohol insoluble solids (Table 16 and Table 17). The statistical analysis demonstrated the same trend as shown on the correlation study.

Table 16. T-test for variables grouped by brine charcteristics based on total solids and alcohol insoluble solids

			Total Solids			Alcohol Insoluble Solids		
	Viscosity of Brine	N	Mean	Standard Deviation	Significance (2-tailed)	Mean	Standard Deviation	Significance (2-tailed)
Drained Weight	Thin	4	280.8	3.6	0.041**	280.8	3.6	0.041**
	Thick	4	291.4	7.3		291.4	7.3	
Soaked Weight	Thin	4	218.4	4.8	0.095	218.4	4.8	0.095
	Thick	4	211.4	5.3		211.4	5.3	
Texture	Thin	4	824.2	137.4	0.842	824.2	137.4	0.842
	Thick	4	806.1	105.7		806.1	105.7	
Total Solids	Thin	4	31.8	1.0	0.761	31.8	1.0	0.761
	Thick	4	31.5	1.0		31.5	1.0	
Solids in brine	Thin	4	7.5	0.6	0.024**	5.4	0.9	0.034**
	Thick	4	8.8	0.5		6.8	0.6	
Protein	Thin	4	8.8	1.2	0.019**	12.6	3.4	0.195
	Thick	4	12.3	1.9		15.9	3.0	
Starch	Thin	4	20.5	3.5	0.563	28.7	2.7	0.828
	Thick	4	21.9	3.0		28.1	4.0	
SDF	Thin	4	27.8	6.8	0.181	39.0	8.6	0.363
	Thick	4	35.1	6.9		44.7	7.9	
IDF	Thin	4	0.7	0.8	0.107	0.9	1.0	0.116
	Thick	4	1.5	0.4		1.9	0.4	
Pectin	Thin	4	6.0	0.6	0.410	8.4	0.3	1.000
	Thick	4	6.6	1.2		8.4	1.4	

** Significant difference at 95% confidence level ($p<0.05$).

Table 17. T-test for variables grouped by bean integrity based on total solids and alcohol insoluble solids

			Total Solids			Alcohol Insoluble Solids		
	Integrity of Bean	N	Mean	Standard Deviation	Significance (2-tailed)	Mean	Standard Deviation	Significance (2-tailed)
Drained Weight	Intact	3	287.5	12.3	0.724	287.5	12.3	0.724
	Split	5	285.2	5.3		285.2	5.3	
Soaked Weight	Intact	3	213.2	6.7	0.569	213.2	6.7	0.569
	Split	5	215.9	6.0		215.9	6.0	
Texture	Intact	3	818.1	149.9	0.959	818.1	149.9	0.959
	Split	5	813.4	107.1		813.4	107.1	
Total Solids	Intact	3	32.1	1.4	0.313	32.1	1.4	0.313
	Split	5	31.4	0.6		31.4	0.6	
Solids in brine	Intact	3	8.5	1.1	0.342	6.3	1.7	0.660
	Split	5	7.9	0.7		6.0	0.6	
Protein	Intact	3	11.1	2.0	0.647	15.3	3.4	0.516
	Split	5	10.2	2.8		13.5	3.6	
Starch	Intact	3	19.2	4.5	0.172	26.0	3.1	0.109
	Split	5	22.4	1.2		29.8	2.5	
SDF	Intact	3	34.4	7.1	0.420	46.6	4.3	0.231
	Split	5	29.6	7.8		39.0	9.0	
IDF	Intact	3	1.0	0.8	0.834	1.2	1.0	0.700
	Split	5	1.1	0.7		1.5	0.9	
Pectin	Intact	3	6.5	1.1	0.553	8.9	0.5	0.238
	Split	5	6.1	0.9		8.1	1.0	

** Significant difference at 95% confidence level ($p<0.05$).

Conclusions

Brine characteristics (thick / viscous / cloudy and thin / fluid / clear) were not related with integrity (intact and split) of cooked beans. The viscous brine contained more SDF and protein than did the fluid brine. There was no one specific component identified which adequately served as a single determinant of bean brine viscosity. No strong correlation was found between cooked bean integrity and canning quality characteristics. Relationships between brine characteristics and canning quality characteristics demonstrated that drained weight was positively correlated with brine thickness. Soaked weight of beans was negatively correlated with brine viscosity. The amount of protein and IDF in brine was strongly correlated with increased brine viscosity.

H_o: the compositional components of brine and physical bean quality after processing are not controlled by genetic background.

Reject the H_o as stated and conclude that the compositional components of brine and physical quality after processing are controlled by genetic background.

SUMMARY AND CONCLUSIONS

This research was conducted to provide a greater understanding of structural changes in components within seeds during thermal processing to improve starch digestibility of dry bean.

The degree of starch gelatinization in cooked beans is of importance to their complete digestibility. An enzymatic procedure using β-amylase and pullulanase was used to selectively digest the gelatinized starch fractions. The effects of selected heat treatments on cell wall structural appearance and starch bioavailability were enzymatically and microscopically examined.

The degree of gelatinization measured by the enzymatic method showed lower scores than that obtained by the microscopic birefringent method on differentially cooked beans. The lower values measured by the enzymatic method resulted from inaccessibility of the enzyme within gelatinized starch. Cooking whole beans for 10 min or longer caused crystallization within cell wall. The crystalline structure was observed under polarized microscope and appeared as a white band around cell walls. The crystallized cell walls of cooked bean was mechanically resistant and acted as a physical barrier not only to enzymatic hydrolysis but also to swelling of starch granules leading to a residual ungelatinized starch during cooking. The rigidity of the crystallized cell wall that changed during cooking was investigated by measuring available starch after reheating of cooked, freeze-dried and milled (CFM) flours. The longer the cooking time, the greater the rigidity of the crystallized cell wall of the cooked beans. Reheating of CFM_{10} enabled

80% of starch to be hydrolyzed by the enzymes while reheating of CFM_{40} allowed only 40% starch hydrolysis for the navy bean (experimental line, N84004). The cell wall rigidity varied between bean types and among the process conditions studied. Differential scanning calorimetry (DSC) thermography demonstrated that the crystalline structure formed during cooking was more stable than starch that is commonly referred to as "resistant starch fraction". Starches of cooked sweet potato and wheat were readily available to undergo enzymatic degradation since no crystallization occurred within cell walls to limit enzyme accessibility.

The attributes of physical appearance, palatability and nutritional bioavailability are essential components associated with food quality of cooked dry beans. Dry bean cell walls undergo structural changes during cooking that limit starch digestibility. The crystallized cells encapsulating starch granules were observed in cooked bean exudate (brine). The amount of starch in brine was not correlated with brine viscosity however total protein and insoluble dietary fiber in brine were strongly correlated with increased brine viscosity.

These studies have provided evidence for mechanisms limiting overall potential for maximum nutritional bioavailability and digestibility of cooked bean products. The development and implementation of appropriate process conditions for preparing dry bean could increase available caloric value for populations who consume beans as a dietary staple, reduce flatulence and perhaps decrease cooking time required to render a palatable product. To enhance understanding of the mechanisms associated with cell wall complexes, further research is required to separate and characterize the crystallized cell

walls and to assess the effect of salts in soaking solution on the rigidity of the crystallized cell wall structures which were demonstrated through these studies.

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