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By

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ABSTRACT

CHARACTERIZATION OF NAVY, PINTO AND BLACK BEAN STARCHES

By

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Pasting characteristics of air-classified high starch flours and purified starches from navy, pinto and black beans were determined using a Brabender Viscoamylograph. Restricted swelling characteristics, showing increased viscosity with no peak during heating and some tendency to retrograde during cooling, were observed. Navy and black bean starch pasting curves were similar and more viscous than those of pinto starch; pH variation (4 to 8) produced only minor effects on pasting properties. Extended high temperature holding (95°C, 30 min) resulted in steady viscosity increase without breakdown. Extended low temperature holding (50°C, 30 min) produced minimal shear breakdown of purified starches; air-classified flours were less stable.

Gel permeation high performance liquid chromatography (HPLC) of bean starches produced similar elution patterns for all three bean types. After hydrolysis with beta amylase, the elution patterns of navy and black bean starches remained similar. Elution pattern differences of pinto bean starch hydrolysates supported pasting characteristic differences.

To my parents and family
for their love, patience and moral support

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INTRODUCTION

Dry edible beans (*Phaseolus vulgaris* L.), as well as other legumes, constitute traditional foods for populations of subtropical and tropical areas. They provide significant amounts of protein, calories, vitamins and minerals. Their primary importance is as an inexpensive source of protein for human consumption (FAO, 1977), particularly for the lower-income groups in many developing countries of the world.

Although they are nutritious and inexpensive, the per capita consumption of dry beans in the United States has declined from about 8.4 lb in 1940 to 4.1 lb in 1981 (USDA, 1981). Several reasons for declining consumption have been proposed, including unavailability of bean-based food ingredients, the presence of antinutritional factors, flatulence production, and especially, time consuming preparation due to long soaking and cooking requirements. Several researchers have studied these problems to find innovative alternatives to increase bean utilization. For example, whole bean flour was air-classified into high protein and high starch fractions (Vose et al., 1976). These fractions have unique functional and sensory characteristics which make them useful as ingredients in specific foods or as nutritional supplements. The protein quality and digestibility (Charley, 1970; Bressani, 1975; Tobin and Carpenter, 1978; Patel et al., 1980; Carpenter, 1981; Sahasrabudhe et al., 1981), the flatulence producing factors (Calloway et al., 1971; Olson et al., 1975; Wagner et al., 1976; Fleming,

1981a,b; Fleming and Reichert, 1983; Sathe et al., 1983), and the antinutritional factors (Honavar et al., 1962; Liener, 1962; Kakade et al., 1969; Liener, 1975; Johnson et al., 1980; Ellenrieder et al., 1980 and 1981; Chang and Tsen, 1981) have also received considerable study in order to improve the nutritional quality of dry beans. On the other hand, the starches have been somewhat neglected.

Dry beans contain up to 60% carbohydrates (Adams, 1972), composed mainly of starch. Only in recent years have detailed investigations of the characteristics and functional properties of bean starch been conducted (Naivikul and D'Appolonia, 1979; Salimath and Tharanathan, 1982; Reddy et al., 1984). Nevertheless, further studies on bean starch are still needed to provide more useful technical data for bean product development and industrial applications.

This study was undertaken to determine the starch characteristics of navy, pinto and black beans. The purified starches were prepared from air-classified high starch bean flours. The general chemical analyses and the sugar and amylose contents of the air-classified flours and purified starches were determined. The pasting characteristics of these starches at varying pH values, holding periods and concentrations were also studied. Finally, the molecular structural characteristics of bean starches were investigated by using the hydrolytic enzymes, alpha and beta amylases, in conjunction with gel permeation high performance liquid chromatography (HPLC).

LITERATURE REVIEW

Dry Beans As A Food Resource

Description of Beans

Legumes are the edible dicotyledonous seed of leguminous plants that belong to the *Leguminosae* family. These plants commonly grow in a wide range of conditions and climates. The family *Leguminosae* is second only to *Gramineae* in importance as a source of food and fodder. The *Phaseoleae* subfamily of the *Leguminosae* contains 47 genera including *Phaseolus*. The genus *Phaseolus* comprises about 200 species of which only 20 are cultivated for their edible pods and seeds.

Navy, pinto and black beans are major commercial beans grown in Michigan and are classified as *Phaseolus vulgaris* L. (Deschamps, 1958). Navy beans are a mid-season bush type plant with white flowers. The seeds are chalky white, roundish to ovoid in shape, weighing in the range of 17 g to 19 g per 100 seeds. Prevalent varieties in Michigan are Sanilac, Gratiot, Seafarer, Kentwood, Fleetwood, Charity, Upland, Snow Flake and Snow Bunting. Pinto beans are a vine type plant. The seeds are medium sized, irregular and flat with brown variegated pattern against light tan background. The major variety is Pinto 114. Black beans are a high yielding mid-to-full season plant with small seeded, purple flowers on an erect vine. Prevalent varieties are Black Turtle Soup, Sanfernando, Icapijao, Jamapa and Tamazulapa.

Bean Handling and Storage

The handling and storage of beans in the field critically affect the ultimate quality of beans and bean products. The moisture content is the most important consideration following harvest. Seeds with a moisture content above 18% are subjected to excessive damage during storage and then in the processing line, due to physical susceptibility to mechanical forces and microbial spoilage from mold (Weston and Morris, 1954). On the other hand, seeds with a moisture content below 15% are sensitive to impact damage.

Edible dry beans have a thin seed coat layer which is easily damaged in handling or drying. Belt conveyors are usually used to move beans into and out of storage in order to reduce the damage. Dry beans can be stored in wood, concrete or steel bins. The choice depends upon the quantity of beans and the processing or drying that might be done in the field or in existing storage structures. Maddex (1978) suggested not using existing silos because filling equipment for loading beans into a silo usually causes bean damage. He recommended flat-bottomed, overhead wooden bins, round steel bins (recently the most popular form of storage) or concrete bins equipped with an aeration system. During storage, seed deterioration can be retarded by providing proper storage conditions. The two major controlling factors are moisture content and temperature.

Moisture Content. Beans stored at too low moisture exhibit clumping and splitting due to seed coat and cotyledon rupture, while storage at high initial moisture encourages discoloration, off-flavor development, loss of water uptake capacity and mold growth. Morris and Wood (1956)

reported that beans with moisture content above 13% deteriorated significantly in both flavor and texture after six months at 77°F and became unpalatable within 12 months. Morris (1963 and 1964), Burr et al., (1968) and Bedford (1972) reported that beans stored at high moisture showed a significant increase in their required cooking time while low moisture beans did not lose their cooking quality.

Temperature. Quality degradation is faster at high temperature than at low temperature. Beans stored at high temperatures get darker in color and require longer cooking times. The deteriorative effect of high moisture on bean quality is increased by high temperature. Long cooking time of beans from high temperature storage was observed by Dawson et al. (1952), Morris (1963) and Burr et al. (1968). Morris (1964) stated that the reduction of 15°F in storage temperature had the same effect as the decrease of 0.6% moisture content to yield an equivalent short cooking time. Uebersax (1972) reported that deterioration rate both in discoloration and mold growth was minimized in beans stored at 55°F under relative humidities ranging from 75% to 86%. The influence of increased storage temperature became greater at higher relative humidity.

Vongsarnpigoon et al. (1981) suggested that optimum bean quality was obtained from dry beans stored at 14% moisture at 70°F. Hard-to-cook phenomenon can develop due to improper storage conditions (Morris and Wood, 1956; Muneta, 1964). Recommendations to prevent storage loss of dry beans from hardening (Mejia, 1980) include: 1) beans should be stored at the lowest possible moisture content and 2) beans should be stored in a dry and cool environment. Aeration with the proper flow rate, relative humidity and temperature improve the stored bean quality.

Aeration, which is the practice of moving air at low flow rates to cool all beans in a bin, prevents moisture migration and also reduces mold growth and development of musty odors and off flavors (Maddex, 1978). Vongsarnpigoon et al. (1981) observed that organic acid treatment provided limited mold inhibition and resulted in beans with brown discoloration and firm texture, whereas $NaHSO_3$ treatment provided limited color stability without adversely influencing processing characteristics. In addition, they reported that vacuum and CO_2 storage did not significantly improve bean quality.

Bean Composition

Legumes have been used as the main source of protein and calories in many developing countries. Besides the major constituents of proteins, carbohydrates and lipids, all grain legumes contain substantial quantities of minerals and vitamins. Their chemical and nutritional composition vary with genetic as well as environmental conditions under which the crop is grown.

Protein. Beans have a high protein content, which is twice greater than cereals, ranging from 17% to 25% on a dry weight basis (Bremner, 1965; Goodhart and Shils, 1980). However, it is not generally recognized that beans contain proteins equivalent to milk, beef and other animal proteins in respect to their content of essential amino acids and their availability via digestion. Bean proteins are relatively rich in essential amino acids, particularly lysine and threonine (Patel et al., 1980; Carpenter, 1981; Sahasrabudhe et al., 1981), whereas cereals are deficient in lysine (Sathe et al., 1981). In addition to having twice as much protein, beans are also better sources of isoleucine, leucine,

phenylalanine, and valine than are cereals (Charley, 1970; Bressani, 1975; Tobin and Carpenter, 1978). They are deficient, however, in sulfur amino acids, particularly methionine and cystine (Patel et al., 1980; Sahasrabudhe et al., 1981). Tobin and Carpenter (1978) reviewed 47 papers on the amino acid composition of dry beans. Thirty-one papers describe the protein quality as a result of studies with rats and humans. This research has indicated that methionine is always the first limiting amino acid. In addition, this research has demonstrated the low digestibility of bean protein (76%). This was confirmed by Graham et al. (1979) and also by other studies where beans have been fed in mixtures.

Bressani (1975) and Kay (1979) reported that the predominant class of proteins present in *Phaseolus* beans is salt soluble globulins of which three distinct proteins have been identified: phaseolin, phaselin and conphaseolin. Liener and Thompson (1980), Geervani and Theophilus (1982) and Sathe et al. (1981) reported that the Great Northern (*Phaseolus vulgaris* L.) proteins, albumins and protein isolates were characterized by high acidic amino acid content, while globulins and protein concentrates had a high proportion of hydrophobic amino acids. They also found that the bean proteins were resistant to in vitro enzymatic attack; however, heating improved in vitro susceptibility to enzymatic hydrolysis.

Carbohydrates. The total carbohydrates of dry beans range from 24% to 68%. These carbohydrates include mono- and oligosaccharides, starch and other polysaccharides. Starch is the most abundant legume carbohydrate and varies from 24% in peas to 56.5% in beans. The variations observed are due to different cultivars and analytical procedures (Pritchard et al., 1973; Cerning-Beroard et al., 1975).

Total sugars (mono- and oligosaccharides) represent only a small percentage of total carbohydrates in dry legume seeds. Among the sugars, oligosaccharides of the raffinose family (raffinose, stachyose, verbascose and ajugose) predominate in most legumes and account for a significant percentage (31.1% to 76%) of the total sugars in several others (Nene et al., 1975; Hymowitz et al., 1972; Cerning-Beroard and Filiatre, 1976; Naivikul and D'Appolonia, 1978; Becker et al., 1974; Kon, 1979; Rockland et al., 1979; Akpapunam and Markakis, 1979; Ekpenyong and Borchers, 1980; Reddy and Salunkhe, 1980; Fleming, 1981 a, b; Sathe and Salunkhe, 1981a). Walker and Hymowitz (1972) found that the sugar content ranged from 4.4% to 9.2% in the 28 varieties of *Phaseolus vulgaris*. Of total sugars present, sucrose accounted for 46.4%; raffinose, 10.4%; and stachyose, 43%. The predominance of a particular oligosaccharide seems to depend on the type of legume. For example, verbascose is a major oligosaccharide in black gram, red gram, mung bean and broad beans (faba beans), and stachyose is the major oligosaccharide in smooth and wrinkled peas, Great Northern beans, California small white beans, red kidney beans, navy beans, pinto beans, pink beans, black eye beans, Bengal gram, soybeans, lentils, cowpeas and lupine seeds.

Crude fiber consists of cellulose, hemicellulose (a heterogeneous group in which pentosans usually predominate), lignin (an aromatic polymer), pectin and cutin substances. Legumes contain appreciable amounts of crude fiber (1.2% to 13.5%). Rather large variations in crude fiber content have been observed in black gram, Bengal gram, mung bean and red gram. Cellulose is the major component of crude fiber in smooth and wrinkled peas, red kidney beans, navy beans, pinto beans, pink beans and black eye beans, while in other legumes (lupine seeds,

lentils, broad beans, red gram, black gram), hemicellulose is the major component of fiber. Deschamps (1958), Tobin and Carpenter (1978), and Kay (1979) reported that the crude fiber content of the common varieties of the genus *Phaseolus* was less than 6%. Preliminary evidence suggests that dietary fiber may contribute positively to health and well-being (Burkitt, 1971; Painter and Burkitt, 1971; Trowell, 1972).

Lipids. The highly unsaturated lipid content of dry beans is generally in the range of 1% to 2%. Deschamps (1958) and Watt and Merrill (1963) reported that *Phaseolus vulgaris* shows a low fat content, generally below 2% with 63.3% being unsaturated fatty acids.

Vitamins. Dry edible beans provide some water soluble vitamins: thiamine, riboflavin, niacin and folic acid, but very little ascorbic acid (Watt and Merrill, 1963; Forham et al., 1975; Tobin and Carpenter, 1978). Common commercial methods of preparation of canned beans cause a significant loss of water soluble vitamins. Therefore, many workers have studied the retention values of water soluble vitamins in order to optimize the quality of bean products (Augustin et al., 1981; Carpenter, 1981). There is no evidence in the literature which indicates that dry beans contain appreciable amounts of fat soluble vitamins. Watt and Merrill (1963) and Kay (1979) reported that *Phaseolus vulgaris* provides less than 30 International Units of vitamin A per 100 grams of raw beans. Variability of vitamin content is high. Augustin et al. (1981) suggested that geographic location of growth appeared to have had a significant effect on this content.

Ash and Minerals. The total ash content of *Phaseolus vulgaris* ranges from 3.5% to 4.1% (Fordham et al., 1975; Tobin and Carpenter, 1978; Kay, 1979). Beans are generally considered to be a good source of

some minerals, such as calcium and iron, but they also contain significant amounts of phosphorus and potassium. Adams (1972) and Patel et al. (1980) observed that navy bean flour had 2 to 17 times as many minerals as wheat flour. The specific mineral content in mature, raw legumes has been reported by several researchers in recent years; however, most values show large variability. Augustin et al. (1981) pointed out that bean classes and environmental factors greatly influence this variability.

Antinutritional Factors and Flatulence Production. The two primary concerns with the use of legumes are the presence of antinutritional factors and flatulence production. Beans contain a number of "antinutrients" and potentially toxic substances. Bressani (1975) categorized the toxic substances present in legumes into seven groups: trypsin inhibitors, hemagglutinins or lectins, goitrogenic factors, cyanogenic glucosides, lathyric factors, compounds that cause favism, and other factors about which less information is known. Of these factors, trypsin inhibitors and hemagglutinins are considered primarily responsible for causing the growth retardation observed in laboratory animals fed raw beans (Honavar et al., 1962; Liener, 1962; Kakade and Evans, 1965, 1966; Jaffe, 1969; Liener and Kakade, 1969; Liener, 1975; Liener and Thompson, 1980).

Trypsin inhibitors are found in several legumes including the species of *Phaseolus*. As the name implies, they are protein fractions that strongly inhibit the enzymatic activity of trypsin in the intestine (Gomes et al., 1979), thereby reducing the digestion of proteins and hence, the absorption of their constituent amino acids. Besides the inhibition of the enzyme trypsin, the poor digestibility of bean proteins may result from the refractory nature of native bean protein to enzyme trypsin (Liener, 1975). Fortunately, soaking (Kakade and Evans, 1966;

Tyler et al., 1981) or germination (Gupta and Wagle, 1980) plus heating (Ellenrieder et al., 1980 and 1981; Johnson et al., 1980; Chang and Tsen, 1981) have been found to improve the digestibility of bean protein in two ways: 1) by denaturation which makes the proteins more susceptible to the action of enzymes, and 2) by destroying trypsin inhibitors. Liener (1962 and 1975) and Liener and Thompson (1980) also reported that the protein of unheated beans resists proteolysis in the intestine; however, after heating, the true digestibility increases and trypsin inhibitory activity decreases.

Hemagglutinins are also present in beans and are destroyed by heat (Thompson et al., 1983). These compounds cause agglutination of red cells and impair absorption (Liener, 1975). Kakade and Evans (1966) found that merely soaking the beans did not result in any loss of hemagglutinating activity. Aguilera et al. (1981 and 1982) proposed that special grinding control during processing may aid inactivation and reduction of both hemagglutinin and trypsin activity.

Some evidence that seed coat color may affect protein digestibility has been offered (Marquardt et al., 1978; Elias et al., 1979), such that the protein in white-coated dry beans showed greater digestibility than that of black-coated dry beans. Extensive chemical analyses, including trypsin inhibitor activity, hemagglutinin concentration, and amino acid analysis showed both black and white dry beans to be essentially alike. This suggested that the pigments of the seed coat were responsible for the decreased protein digestibility. These pigments are tannins, which could react with bean proteins, decreasing their digestibility. Tannins inhibit the digestive enzymes by complexing the enzymes (Goldstein and Swain, 1965; Haslem, 1974; Griffith and Jones, 1977) and also with protein substrates. In vitro studies have

indicated that isolated tannins have high inhibitory action against digestive enzymes (Morris and Wood, 1956; Milic et al., 1972). It has been shown that high tannin content reduces the nutritional value, and at enzyme inhibition, is associated with grain that has a brown or colored pericarp. Protein binding by tannins (polyphenols) is believed to be responsible for the growth depression observed in rats and chickens.

In addition, legumes contain relatively large amounts of phytic acid, up to 5% by weight (DeBoland et al., 1975). This compound can effectively bind nutritionally important minerals, reducing their availablility for absorption (Sathe and Krishnamurthy, 1953; O'Dell and Savage, 1960; Roberts and Yudkin, 1960; O'Dell et al., 1972; Davies and Nightingale, 1975; Maga, 1982).

The ability of legume seeds to stimulate intestinal gas formation (flatulence production) is another factor limiting their consumption by humans (Protein Advisory Group, 1973). It has frequently been suggested that the galactose-containing oligosaccharides including raffinose, stachyose and verbascose are the components in the legume seeds which are responsible for flatulence (Fleming, 1981a; Fleming and Reichert, 1983; Sathe et al., 1983). These sugars have been considered fermentable since the alpha galactosidase enzyme, although not produced by the human digestive system, is secreted by the indigenous gut microflora. Therefore, these substrates are expected to be fermented within the large intestine and hence, cause gas production (Calloway et al., 1971; Olson et al., 1975; Wagner et al., 1976). Fleming (1981b) studied the flatus potential of seven types of legume seeds and reported that hydrogen production was significantly and positively correlated to oligosaccharides and acid hydrolyzable

pentosan contents, but negatively correlated to the starch and lignin contents.

In view of increasing dry bean utilization and nutritional significance, greater research efforts should be directed to understanding and minimizing the effects of these antinutritional and flatulence-producing factors.

Bean Flour and Bean Starch

Dried beans traditionally have been utilized by soaking and cooking in the home or consumed as commercially processed canned beans. Many researchers have searched for innovative alternatives to increase bean consumption. A shelf-stable flour has been proposed as a food ingredient to provide increased versatility and to improve bean utilization.

Initial efforts to produce bean ingredients were aimed at keeping cell rupture at a minimum in order to retain the same texture, appearance and taste as conventionally prepared bean soups. Instant precooked bean powders have been prepared by soaking, cooking, slurrying, and drum or spray drying (Bakker et al., 1973). The second generation of bean ingredients are based on fractionation of the two major components, starch and protein. Chang and Satterlee (1979) produced bean protein concentrates containing 72% to 81% protein by wet processing using water extraction techniques. Molina and Bressani (1973) prepared protein isolates containing about 90% protein and starch products with almost 50% starch. Several disadvantages exist in using wet processing for fractionation and concentration: 1) significant energy is used in drying the final products; 2) waste by-products are produced which contain significant amounts of organic matter; and 3) yields are reduced by the losses in wet by-product streams.

In recent years, dry milling of beans into whole bean flour and air-classifying into high starch and high protein fractions have received increased interest as efficient processing methods. Compartmentalization of protein in friable protein bodies, and starch in denser, less brittle granules, enables size reduction and fractionation of dry beans into a coarse fraction which contains most of the starch and a fine fraction which contains most of the protein. Because of size distribution, sieving, a simple fractionation technique, had been proposed. However, it becomes progressively more difficult as the size separation point is reduced. Very fine materials have a tendency to agglomerate especially if oil is present. Such powders tend to build-up on the screen causing blinding of the sieve and a loss of separation efficiency. Air-classification has been applied to wheat (Stringfellow and Peplinski, 1964); to corn, sorghum and soy (Pfeifer et al., 1960); to rice (Stringfellow et al., 1961); to barley (Pomeranz et al., 1971); and to triticale flours (Stringfellow et al., 1976). Air-classification works on the principle of difference in terminal settling velocity among different particle sizes.

It has been observed that, when raw legumes are ground without any pretreatments, they develop undesirable odors and flavors. Lipoxidase has been held responsible for the appearance of off-flavors by catalyzing formation of hydrogen peroxides from unsaturated fatty acids (Kon et al., 1970). However, treatment with dry heat for six to eight minutes at 104°C to 105°C completely inactivates this enzyme (Smith and Circle, 1972). Aguilera et al. (1982) found that heat treatment also destroys trypsin inhibitor and hemagglutinin activities. Therefore, it is advantageous to apply a heat treatment to beans prior to bean flour production.

The conventional heating method, using air as the heat transfer medium, is attractive due to ease of handling and lack of product contamination. However, air does not give a high rate of heat transfer. Khan (1972) reported high temperature drying of paddy rice by using heated sand. Harper and Lorenz (1974) prepared the full-fat soy flour by roasting soy beans in a bed of heated salt. Similarly, Carvalho et al. (1977) produced instant navy bean powders by roasting dry beans in a bed of salt, then grinding. They also reported a reduction of trypsin inhibitory activity by this method. In 1978, Peterson and Harper developed the new heated granular bed roaster. Based on this previous work, Aguilera et al. (1982) developed a new particle-to-particle heat exchanger for roasting navy beans and observed the functional characteristics of several roasted bean products. These roasted products showed reduced water-soluble nitrogen content, gel forming capacity, trypsin inhibitor and hemagglutinin activities, increased water holding capacity and cold paste viscosities, and no changes in available lysine and degree of starch damage. In addition, roasting causes fracture and separation of hulls, and facilitates their removal.

Dry beans contain up to 60% carbohydrates, most of which is starch. Chemically, bean starch is similar to starches from other sources (Staudinger, 1932). However, their physico-chemical properties and internal molecular structures are different depending on the original source, maturation and environmental factors (Rosenthal and Takeko, 1972).

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. In the linear fraction, the glucose units are joined exclusively by α-1,4 glucosidic bonds. The number of

glucose units may range from a few hundred to several thousand. The amylose stains bright blue with iodine. Rundle and French (1943) demonstrated that the polymer chain takes the form of a helix which may form inclusion compounds with a variety of materials, such as iodine (Harris et al., 1928; Hanes, 1937; Freudenberg et al., 1939). Amylopectin is a branched molecule containing both α-1,4 and α-1,6 glucosidic bonds and does not stain blue with iodine. Because it is a branched polymer, various workers attempted to describe the molecular structure of amylopectin. 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, B chains are linked to two or more other chains, and 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 A chains. Meyer (1952) originated the "tree type" structure. Whelan (1970) 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.

The linear (amylose) and branched (amylopectin) molecules are linked together by hydrogen bonds and form a micellar network. The starch granules are completely insoluble in cold water; however, they exhibit a limited capacity for absorbing cold water and swelling reversibly.

When heat or appropriate chemicals are applied to disrupt the hydrogen bonds that link the molecule in the presence of surplus water, the hydration of the network is encouraged, and an irreversible process of swelling is started. The critical temperature at which this process starts is known as gelatinization temperature, which is a characteristic for starches of different origins. Attaining the gelatinization temperature range with subsequent swelling of the granules is accompanied by an increase in viscosity. This phenomenon may be explained by a greater chance of swollen granules coming into contact with each other, which probably could be considered a measure of the work required to move the granules past each other as they continue to swell (Schoch, 1965 and 1969). In 1973, Miller and co-workers, however, indicated that granule swelling does not account for the rapid rise in viscosity of a wheat starch suspension heated in excess water, but that the exudate principally causes the large increase in viscosity at higher temperatures. They indicated that a correlation between starch paste viscosity and granule size was almost nonexistent. This exudate contains colloidally and molecularly dispersed starch molecules leached from the granules into the surrounding aqueous phase during the initial free swelling stage. As suggested by Meyer and Gibbons (1957), this process of leaching starts with dissolving molecules of low molecular weight from the surface of the granules, whereas those of higher molecular weight are still left in swollen, elastic meshes. Banks and Greenwood (1967a) and Ghiasi et al. (1982) studied the leaching exudate during the heating of starch granules and reported that at a low temperature, the lower molecular weight linear amylose exudate is preferentially leached from the granules. As the temperature increases, the higher molecular weight branch amylose and amylopectin are gradually obtained. Water penetrates the granules

through these meshes and continues to dissolve molecules of low molecular weight within the granules. At this point, the granules are very swollen making the paste very viscous. If this process of swelling reaches the point when the granules occupy the entire volume, the solubles in the exudate most likely rediffuse back into the swollen granules and the system becomes a gel-like continuum (Leach, 1965). At this stage, the granules are highly susceptible to thermal or mechanical breakdown due both to the highly swollen condition and progressive weakening of bonding forces within the micellar lattice. Prolonged heating of the paste results in the disintegration of the granules, which leads to decrease in paste viscosity. Continued heating, especially while stirring, produces a paste that is a mixture of swollen granules, granule fragments, and dispersed starch molecules from the granules. If the temperature of the paste is subsequently decreased, the elements present in the paste start to associate or retrograde, then increase in viscosity. Starches from different origins or having been pretreated exhibit a different rate of viscosity increase at this stage of the pasting cycle, reflecting their different retrogradation tendencies.

The practical importance of all these viscosity changes during the above-described pasting cycle was recognized by Caesar (1932). Caesar and Moore (1935), Anker and Geddes (1944) and Mazurs et al. (1957) stressed the practical significance of the following points: the gelatinization temperature, the maximum viscosity during heating, the paste viscosity at the end of cooking, the viscosity increase during cooling, and the viscosity of the cold paste. Several instruments and techniques have been devised for viscosity measurements at these points. The Brabender Viscoamylograph has become the most commonly used for starch and starch containing products. It measures and records the

gelatinizing properties with relation to time, temperature and rate of shear. It is essentially a torsion viscosimeter in which the sample is kept at a constant temperature, heated or cooled at a constant rate, and the torque continuously plotted on the strip-chart recorder. Table 1 (Mazurs et al., 1957) interprets the Brabender amylogram relative to the functional properties of starch.

Bean starches, which are of interest in this study, have only recently been characterized by various investigators. Even though the starch analysis techniques may be advanced, analytical difficulties still very much exist. Greenwood (1979) suggested that one of the inherent difficulties of studying starch is its microheterogeneity. Because of this complication, it is not possible to make many generalizations about starch. However, this literature review has summarized the current information available on the characteristics of bean starches.

Physical-Chemical Properties

Granule Size, Shape, and Microscopic Appearance. Granule size of bean starches has been investigated by several researchers (Kawamura et al., 1955; Rosenthal et al., 1970; Hall and Sayre, 1971; McEwen et al., 1974; Rockland and Jones, 1974; Lineback and Ke, 1975; Lai and Varriano-Marston, 1979; Naivikul and D'Appolonia, 1979; Lii and Chang, 1981; Sathe and Salunkhe, 1981a). The granule size is quite variable and the granule dimensions range from about 1 to 80 μm depending on the source. Most bean starch granules are slender (greater length than width), although spherical, ovoid, elliptical, kidney-shaped, and irregular granules are also found. This wide variation in granule size and shape could be due to genetic control and seed maturity. For dry

Table 1. Functional and molecular properties associated with pasting characteristics of starches

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

(Mazurs et al., 1957)

beans, however, a wide variability in shape is found in starch granules from the same source (Reddy et al., 1984).

Light microscopic studies of legume starches clearly reveal two distinct starch granule characteristics: the presence of a hylum and the presence of lamellae. The hyla 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 hylum and lamellae observed under the light microscope are not seen, however, 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, or both.

Molecular Weights. Starches are high molecular weight compounds since they are polymeric monosaccharides. Biliaderis et al. (1979) reported that the major portion of legume starches have molecular weights higher than 2×10^6, and over 90% of them have molecular weights above 4×10^4.

Amylose and Amylopectin. In legumes, the amount of amylose, ranging from 10% to 66%, may influence starch solubility, lipid binding, and other functional properties. Amylopectin is thought to be responsible for the structural form of starch granules. Recently, Biliaderis et al. (1981) fractionated legume starches into amylose and amylopectin and determined certain molecular properties. They reported that the variability between amyloses from different legumes may be due to: 1) maturity of seed, 2) genetic control of amylose synthesis, 3) cultivar differences, and/or 4) seed history. The range for degree of polymerization of amylose is quite variable. A high degree of amylose polymerization may confer

structural stability on the granule and also may be partially responsible for its resistance toward in vitro alpha amylolysis.

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). From X ray diffraction studies, most legume granules exhibit crystalline structures in the Type C pattern (Rosenthal et al., 1974).

Functional Properties

Swelling 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. Most legume starches have a gelatinization temperature of 60°C to 90°C, with the exception of wrinkled peas (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'Appolonia, 1979; Lii and Chang, 1981; Sathe and Salunkhe, 1981a). 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 cooking. Most bean starches show some tendency to retrograde during cooling and have relatively constant cold-paste viscosity during a holding period at 50°C. Kawamura and Fukuba (1957) classified legume starches 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, 63.8°C to 76°C, which is considerably higher than that of wheat starch, 55.6°C to 63°C. This result is similar to that 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

Table 2. Gelatinization temperature of legume starches

Starch Source	Gelatinization Temperature 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)

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 program, 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 (1981a,c) indicated that the gelation of purified bean starch could yield stable a gel at concentrations of 7% or above (w/v).

In recent years, more interest has focused on the relationship between the molecular structure and the functional properties of starch. Biliaderis et al. (1979) reported that the average molecular weight of legume amylopectin, determined by gel chromatography, was greater than 20×10^6, and there was significant correlation ($P < 0.05$) between set-back viscosities and percentage of starch components fractionated between 0.2 and 0.9 Kav ($Kav = [V_e - V_o] / [V_t - V_o]$), where V_e is the elution volume, V_o is the exclusion volume, and V_t is the total volume. In the same year, Naivikul and D'Appolonia observed that the intrinsic viscosity was directly proportional to the molecular weight of amylose fraction from various legume starches. In 1981, Biliaderis et al. developed an hypothesis to explain starch gelatinization and granule rigidity of legume starches based on the molecular characteristics of the branched polysaccharide fractions (amylopectin). On the other hand, Suzuki et al. (1981) suggested that the gelatinization and retrogradation properties may be influenced by the structure of amylose. Recently,

Takeda et al. (1983) confirmed that the chain length distribution of amylopectin affects the pasting properties of starch.

Starch Analysis

Extraction. In order to study the properties of bean starches, extraction and purification are essential to isolate starch granules from beans and/or bean flours. Schoch and Maywald (1968) found that the separation of pure starch was difficult from certain legumes because of the presence of a highly hydrated fine fiber fraction and also a high content of insoluble protein. Kawamura et al. (1955) proposed a method to isolate starch from various legumes by treatment with a 0.2% NaOH solution, washing with water, and dehydration with ethanol and ether. Schoch and Maywald (1968) simply isolated legume flour by repeated washings with distilled water and air-drying at room temperature. Recently, Sathe and Salunkhe (1981a) isolated starch from Great Northern beans (*Phaseolus vulgaris* L.) by extracting sequentially with different solvents: water, 2.0% NaCl, 0.1N NaOH, and aqueous ethanol.

Quantitation. Various methods for quantitative determination of starch have been reported. Those based upon optical rotation were unsatisfactory because of turbidity and the relatively large quantities of materials required. The methods involving acid hydrolysis followed by reducing sugar determinations were slow and limited to samples which were free from other cellulosic materials which could also yield glucose on treatment with boiling acid. In 1970, the AACC Starch and Pentosans Committee began an investigation into new and improved methods for determining starch in cereal products. After surveying the literature,

it was decided to examine a procedure that incorporated the use of enzymes specific in their catalytic action on starch and glucose. For instance, the use of glucoamylase to hydrolyze starch to glucose could eliminate the problem of inversion caused by acid hydrolysis (AACC Method 76-20). Also, the use of glucose oxidase would reduce the uncertainties associated with empirical methods of measuring glucose, such as reducing sugars (AACC Methods 76-30A, 80-60, 80-68; Underkofler et al., 1943), and certain colorimetric techniques (Dubowski, 1962; Saunders et al., 1970). The use of enzymes could eliminate the problem of materials other than starch contributing to the angle of rotation as measured by polarimetric procedures (AACC Method 76-20).

The use of some pretreatments before starch determination has been proposed by many investigators. Banks et al. (1970) proposed a method of initial extraction with aqueous methanol or ethanol to remove the low molecular weight carbohydrates. Gelatinization of starch is accomplished by permitting rapid and complete enzymatic hydrolysis. This gelatinization process may be effected by autoclaving (Thivend et al., 1965; Shetty et al., 1974) or by chemical treatments using dimethyl sulfoxide (McGuire and Erlander, 1966; Libby, 1970) or concentrated calcium chloride (Earle and Milner, 1944; Banks et al., 1970). Libby (1970) and Vose (1977) suggested the combination of alpha amylase and glucoamylase enzymes for digesting the starch to yield glucose. In 1979, Baur and Alexander developed the enzymatic hydrolysis method by using an ordinary water bath for gelatinizing starch followed by hydrolysis with both alpha amylase and glucoamylase. Fleming and Reichert (1980) reported that calcium chloride dispersion provides higher yield of starch and more reproducible analytical results than does gelatinization by autoclaving.

Although sensitive, these enzymatic methods are not sufficiently accurate for all purposes. Enzyme purity is a limiting factor. Glucoamylase preparations are usually contaminated with beta glucanase, which can release glucose from the cell wall. Recently, Lustinec et al. (1983) presented a new, accurate and rapid method which is based on simplified extraction of the starch-iodine complex on a glass fiber disk. This method was designed to overcome the disadvantages of enzymatic methodology; moreover, it is faster as well as more starch-specific.

Linear starch components, amylose, can be determined directly from starch materials by either of the four general techniques: 1) potentiometric titration of the dissolved starch with standard iodine, 2) similar amperometric titration, 3) spectrophotometric determination of intensity of blue coloration with iodine, or 4) absorption of congo red (Schoch, 1943; Larston et al., 1953; Gilbert and Spragg, 1964; Carroll and Cheung, 1960). The first three methods are based on the iodine affinity of amylose; the fourth procedure is based on the reduction of dye affinity due to the presence of branching. Whistler et al. (1964) suggested that the precision of absorption of the congo red method was inferior to the iodine binding methods. Therefore, the determination of the iodine bound by amylose is the fundamental, basic method used to determine the amount of amylose in starches. Quantitative isolation of amylose is reported to give the more accurate results.

Methods of fractionating starch have been extensively reviewed since 1945 by Schoch. The Schoch method is based on dispersing starch granules into an aqueous solution, followed by the addition of a polar, organic substance, resulting in the formation of an insoluble amylose complex. Amylose is then recovered and determined by the fundamental

methods. Several investigators developed and modified Schoch's method (Greenwood and Thompson, 1962; Montogomery and Senti, 1964; Banks and Greenwood, 1967a,b).

Size exclusion chromatography is another useful technique used for fractionating starch components. This gel filtration technique was originally developed for the separation of biological materials in aqueous media on bead-formed gels consisting of dextran cross-linked with epichlorohydrin. A significant breakthrough in the evolution of the size exclusion chromatography was the development of polystyrene beads. These allow for the separation of polymers dissolved in organic solvents, and this method is known as gel permeation chromatography (GPC). Since the basis of the two techniques is the same, they are combined under the name of size exclusion chromatography separation (according to molecule size). Because of the different molecule size of amylose and amylopectin, size exclusion chromatography has been applied by many researchers to separate the starch components. In addition, commercially available dextrans, with known molecular weights, can be used as size-exclusion calibration standards to obtain the molecular weight distribution (MWD) patterns.

Barker et al. (1969) have applied chromatography over porous glass beads for the fractionation of dextran and hyaluronic acid. Patil and Kale (1973) developed a cellulose column equilibrated with ethanol-urea used for the quantitative separation of amylose and amylopectin. Dintzis and Tobin (1974) fractionated amylose and dextran over porous glass. Ebermann and Schwarz (1975) separated the starch components by gel filtration on agarose beads. Yamada and Taki (1976) adopted perchloric acid as the solvent in a gel chromatography system. Aqueous dimethyl-sulfoxide (DMSO) is able to dissolve carbohydrates without damaging their

primary structure and the activities of hydrolytic enzymes are unaffected by concentrations of DMSO up to 20% (Lineback and Sayeed, 1971). Therefore, DMSO was suggested to be used in gel permeation chromatography (GPC) systems to give better resolution, although it is a viscous solvent (Minor, 1979). Papantonakis (1980) used the aqueous DMSO as solvent in micro-BONDAGEL GPC columns and suggested that the ratio of DMSO and H_2O had an effect on resolution. Because DMSO is a viscous solvent, it is not particularly well suited for use in conventional chromatography techniques. High performance liquid chromatography (HPLC), however, can overcome the disadvantage of using DMSO by supplying enough pressure to counteract the viscosity of the DMSO.

High performance liquid chromatography facilitates more rapid separations than conventional column chromatography. The main difference between HPLC and conventional chromatography is that HPLC uses a pressurized eluent as a driving force for separating samples. Development of improved pumping systems capable of maintaining a constant liquid flow rate over a period of time and improved columns able to separate closely related compounds in a mixture have made HPLC more valuable in the past few years, especially for the anlysis of carbohydrates. The combination of size exclusion with HPLC techniques has been successfully used and reported by many workers (Papantonakis, 1980; Kruger and Marchylo, 1982).

MATERIALS AND METHODS

Dry Bean Products

Bean Source

Prime, handpicked navy, black (1981 Michigan crop) and pinto (1982 Michigan crop) beans (*Phaseolus vulgaris* L.) were used in the production of dry roasted fractionated bean flours. Ninety-one kilograms of each bean type were shipped to the Food Protein Research and Development Center at Texas A & M University for further processing.

Preparation of Samples

Air-Classified High Starch Flours. Forty-five kilograms of each bean type were roasted in a solid-to-solid heat exchanger as shown in Figure 1 (custom built by Food Processes Inc., Saginaw, Michigan). The heat exchanger's heat transfer medium consisted of 1.6 mm (1/16 in) diameter type A 90% aluminum oxide ceramic beads (Coors Ceramic Co., Golden City, Colorado) with a specific gravity of 3.6 g/cm^2. The beads were heated to 240°C and were maintained in the chamber with the raw beans for 100 seconds in a 1:5 ratio of beans to beads. These processing conditions resulted in a bean exit temperature of 113°C. Roasted beans were then cracked through a corrugated roller mill (Ferrell Ross, Oklahoma City, Oklahome) into six to eight pieces. The hulls were removed using a zig-zag aspirator (Kice Metal Products, Wichita, Kansas).

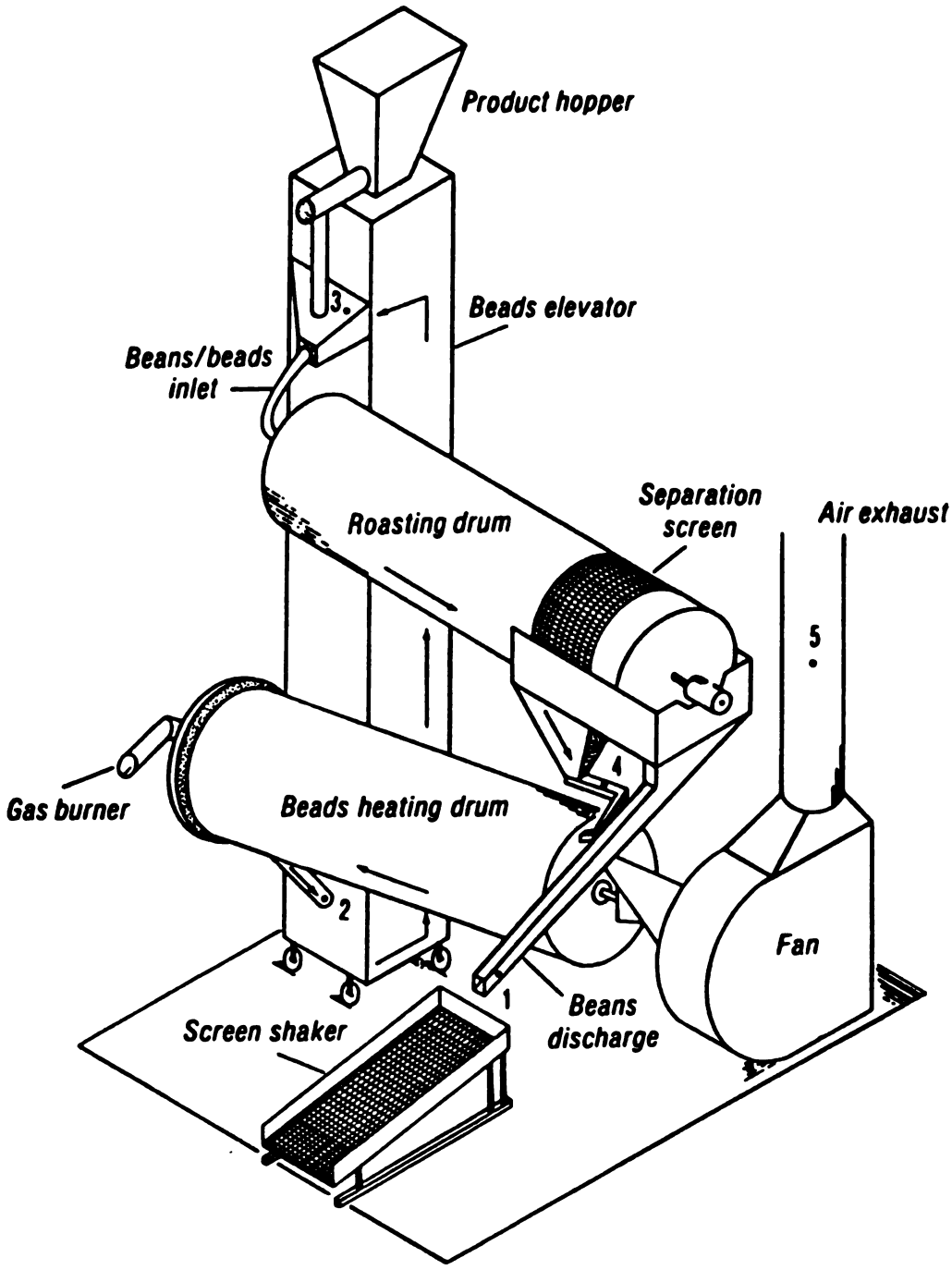

Figure 1. Schematic diagram of the particle-to-particle dry roaster. Points 1 to 5 indicate temperature measurement sites: 1) product, 2) beads out of heating drum, 3) beads into upper drum, 4) beads return, and 5) air. Arrows indicate flow of beads.

After hull removal was completed at the Food Protein Research and Development Center, Texas A & M University, the cracked cotyledons were shipped to Alpine American Corporation, Natick, Massachusetts, for milling and air-classification. The cracked cotyledons were finely ground in a Model 250 CW stud impact mill at a speed of 11,789 rpm and a door speed of 5,647 rpm. The resulting flours were air-classified in a Model 410 MPVI air-classifier at a rotor speed of 2,200 rpm and a brake ring setting of 3, using a 7.62 cm (3 in) screw feeder operating at 25 rpm. At this point, two flour fractions were obtained: an intermediate starch fraction (coarse I) and an intermediate protein fraction (fines I). The intermediate protein fraction was reclassified under the following conditions: rotor speed of 2,200 rpm, brake ring setting of 0, 7.62 cm (3 in) screw feeder operating at 25 rpm. As a result of this second air-classification step, a high starch fraction (coarse II) and a high protein fraction (fines II) were obtained. A flow diagram of the flour processing scheme and the fractions produced is presented in Figure 2. All flour fractions were packaged in polyethylene bags and shipped to Michigan State University in fiber drums at ambient temperature. Upon arrival at Michigan State University, the flour fractions were stored at room temperature. The high starch flours from each bean type were used in this study.

Purified Starch. Bean starches were purified according to the method modified by Naivikul and D'Appolonia (1979). Approximately five kilograms of air-classified high starch flour of each bean type were slurried in 20 Kg of 0.016 N NaOH solution with continuous mixing for 20 min. The starch was recovered by centrifugation (2,000 x g, 20 min). The lighter fine fiber and any undissolved protein, which remained

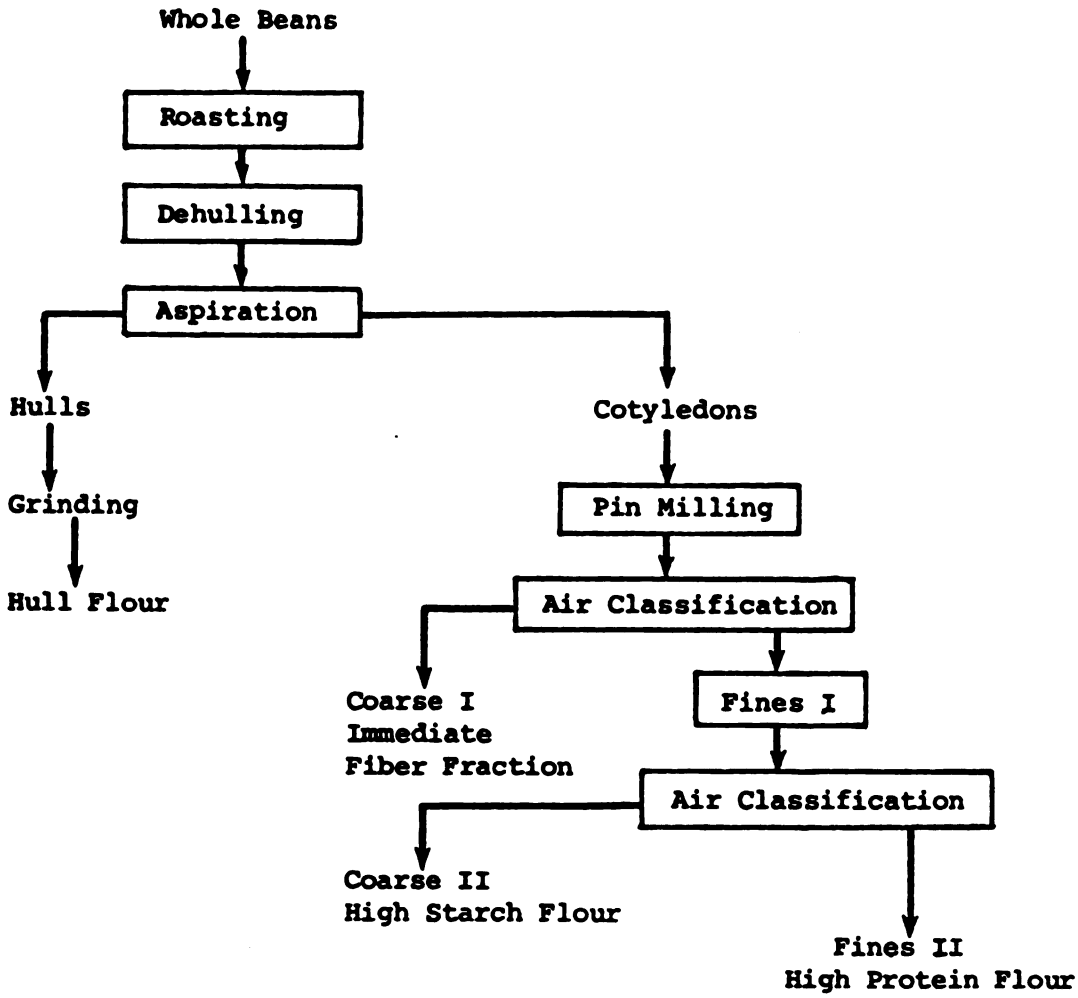

Figure 2. Flow diagram of processing navy, pinto and black beans

suspended in the supernatant, were decanted, and the spongy gray upper layer of starch tailing was skimmed off. The dense white bottom layer was crude starch which was washed three times with distilled deionized water, two times with 70% ethanol, and two additional times with distilled deionized water, respectively. The supernatant and sludge was removed and discarded after each washing. The prime starch was then air-dried at 38°C to 40°C for two days. Finally, the dry starch was ground using a Waring blended (Waring Products Division, New Hartford, Connecticut).

Physico-Chemical Analyses

Analytical Methods

Moisture. The moisture content of samples was determined in triplicate by AACC Method 44-32 (1962). Approximately 5 g samples were weighed into previously dried, cooled and tared crucibles. Samples were dried under a partial vacuum (ca. 25 mm Hg) between 95°C and 100°C for six hours. After cooling in a desiccator, samples were reweighed and percent moisture was calculated based on the fresh weight, as follows:

$$\% \text{ Moisture} = \frac{\text{wet sample wt (g)} - \text{dry sample wt (g)}}{\text{wet sample wt (g)}} \times 100$$

Ash. The ash content of samples was determined in triplicate by AACC Method 08-01 (1962). The dried samples obtained from the moisture determinations were incinerated at 525°C for 24 hours in a Barber-Coleman muffle furnace Model No. 293 C (Thermolyn Corp., Dubuque, Iowa). The uniform white ash was cooled to room temperature in a desiccator

prior to weighing. Percent ash was calculated based on the dry weight, as follows:

$$\% \text{ Ash} = \frac{(\text{crucible wt [g]} + \text{ash wt [g]}) - \text{crucible wt (g)}}{\text{sample wt (g)}} \times 100$$

Protein. The protein content of samples was determined in triplicate by AACC Method 46-23 (1962), a standard micro-Kjeldahl procedure. Percent nitrogen was calculated based on the dry weight, as follows:

$$\% \text{ N} = \frac{(\text{ml HCl} - \text{ml blank}) \times \text{N HCl} \times \text{equiv. wt NaOH}}{\text{sample wt (mg)}}$$

Percent nitrogen was then multipled by 6.25 to obtain percent total protein.

Crude Fat. The crude fat content of samples was determined in triplicate using a modification of AACC Method 30-25 (1962), a Soxhlet extraction procedure. Percent crude fat was calculated based on the wet weight, as follows:

$$\% \text{ Crude Fat} = \frac{(\text{fat wt [g]} + \text{flask wt [g]}) \times \text{filtrate vol (ml)}}{\text{sample wt (g)} \times 50 \text{ ml}} \times 100$$

Dietary Fiber. The enzyme neutral detergent fiber (ENDF) was determined for each sample in triplicate by the method of Robertson and Van Soest (1977). This method was modified to include 1 mg of amyloglucosidase to provide additional digestion to starch.

Sugars. The simple and oligosaccharide sugars (glucose, sucrose, inositol, raffinose and stachyose) commonly found in beans (*Phaseolus*

vulgaris L.) were determined by the new method developed by Agbo (1982) using high performance liquid chromatography (HPLC).

Sugars were analyzed by first extracting a one gram flour sample with 10 ml of 80% ethanol in an 80°C water bath shaker for 15 min. The mixture was then centrifuged at 2,000 rpm for three minutes and the supernatant was collected in a new tube. The extraction was repeated two more times with the same procedure except that 5 ml of 80% ethanol were used in the second extraction. The precipitate was saved for starch analysis. Two ml of lead acetate were added to the extract collected from these three extractions. The mixture was shaken and centrifuged at 2,000 rpm for three minutes to precipitate protein and the supernatant was transferred to another tube. Two ml of 10% oxalic acid were added to the tube and again the mixture was shaken and centrifuged to remove the residual lead acetate. The final supernatant was brought to 25 ml volume and was passed through a SEP-PAK C Cartridge (Waters Associates, Inc., Milford, Massachusetts) prior to analysis. The HPLC consisted of a Solvent Delivery System 6000 A, a Universal Chromatograph Injector U6K, Differential Refractometer R 401, and a Data Module Model 730 (Waters Associates, Inc.). Acetonitrile-water (75:25, v/v) was used as the solvent at a flowrate of 2.0 ml/min. The carbohydrate analysis (micro-BONDAPAK) column was used to resolve the sugars from the aqueous extract. Quantitation and identification of sugars were done using the external standard method programmed in the Data Module to calculate the absolute amounts of the components in the injected sample (25 µl) and to obtain the percent sugar content based on dry weight:

$$\% \text{ Sugar} = \frac{\text{Data Module amt (mg/ml) x original sample (ml)}}{\text{sample wt (mg)}} \times 100$$

Starch. Starch was determined by the enzymatic hydrolysis method, developed by Agbo (1982), in conjunction with HPLC. The precipitate residue obtained from the sugar extraction was air dried to remove ethanol. Ten ml of 0.5 N NaOH were added to the centrifuge tube containing the pellets (approx. 1 g). With a rod, the pellets were colloidally dispersed into the 10 ml of 0.5 N NaOH solution by continuous crushing and stirring. After the pellets were completely dispersed, 10 ml of 0.5 N acetic acid (CH_3COOH) solution were added to the tube to neutralize the NaOH used for dispersion. The neutral colloidal suspensions were allowed to stand for a few minutes in order to settle undissolved particles, mainly cellulose, as well as pectic substances. Then, 1 ml was transferred to a test tube, and 3 ml of amyloglucosidase enzyme solution (5 mg/ml) were added (glucoamylase 1,4-α-D-glucan glucohydrolase from *Rhizopus*, Sigma Chemical Co., St. Louis, Missouri, in 0.02 M acetate buffer, pH 4.9). The sample was continuously shaken in a water bath at 55°C ± 2°C for 30 min. After shaking, the test tube was removed from the water bath and filtered through a SEP-PAK to remove the acetate (Lester, 1980) prior to injection into the HPLC column (the sample system used in the sugar analysis system). Quantitation was the same as for sugars with exception that only glucose was used as a standard solution:

$$\% \text{ Starch} = \frac{\text{Data Module amt (mg/ml)} \times 10 \times \text{original sample (ml)} \times 0.9}{\text{sample wt (mg)}} \times 100,$$

where 0.9 is the factor to account for the water gained during hydrolysis and 10 is the equation correction factor.

Amylose. Amylose content of the purified starch was determined in quadruplicate using the "Blue Value" method of Gilbert and Spragg (1964). To a 50 ml flask 1.0 ml of an aqueous solution containing approximately 0.5 mg of sample and 0.5 ml of 1.0 N NaOH were added. The mixture was heated in a boiling water bath for 3 min. After cooling, 0.5 ml of 1.0 N HCl were added, followed by 0.07 g to 0.1 g of potassium hydrogen tartrate. Water was added to a volume of about 45 ml followed by 0.5 ml of iodine solution (2 mg iodine/ml; 20 mg potassium iodine/ml). This solution was made up to 50 ml, mixed, and allowed to stand 20 min at room temperature. The absorbance was measured at 680 mμ in a spectrophotometer (Bausch & Lomb) using a 1 cm cell.

For the reference solution, an iodine solution of equal concentration was used. The "Blue Value" (B.V.) was calculated from the following equation:

$$\text{"Blue Value"} = \frac{\text{absorbance (mμ)} \times 4}{\text{c (mg/dl)}},$$

where c refers to the carbohydrate content of the solution.

Blue Values of the samples were compared with those of pure amylose (amylose from potato, type III, Sigma Chemical Co.) to determine a measure of amylose content.

Pasting Characteristics

A Brabender Viscoamylograph (C.W. Brabender Instruments, Inc.) equipped with a 700 cm-g sensitivity cartridge was used to determine the pasting characteristics of air-classified high starch flours and purified starches. Ten g/100 ml and 8 g/100 ml concentrations of starch slurry in 400 ml of pH 5.3 phosphate buffer were used as standard conditions for air-classified and purified samples, respectively. Each sample was examined in duplicate following the standard procedure according to the AACC Method 22-10 (1962). The temperature of the sample was raised uniformly from 30°C to 95°C at a rate of 1.5°C/min, held at 95°C for 15 min, and then cooled uniformly to 50°C at the same rate, and held at 50°C for 15 min.

Concentrations of 6, 8, 10 and 12 g/100 ml were used for air-classified high starch flours, and concentrations of 6, 8 and 10 g/100 ml for purified starches. Standard conditions of amylograph operation were utilized throughout these studies.

Phosphate buffers of pH 4.0, 5.3, 6.0 and 8.0 were used for all samples. Standard conditions of amylograph operation were utilized throughout these studies.

Extended high temperature holding at 95°C for 30 min and extended low temperature holding at 50°C for 30 min following standard heating conditions were applied to all samples.

Molecular Structure

The molecular structure of purified starches was characterized by the application of gel permeation and enzymatic method.

Alpha amylase from *Bacillus subtilis* (Sigma Chemical Co.) and beta amylase from sweet potato (Sigma Chemical Co.) were applied to provide better structural information for this study.

The gel permeation chromatography (GPC) system used in this study was comprised of two 4.0 mm I.D. 30 cm micro BONDAGEL E-linear columns connected in series (Waters Associates, Inc.). This type of GPC column contains fully porous silica gel with a bonded organic ether as the stationary phase. Silica particles, approximately 10 μm in diameter, provide resolution for polymers ranging in molecular weight from 2×10^3 to 2×10^6. These two GPC columns were connected to the HPLC system used for sugar analysis. Dimethyl sulfoxide-water (90:10, v/v) was used as the solvent at a flow rate of 0.3 ml/min. The injected sample volume was 50 μl.

Samples prepared for this study included starch dissolved in aqueous DMSO (90:10, v/v) without any enzyme treatments as control, alpha amylase hydrolysates, and beta amylase hydrolysates.

Control. A sample of 0.15 g purified starch was dissolved in 100 ml aqueous DMSO (90:10, v/v). The mixture was continuously shaken in a 55°C water bath for 24 hours (Leach and Schoch, 1962). After shaking, the mixture was centrifuged at 2,000 rpm for 10 min. The supernatant was filtered through the clarification kit (Waters Associates, Inc.) prior to injection onto BONDAGEL E-linear GPC columns.

Alpha and Beta Amylase Hydrolysates. A sample of 0.15 g purified starch was treated separately with 10 ml of two enzyme solutions. Alpha amylase enzyme solution contained 560 units/ml in 0.02 M sodium acetate buffer, pH 5.8. Beta amylase enzyme solution contained 200 units/ml in 0.02 M sodium acetate buffer, pH 4.8. The starch-enzyme mixture was

incubated in a 35°C water bath for 24 hours with continuous shaking. The digestion solution was subsequently heated in boiling water for 20 min to inactivate enzyme activity. DMSO was added to afford the desired solvent composition (DMSO:H_2O, 90:10, v/v). Centrifugation and filtering were also required to prepare the injection samples.

Commercial amylose and amylopectin from potato (Sigma Chemical Co.), maltose, and glucose were used as standards to investigate their elution volumes. Sample preparation of standards followed the procedures for the control sample.

Statistical Analyses

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

Multivariate analyses of variance and covariance were determined by using subprogram ANOVA. Mean squares were reported after rounding. Single classification analyses of variance and Tukey mean separation were determined by using subprogram ONEWAY.

Tukey mean separation was selected for judging the significance of observed differences between each treatment and control. Treatments not significantly different ($P < 0.05$) were indicated with like letters.

RESULTS AND DISCUSSION

Chemical Composition

Proximate Composition Analyses

Mean values and Tukey mean separations for proximate composition of air-classified high starch flours and purified starches from navy, pinto and black beans are presented in Table 3.

Air-Classified High Starch Flours. All three flours show similar composition. This similarity might be a result from not only the same preparation process but also from the same general species of leguminous seeds, *Phaseolus vulgaris* L.

Fat content of navy bean flour (1.47%) was significantly higher than that of pinto (0.83%) and black (0.72%) bean flours. Significant differences in protein content were detected for all three bean flours. Black bean flour showed the lowest protein content (12.42%) and pinto bean flour, the highest protein content (19.06%). Conversely, the starch content of pinto bean flour was the lowest (65.43%) and that of black bean flour, the highest (69.87%). This inverse relationship between starch and protein content has been noticed previously by Tyler et al. (1981) for a number of other bean flours. Navy and black bean flours showed no significant differences in ash (3.63% and 3.74%,

Table 3. Proximate composition of air-classified high starch flours and purified starches from navy, pinto and black beans[1]

Bean Type/ Starch Treatment	Moisture % wb	Fat	Protein	Ash	ENDF	Starch	
		% db				Total	Amylose[2]
Navy							
air-classified	9.79e	1.47c	15.51d	3.63c	1.76b	65.43a	---
purified	7.22c	0.11a	0.98a	0.46a	0.51a	91.17c	39.24ab
Pinto							
air-classified	7.06c	0.72b	19.06e	3.05c	2.53c	64.56a	---
purified	5.60a	0.12a	1.45b	0.26a	0.79a	90.92c	36.09a
Black							
air-classified	8.14d	0.83b	12.42c	3.74c	1.72b	69.87b	---
purified	6.03b	0.12a	0.81a	1.56b	0.81a	87.87c	41.62b

[1]Mean value, n = 3 (Tukey mean separations, like letters within each column indicate no significant differences, $P < 0.05$)

[2]n = 4, based on percent dry weight of purified starch

respectively,) or ENDF (1.76% and 1.72%, respectively) contents, but they were significantly different from those of pinto bean flour (3.05% ash, 2.53% ENDF).

The air-classified high starch flour investigated by Tyler et al. (1981), however, had a higher starch content (71.0% to 75.2%) than any of these studied here. This higher starch separation efficiency might have been caused by differences in bean seeds (seasonal variations among beans), seed hardness, or process conditions.

Purified Starches. The air-classified high starch flours were purified further in order to study the characteristics of only the bean starch. The flours were purified by following the method modified by Naivikul and D'Appolonia (1979), where 0.016 N NaOH, water, and 70% ethanol were used in the purification process as extractant solvents.

Dilute NaOH is used by many investigators and it is suggested as a good medium to dissolve protein, but not to gelatinize the starch (Schoch and Maywald, 1968). In addition, dilute alkali saponifies free fatty acids which are then removed by water washing. Fiber is partially soluble in this solution. Water is used as a neutralized solvent and wash, where water soluble components such as water soluble pentosans and monosaccharides are removed. Ethanol is used to extract sugars from starch and also to decrease the fat content.

The lighter, fine fiber, some undissolved denatured protein, and other insoluble solids remain suspended. Due to differences of density, starch can be recovered by centrifugation. Two distinct layers show after centrifugation: a spongy gray top layer and a dense white bottom layer. The top layer was skimmed off; the bottom layer is the purified starch.

During starch purification, greater difficulties in separation of pinto starch were encountered than for navy or black bean starches. The top layer, a spongy gel-like substance, could not be separated from the starch layer, as it was with the navy and black bean starches. Thus, a higher impurities content was expected. Also as expected, pinto starch showed a significantly higher protein content (1.45%) than that of navy (0.98%) or black (0.81%) bean starches. However, no significant differences in fat content were detected for any of the three bean starches (0.11% to 0.12%). Black bean starch showed significantly higher ash content (1.56%) than that of navy (0.46%) or pinto (0.26%), due, perhaps, to the inclusion of small black particles dispersed in the purified starch fraction of the black bean after centrifugation.

Naivikul and D'Appolonia (1979) reported the chemical composition of navy and pinto bean starches isolated by this same method. Their results show higher starch contents than do the results presented in this study, possibly because they prepared starch on a smaller scale (500 g) than this study did (7 Kg). The washing step could have been more efficient with the smaller sample than with the larger sample size.

The amylose content of navy, pinto and black bean starches, determined by the "Blue Value" method (Gilbert and Spragg, 1964), were 39.24%, 36.09% and 41.62%, respectively. Among these three bean starches, the amylose content of navy bean was not significantly different from that of pinto or black beans, but there was a significant difference between pinto and black beans.

Naivikul and D'Appolonia (1979) reported much lower amylose contents of navy (22.1%) and pinto (25.8%) bean starches. The amylose content of black bean starch was slightly higher than the value of 38.1% reported by Lai and Varriano-Marston (1979). However, all of the amylose contents

from this study fall within the range reported by Reddy et al. (1984) for legume starches. The disparity in results of amylose contents between previous reports and the present study might be explained by differences in the methods used to determine amylose levels. In addition, Duffus and Murdoch (1979) suggested that large variations in amylose contents of individual varieties of legumes depended on not only geographic location of growth but also the maturation of seed.

Sugar Analyses

Mean values and Tukey mean separations for sugar analyses of air-classified high starch flours and purified starches from navy, pinto and black beans are reported in Table 4.

Air-Classified High Starch Flours. All three bean flours show the same sugar order pattern, from high to low: sucrose, stachyose, glucose, raffinose, with no inositol detection. This order of dominance agrees with many previous studies (Schweizer et al., 1977; Naivikul and D'Appolonia, 1978; Fleming and Reichert, 1983); however, the sugar content levels are different.

The stachyose (0.93%) and raffinose (0.17%) levels of navy bean flours are slightly lower than those reported by Fleming and Reichert (1983), 1.02% and 0.51%, respectively. The differences may be explained by the different methods of sugar determination, as well as the variation inherent in beans grown in different locations.

Purified Starches. As expected, no sugar was detected because 70% ethanol was used twice as the solvent to wash the starches during the final step of purification, thereby completely extracting the sugars with ethanol.

Table 4. Percent sugar content (glucose, sucrose, inositol, raffinose and stachyose) of air-classified high starch flours and purified starches from navy, pinto and black beans[1]

Bean Type/ Starch Treatment	Sugar (% db)[2]				
	Glucose	Sucrose	Inositol	Raffinose	Stachyose
Navy					
air-classified	0.21a	1.10a	ND[3]	0.17a	0.93a
purified	ND	ND	ND	ND	ND
Pinto					
air-classified	0.39b	1.49ab	ND	0.23b	0.92a
purified	ND	ND	ND	ND	ND
Black					
air-classified	0.45b	1.69b	ND	0.19ab	0.95a
purified	ND	ND	ND	ND	ND

[1]Mean values (Tukey mean separations, like letters within each column indicate no significant differences, $P < 0.05$)

[2]n = 4 (2 replicates/sample x injections/replicate)

[3]not detectable

No comparison can be made with literature for the purified starches, since there has been no previous study of them with regard to the simple sugars.

Brabender Pasting Characteristics

The Brabender pasting curves of air-classified high starch flours and purified starches from navy, pinto and black beans with varying concentrations, pH values, and holding periods are shown in Figures 3 through 20. As a result from these curves, several practical significant points are expressed in tabular form according to Medcalf and Giles (1966) terminology (Tables 5, 6 and 7).

The initial pasting temperature is defined as the temperature at which an initial increase of ten Brabender units (BU) in viscosity is reached. Normally, peak height is the maximum viscosity obtained; however, since no definite peak was obtained in this study, the values at 95°C were reported. The viscosities of samples after the 15 min holding time at 95°C, after cooling to 50°C, and after 15 min holding time at 50°C are also reported.

The Brabender pasting characteristics of air-classified navy, pinto and black bean flours at standard conditions (10 g/100 ml concentration in pH 5.3 phosphate buffer solution) are shown in Figure 3. Individual points are recorded in Table 5. Navy and black bean flours gave similar initial pasting temperatures (78.5°C) which were slightly higher than that of pinto bean flour (75.5°C). However, they all showed the same viscosity pattern. This pattern showed restricted swelling, containing no pasting peak, and increasing viscosity during heating at 95°C. A tendency to retrograde during cooling was also shown on their pasting

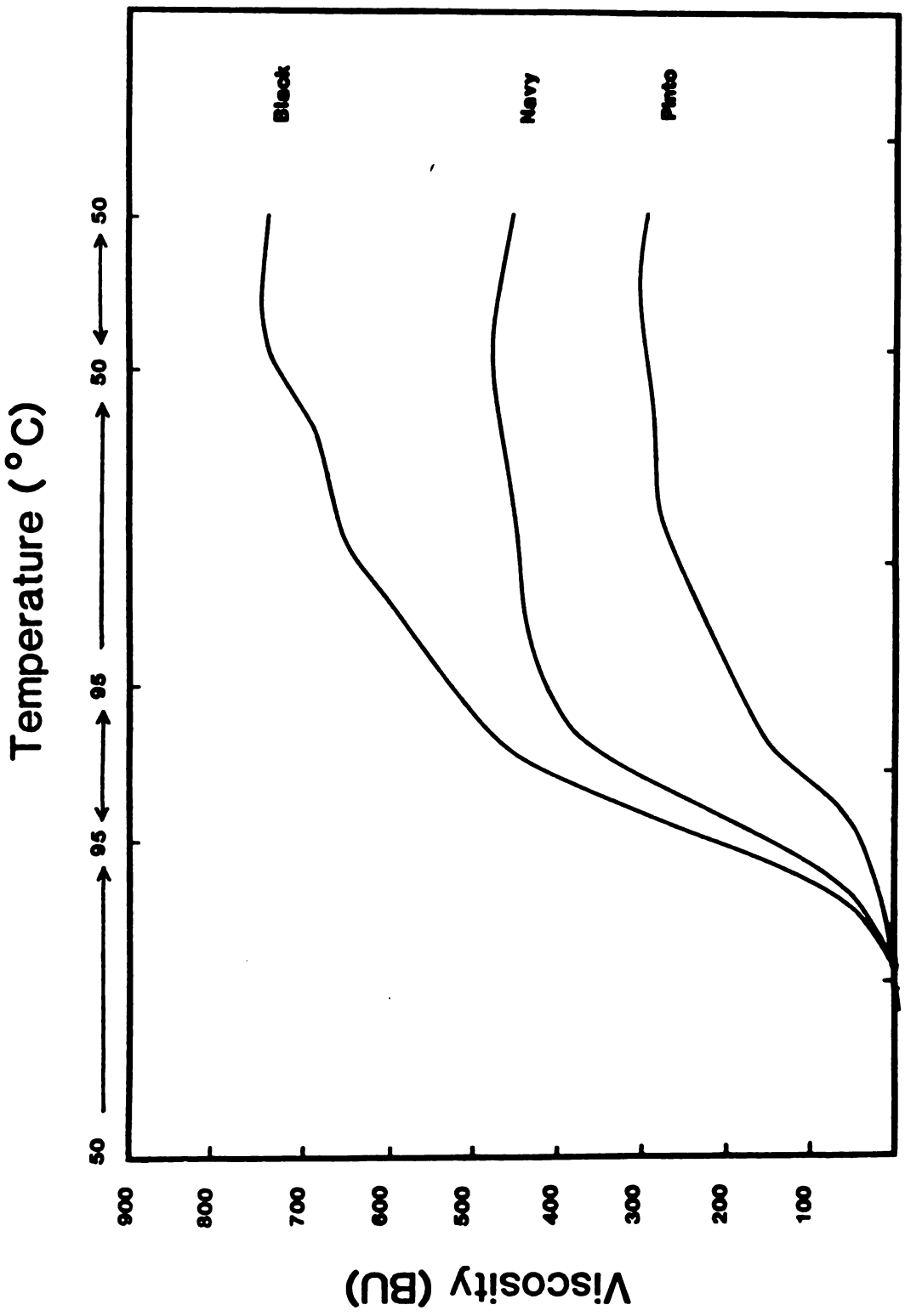

Figure 3. Brabender pasting curves (standard conditions) of air-classified navy, pinto and black bean flours at 10 g/100 ml in pH 5.3 phosphate buffer solution

Table 5. Pasting characteristics of air-classified high starch flours and purified starches from navy, pinto and black beans at various concentrations in pH 5.3 phosphate buffer solution[1]

Bean Type/ Starch Treatment	Concentration (g/100 ml)	Initial Pasting Temp (°C)	Viscosity (BU) at 95°C	Viscosity (BU) after 15 min at 95°C	Viscosity (BU) at 50°C	Viscosity (BU) after 15 min at 50°C
Navy						
air-classified	6	-	-	7	18	12
	8	83.0	15	98	152	145
	10	78.5	165	394	475	445
	12	75.5	530	882	>1000	>1000
purified	6	-	8	20	38	38
	8	79.3	190	425	535	550
	10	78.5	475	719	>1000	>1000
Pinto						
air-classified	6	-	-	4	14	12
	8	80.0	7	32	124	112
	10	75.5	47	180	300	285
	12	75.5	210	498	715	720
purified	6	84.5	7	15	37	38
	8	81.5	30	157	253	274
	10	77.8	335	745	>1000	>1000

Table 5 (cont'd.)

Bean Type/ Starch Treatment	Concentration (g/100 ml)	Initial Pasting Temp (°C)	Viscosity (BU) at 95°C	Viscosity (BU) after 15 min at 95°C	Viscosity (BU) at 50°C	Viscosity (BU) after 15 min at 50°C
Black						
air-classified	6	83.0	6	14	58	62
	8	78.5	24	125	214	202
	10	78.5	230	520	737	734
	12	75.5	640	>1000	>1000	>1000
purified	6	79.3	8	28	62	63
	8	78.5	117	320	449	483
	10	77.0	590	>1000	>1000	>1000

[1]Mean values (n = 2) and standard deviation = ±10.0 BU

curves. Among the three types of beans, black bean flour showed the greater tendency to retrograde than did either pinto or navy bean flour. Later on during the 15 min holding phase at 50°C, the curves showed some decrease in viscosity. This phenomenon is most likely the case of molecular breakdown, resulting from a continuous shear force. At the same concentration, black bean flour developed a more viscous paste than did the navy or pinto bean flours. The reason may not only be due to the individual starch characteristics, but also to some effects from the differences in starch content (Table 3).

The Brabender pasting curves of purified starches of the three bean types at standard conditions (8 g/100 ml in pH 5.3 phosphate buffer solution) are illustrated in Figure 4, with individual points reported in Table 6. A lower solid concentration was used for the purified starches (8.0 g/100 ml) than was used for the air-classified starches (10 g/100 ml), because purified starch contains a higher percentage of starch at the same concentration than the air-classified flour. From these three bean starches, pinto showed the highest pasting temperature (81.5°C) followed by navy (79.3°C) and black (78.5°C), respectively. The initial pasting temperature of navy and black bean starches determined by this investigation has good agreement with previous studies: navy bean, 77.0°C (Naivikul and D'Appolonia, 1979); black bean, 76.0°C (Lai and Varriano-Marston, 1979). However, the initial pasting temperature of pinto starch from this study (81.0°C) was higher than 77.0°C reported by Naivikul and D'Appolonia (1979). All bean starches showed similar viscosity patterns as their flours. Nevertheless, there were some differences, which will be discussed later. The purified starch curves indicated a tendency for the starches to retrograde during cooling. Later on during the 15 min holding phase at 50°C, the viscosity of the

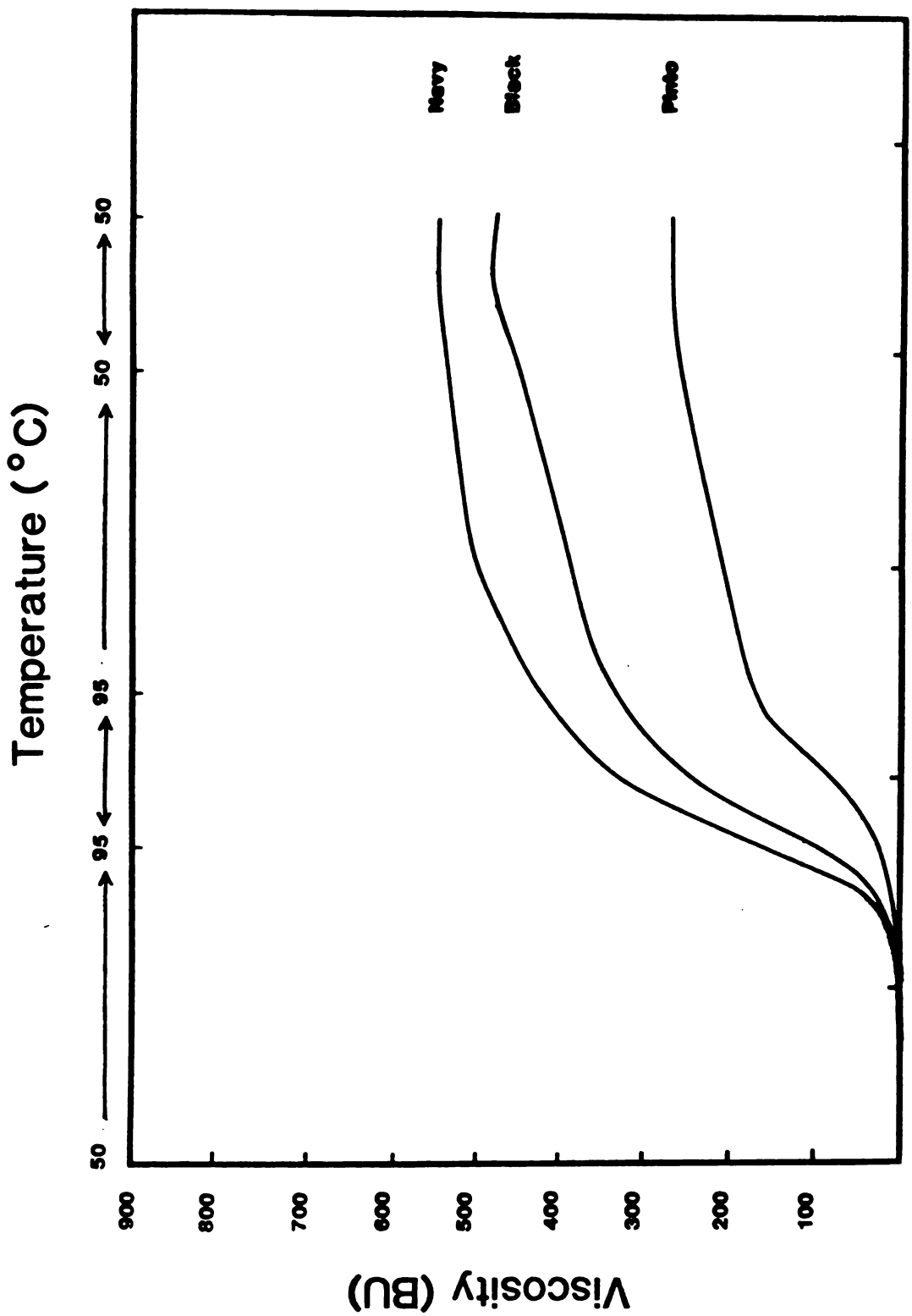

Figure 4. Brabender pasting curves (standard conditions) of purified navy, pinto and black bean starches at 8 g/100 ml in pH 5.3 phosphate buffer solution

Table 6. Pasting characteristics of air-classified high starch flours (10 g/100 ml) and purified starches (8 g/100 ml) from navy, pinto and black beans at different pH values of phosphate buffer solution[1]

Bean Type/ Starch Treatment	pH	Initial Pasting Temp (°C)	Viscosity (BU) at 95°C	Viscosity (BU) after 15 min at 95°C	Viscosity (BU) at 50°C	Viscosity (BU) after 15 min at 50°C
Navy						
air-classified	4.0	78.5	143	374	443	405
	5.3	78.5	165	394	475	445
	6.0	78.5	160	418	513	500
	8.0	77.0	184	448	564	555
purified	4.0	77.0	167	403	487	494
	5.3	79.3	190	425	535	550
	6.0	77.0	184	440	557	565
	8.0	77.0	215	468	588	590
Pinto						
air-classified	4.0	75.5	47	177	323	285
	5.3	75.5	47	180	300	285
	6.0	80.0	45	174	269	255
	8.0	80.0	52	163	232	205
purified	4.0	81.5	26	146	233	240
	5.3	81.5	30	157	253	274
	6.0	81.5	40	174	265	288
	8.0	81.5	50	232	314	334

Table 6 (cont'd.)

Bean Type/ Starch Treatment	pH	Initial Pasting Temp (°C)	Viscosity (BU) at 95°C	Viscosity (BU) after 15 min at 95°C	Viscosity (BU) at 50°C	Viscosity (BU) after 15 min at 50°C
Black						
air-classified	4.0	80.0	190	470	694	680
	5.3	78.5	230	520	737	734
	6.0	78.5	250	535	761	760
	8.0	78.5	314	634	794	793
purified	4.0	77.0	120	313	422	460
	5.3	78.5	117	320	449	483
	6.0	78.5	120	350	464	495
	8.0	80.0	138	395	525	575

[1]Mean values (n = 2) and standard deviation = ±10.0 BU

starches steadily increased which indicated that starch granules are very resistant to swelling and fragmentation. Most legume starches show these pasting characteristics (Schoch and Maywald, 1968; Kawamura, 1969; Lineback and Ke, 1975; Vose, 1977; Comer and Fry, 1978; Billiaderis et al., 1979; Lai and Varriano-Marston, 1979; Naivikul and D'Appolonia, 1979; Lii and Chang, 1981; Sathe and Salunkhe, 1981a). For the same concentration, navy bean starch was more viscous than either black or pinto bean starches. In this case, the effect of individual starch characteristics was a predominant factor, because among three purified bean starches, there was no significant difference in starch content (Table 3).

When the pasting characteristics of air-classified flours and purified starches were compared (Figures 3 and 4), it could be seen that there were other factors affecting pasting characteristics of starch. There was no significant change in initial pasting temperature of black bean flour after purification; on the other hand, purified navy bean starch showed a small increase in this temperature, and purified pinto bean starch showed a significant increase. This significant increase in initial pasting temperature of pinto bean starch might have been due to the effect of its protein: the higher protein content, the lower initial pasting temperature. Because proteins can absorb water and display a tendency to swell without disintegration, this swelling from water uptake might cause the lower initial pasting temperature and increase in viscosity (Edsall, 1965; Hermansson, 1979). Gaucher-Choquette and Boulet (1982) also reported that the concentration of protein can affect the viscosity change. From both Figures 3 and 4, it can be noticed that the flours show a higher rate of viscosity increase during the pasting cycle than do the purified starches.

The above results confirm that protein plays a significant role in viscosity changes. In an aqueous environment, proteins are generally folded in compact state with the polar groups exposed to the aqueous phase and the hydrophobic groups located internally (Fox and Condon, 1981). With the temperature increase and the applied shear force, the bean flour proteins may change their physical structure to an unfolding form, which is a prerequisite for the viscosity increase. As the protein molecules unfold and their hydrophobic interior is exposed to the environment, they tend to associate with themselves at a rate related to the hydrophobic character of the protein (Salimath and Tharanathan, 1982) and thus, the viscosity steadily increases. Even though the protein level of the flour affects the initial pasting temperature, it still does not cause any change in the flour viscosity pattern. The decrease in viscosity of flours during the 15 min holding phase at 50°C indicated that protein molecules were more susceptible to shear breakdown than were starch granules.

Figures 5, 6 and 7 show the Brabender pasting curves of air-classified navy, pinto and black bean flours at various concentrations with the individual points presented in Table 5. The viscosity patterns for the various concentrations did not change. At low concentrations (6 g and 8 g/100 ml), viscosity stability was evident throughout the heating and cooling cycles. At low concentration 6 g/100 ml, the detected initial pasting temperatures were rather high. At this low concentration, the starch content may be too low to pick up the early stages of gelatinization. At high concentration, an increase in viscosity during heating was evident. A higher tendency to retrograde was also observed for the high concentration conditions, because of the greater probability for amylose molecules to associate and form hydrogen bonds among themselves.

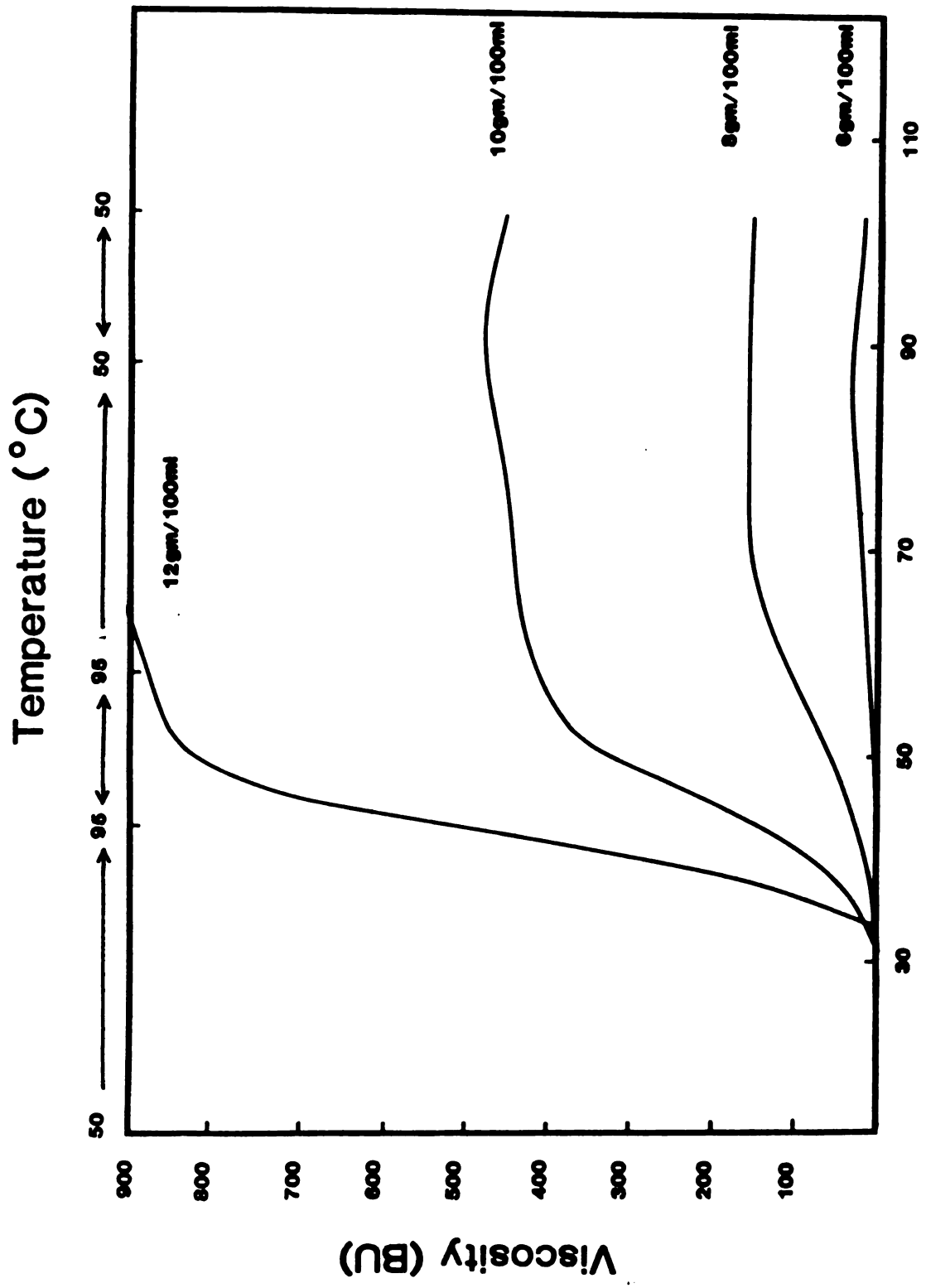

Figure 5. Brabender pasting curves (standard conditions) of air-classified navy bean flour at various concentrations in pH 5.3 phosphate buffer solution

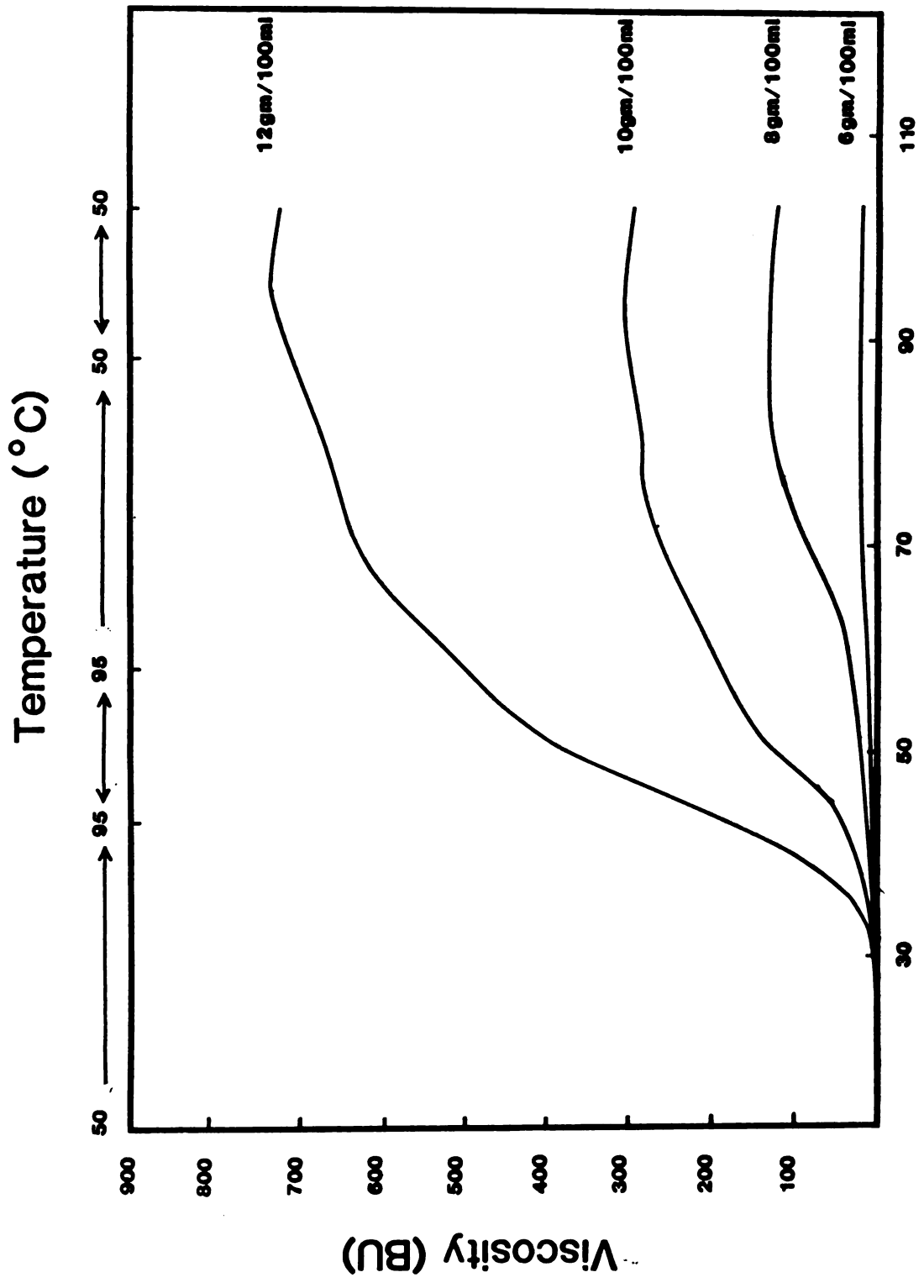

Figure 6. Brabender pasting curves (standard conditions) of air-classified pinto bean flour at various concentrations in pH 5.3 phosphate buffer solution

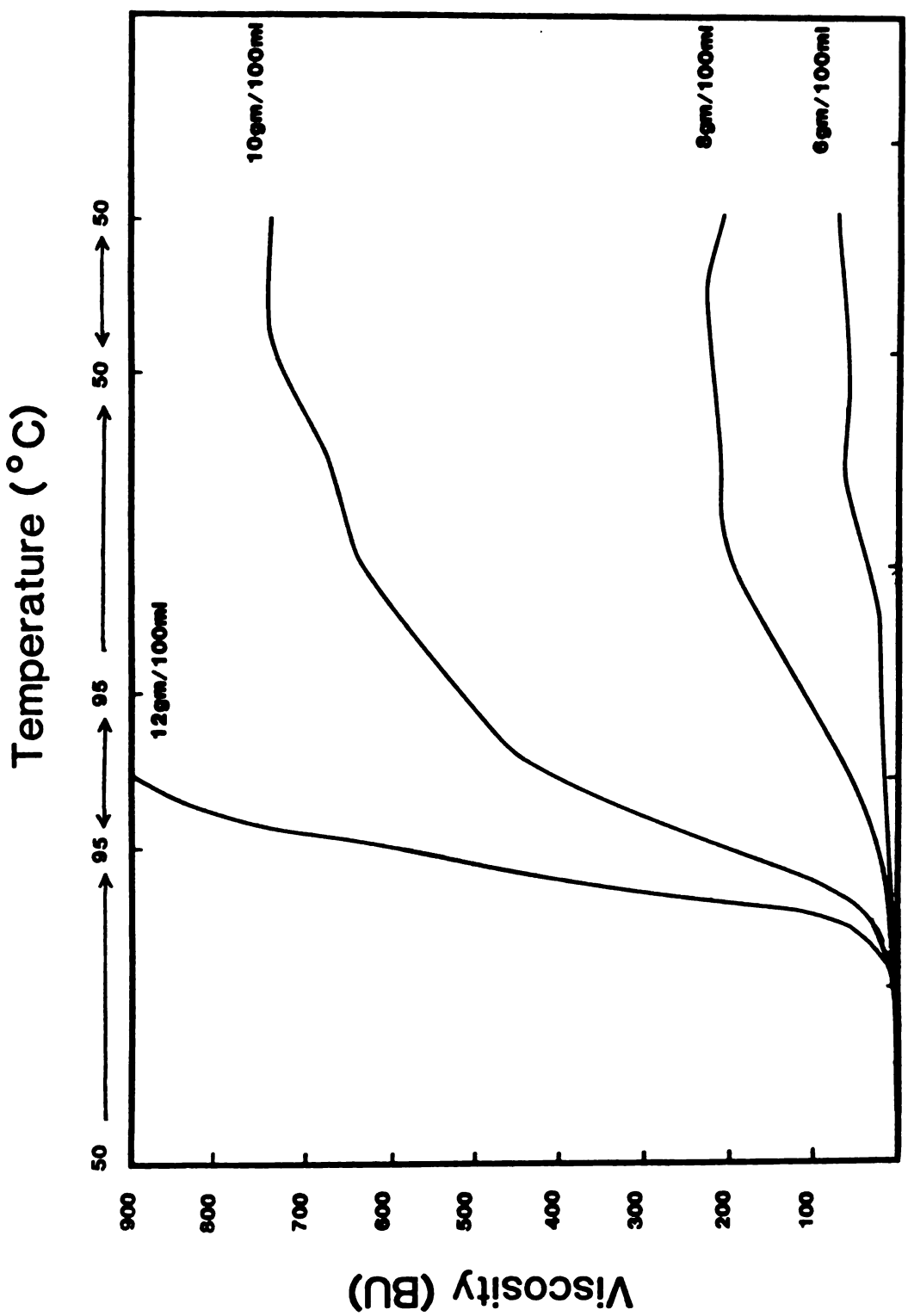

Figure 7. Brabender pasting curves (standard conditions) of air-classified black bean flour at various concentrations in pH 5.3 phosphate buffer solution

Figures 8, 9 and 10 show Brabender pasting characteristics of purified navy, pinto and black bean starches at various concentrations with their specific points presented in Table 5. Their results are quite similar to those of air-classified flours, i.e., no change in viscosity pattern, more stable paste through pasting cycle at low concentration, and a high tendency to retrograde at higher concentration. The difference between air-classified flours and purified starches is that the rate of increasing viscosity of purified starches is higher than that of air-classified flours at the same increasing rate of concentration. The reason is that the purified starch contains a higher starch content than does air-classified flour (Table 3).

Figures 11, 12 and 13 show the Brabender pasting curves of air-classified flours at different pH values of phosphate buffer solution, with their individual points presented in Table 6. Because most foods have pH values in the range of 4 to 8, this pH range was selected for this study. Variation within this pH range produced only minor effects on pasting characteristics and had no effect on the viscosity pattern. Most changes occurred after the temperature reached 95°C. This minor effect of variation in pH on pasting properties might be explained by the buffer capacity property of specific type of proteins (Sathe and Salunkhe, 1981b). Significant changes in pasting characteristics were noticed only in the pH 8 and the pH 4 solutions. There were no significant changes in initial pasting temperature of navy and black bean flours in the pH range of 4 to 8; however, one can see a trend to lower this temperature as the pH increases. Pinto bean flour gave the opposite result: the higher the pH, the higher the initial pasting temperature. An increase in pasting viscosity was observed in navy and black bean flours with increasing pH values, while pinto bean flour showed the

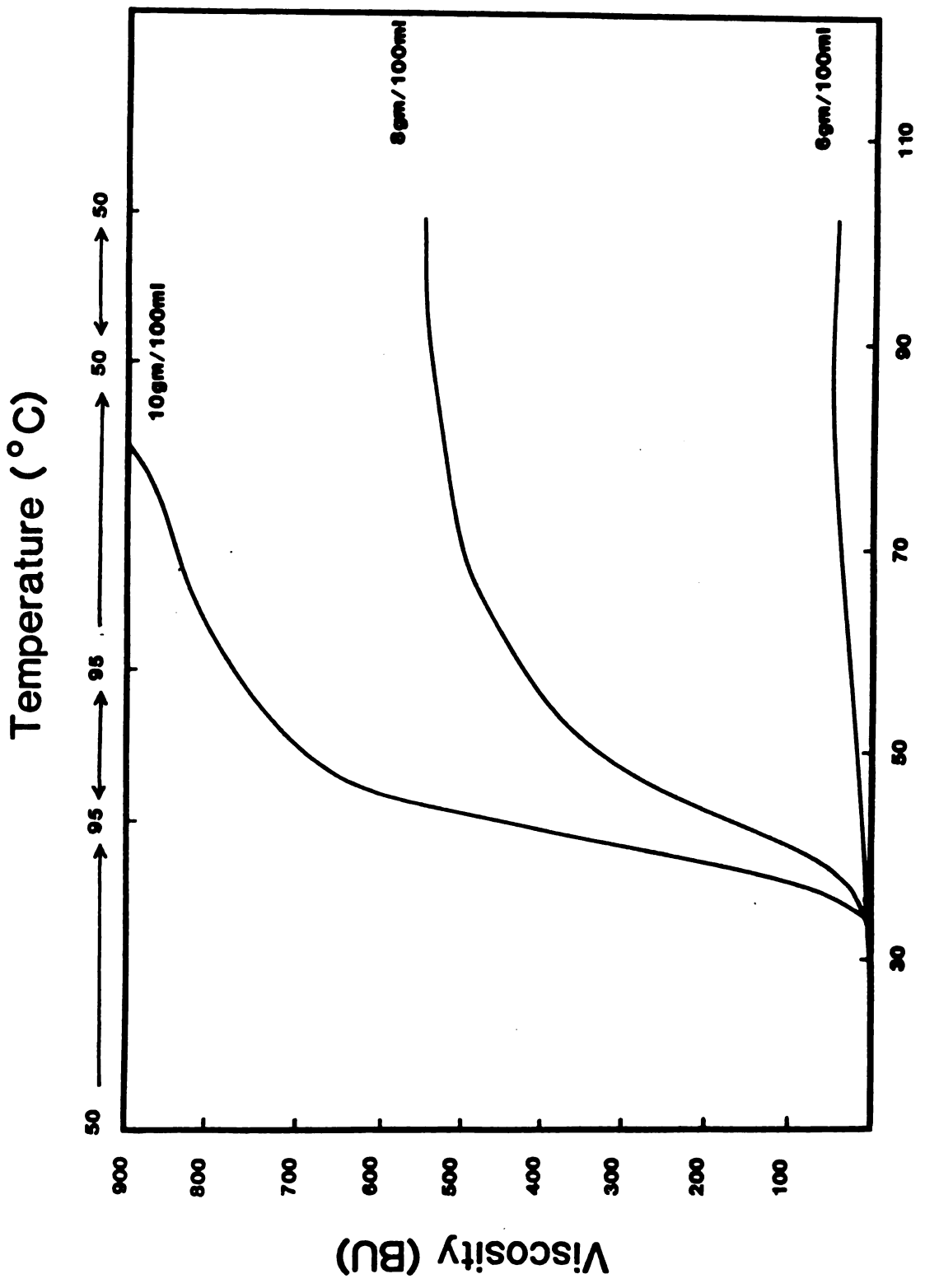

Figure 8. Brabender pasting curves (standard conditions) of purified navy bean starch at various concentrations in pH 5.3 phosphate buffer solution

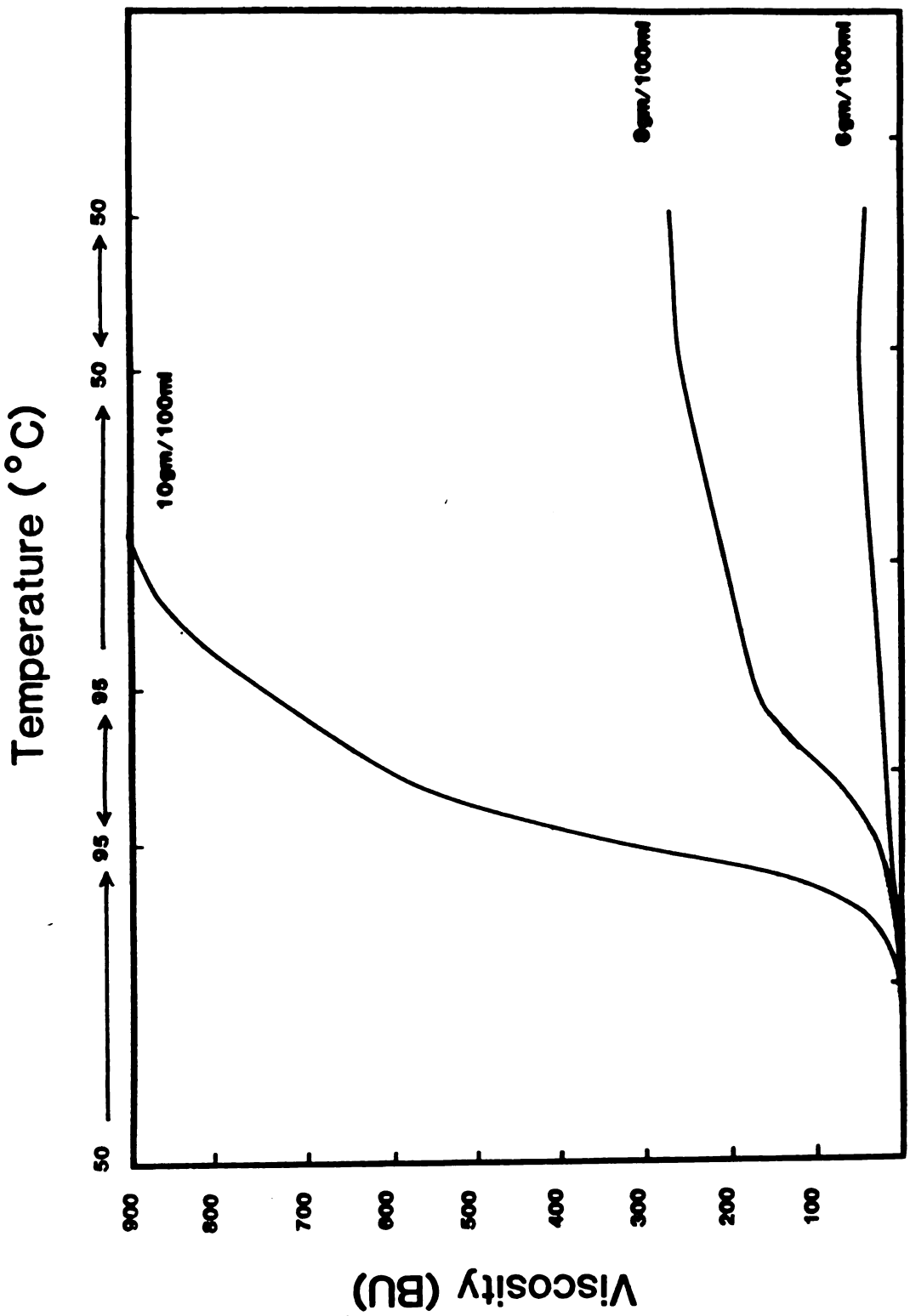

Figure 9. Brabender pasting curves (standard conditions) of purified pinto bean starch at various concentrations in pH 5.3 phosphate buffer solution

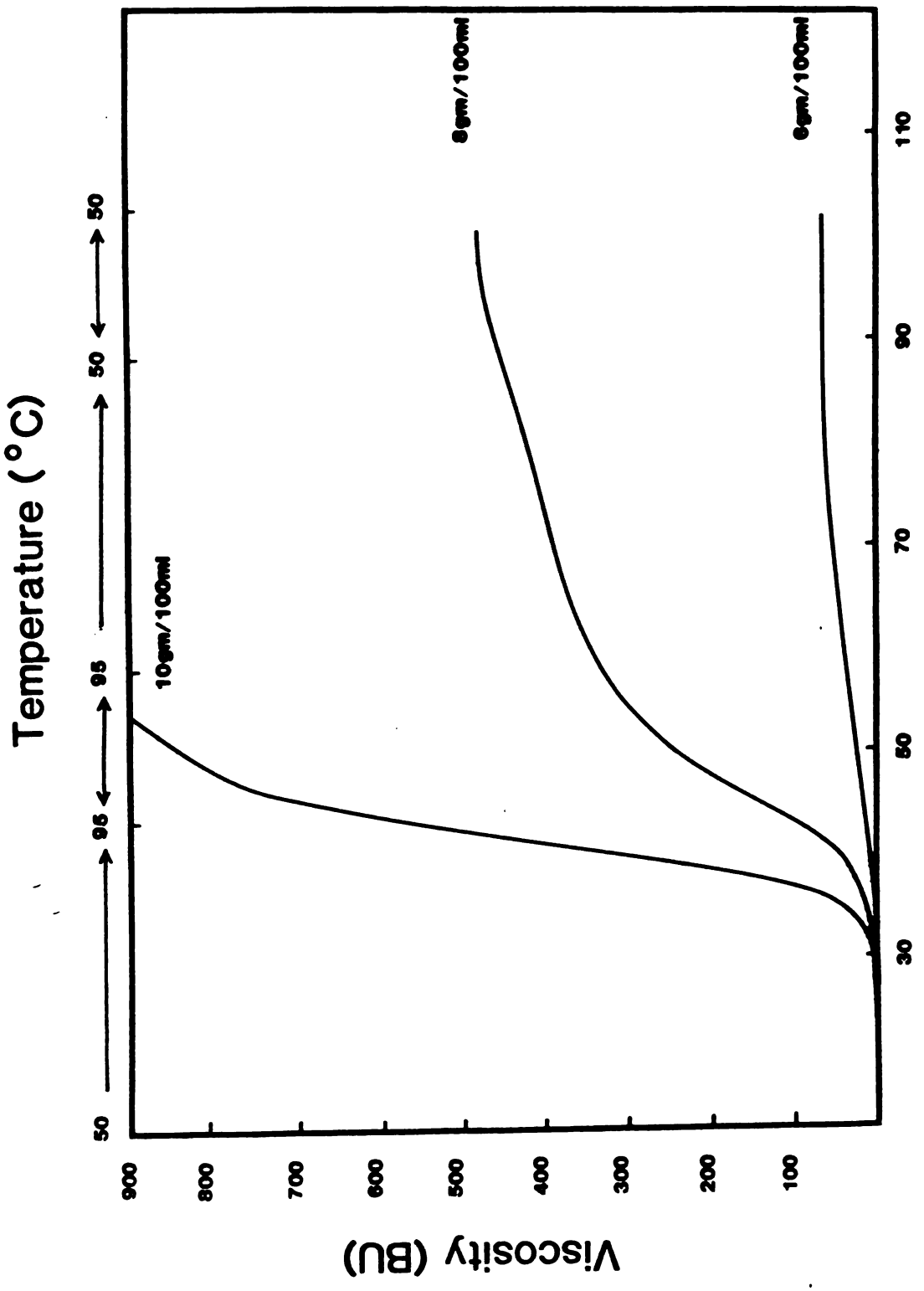

Figure 10. Brabender pasting curves (standard conditions) of purified black beam starch at various concentrations in pH 5.3 phosphate buffer solution

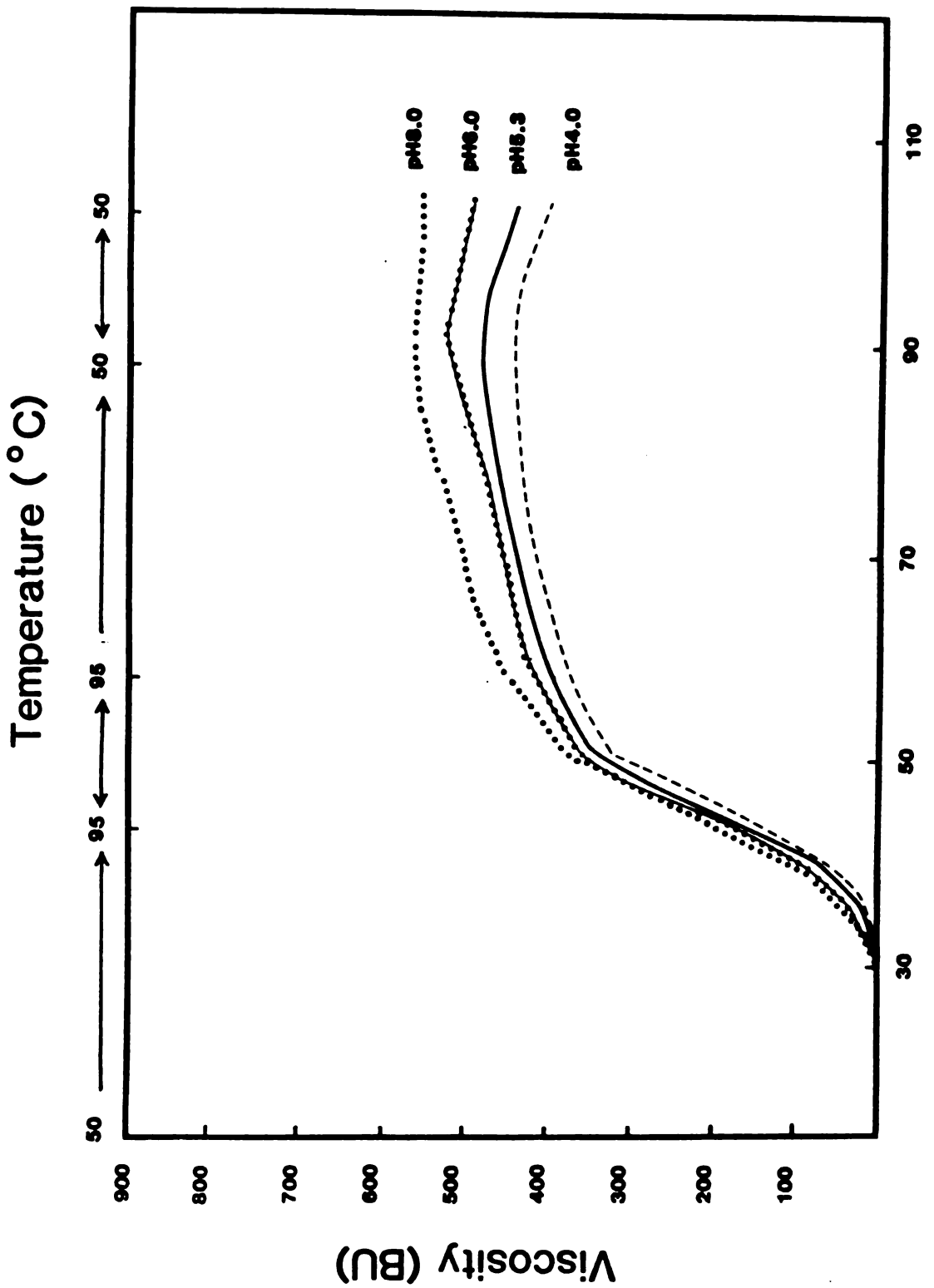

Figure 11. Brabender pasting curves (standard conditions) of air-classified navy bean flour at different pH values of phosphate buffer solution at 10 g/100 ml concentration

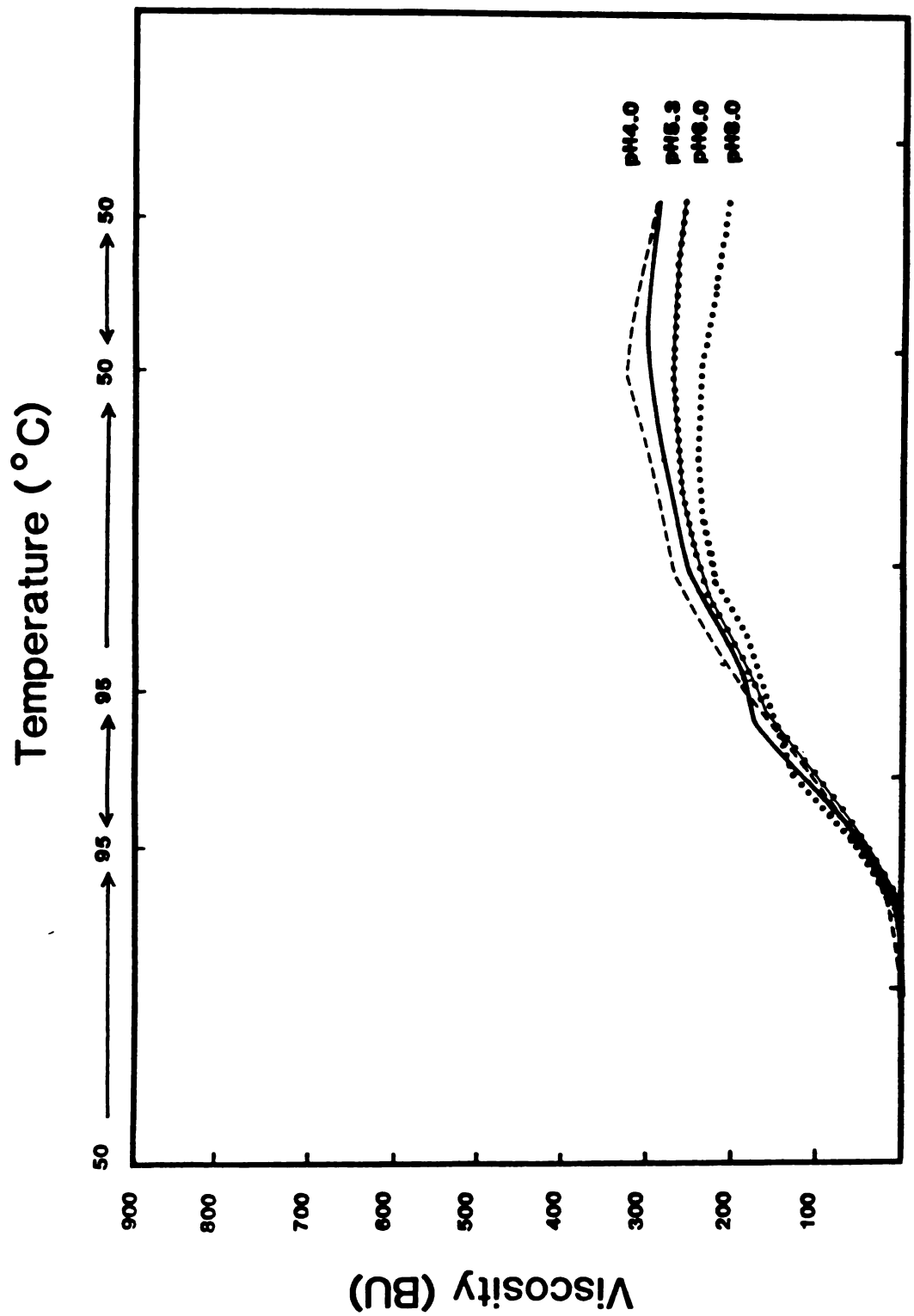

Figure 12. Brabender pasting curves (standard conditions) of air-classified pinto bean flour at different pH values of phosphate buffer solution at 10 g/100 ml concentration

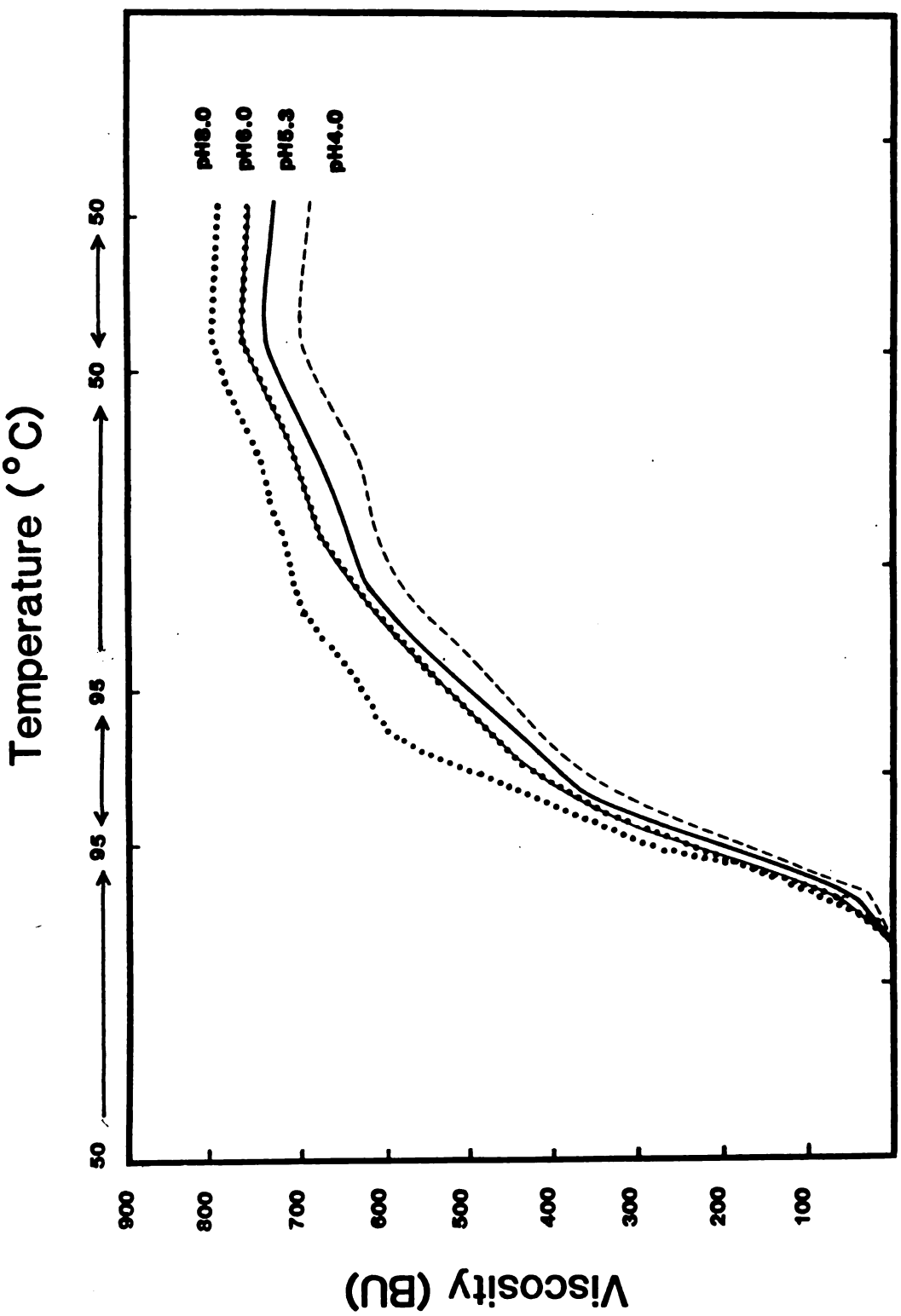

Figure 13. Brabender pasting curves (standard conditions) of air-classified black bean flour at different pH values of phosphate buffer solution at 10 g/100 ml concentration

opposite effect. It may have been the effect of protein type or content. Edsall (1965) reported that the viscosity of a protein dispersion is a function of protein type (molecular size, shape, surface charge, concentration), pH, temperature, and shear rate.

Figures 14, 15 and 16 show the Brabender pasting curves of purified starches at different pH values of phosphate buffer solution with individual points recorded in Table 6. As can be seen from these curves, changing pH values from pH 4 to pH 8 caused only small effects on pasting properties of bean starches. There were no changes in the viscosity patterns, in the initial pasting temperature, or in the pasting properties during heating up to 95°C. However, all bean starches showed the tendency of increasing viscosity with increasing pH. These characteristics indicated that bean starch granules were quite resistant to variations in pH. Vose (1977) and Comer and Fry (1978) reported a reverse viscosity pattern for pea starch: the higher pH gave the lower viscosity.

When air-classified flours were compared to their purified starches (Table 6), neither showed significant differences in viscosity pattern, pasting properties, or pH sensitivity, except for the air-classified pinto flour which showed a decreased viscosity with increasing pH values. This effect might have been due to the high protein content in pinto flour.

The Brabender pasting curves of air-classified high starch flours of three bean types at an extended high temperature (95°C) holding period (30 min) are shown in Figure 17, with their specific points reported in Table 7. Prolonged heating tended to increase viscosity of flours. There was no change in the viscosity pattern. The curves steadily increased and showed less shear force breakdown during the 15 min holding period at 50°C as compared to bean flours at standard

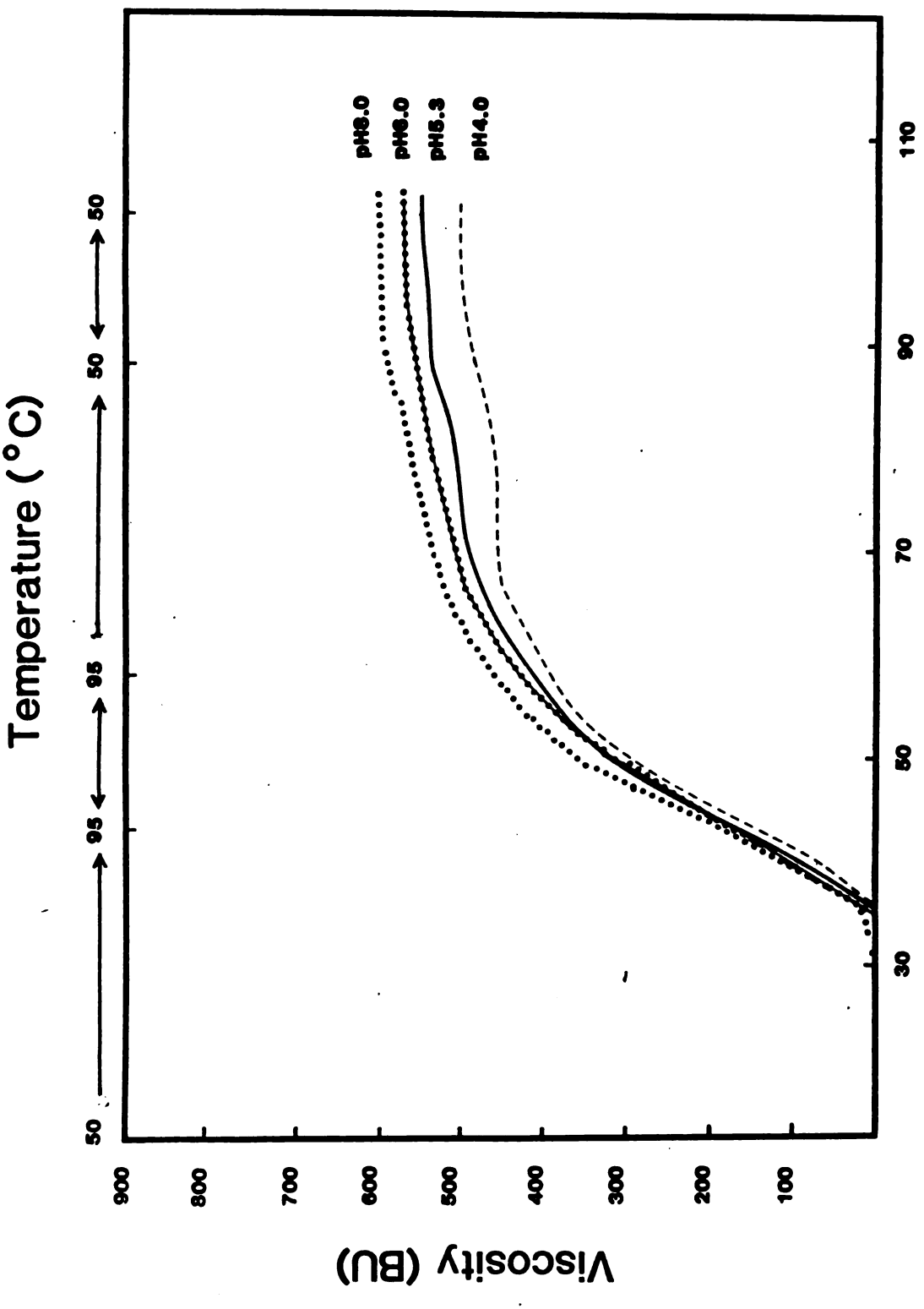

Figure 14. Brabender pasting curves (standard conditions) of purified navy bean starch at different pH values of phosphate buffer solution at 8 g/100 ml concentration

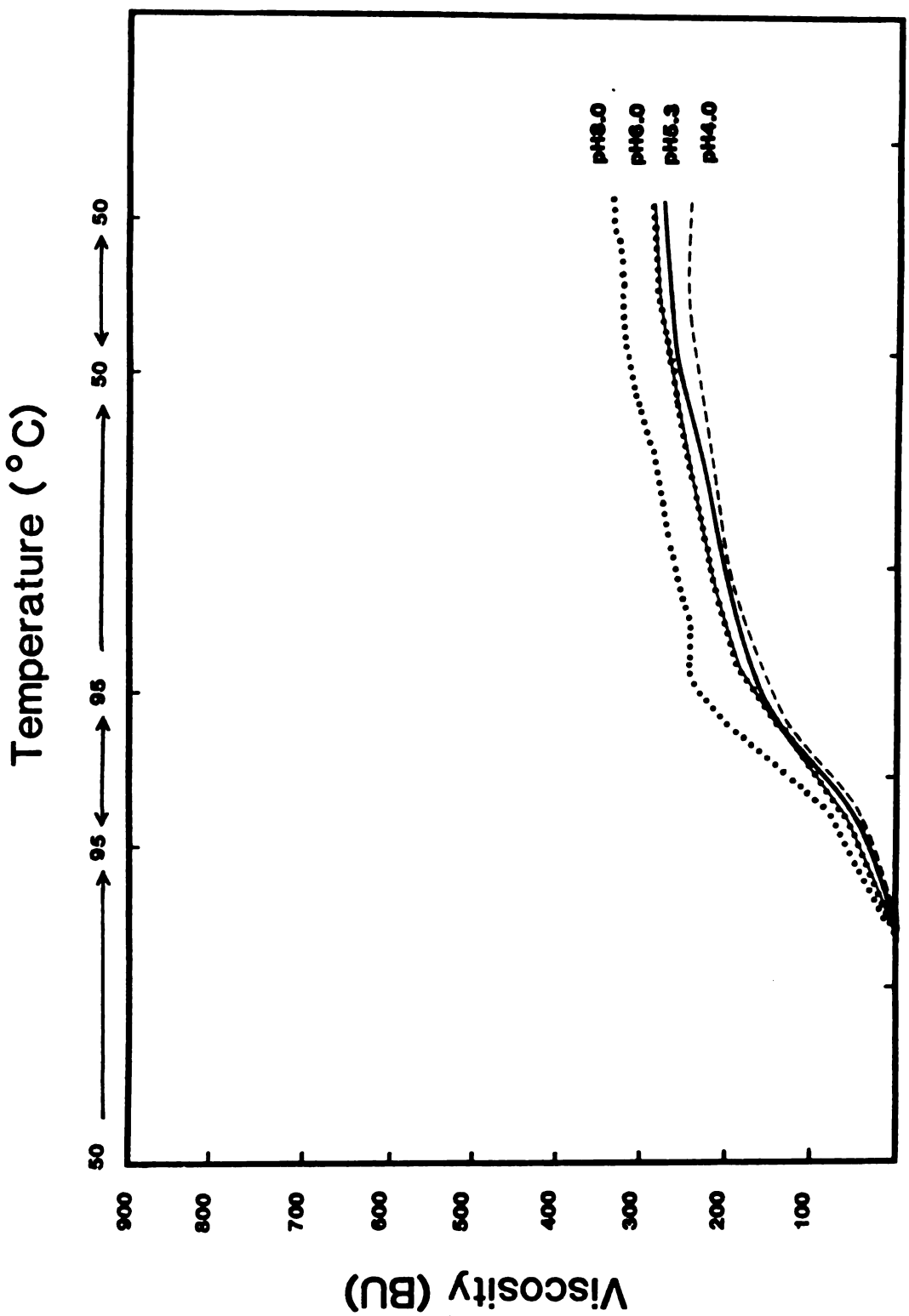

Figure 15. Brabender pasting curves (standard conditions) of purified pinto bean starch at different pH values of phosphate buffer solution at 8 g/100 ml concentration

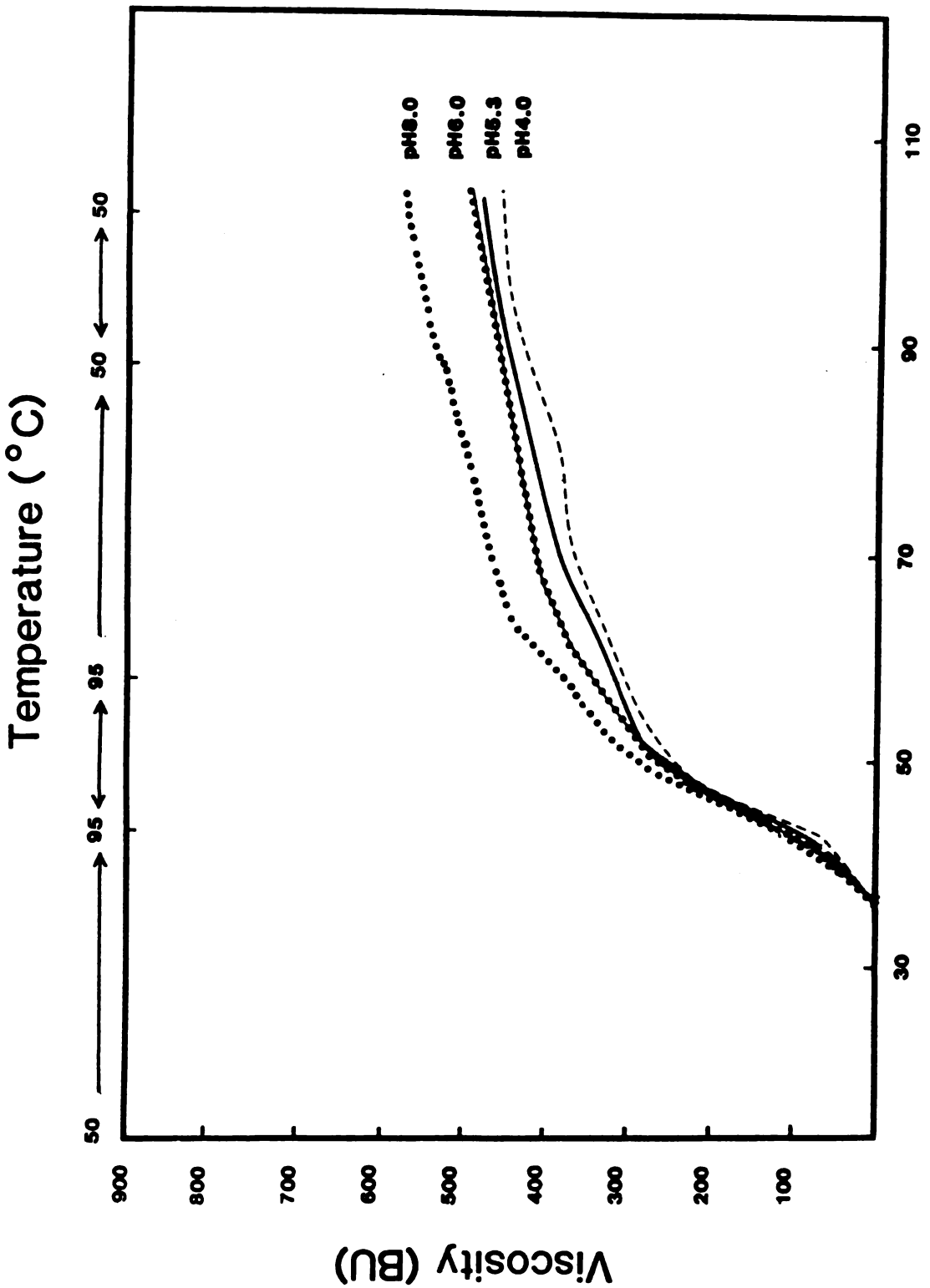

Figure 16. Brabender pasting curves (standard conditions) of purified black bean starch at different pH values of phosphate buffer solution at 8 g/100 ml concentration

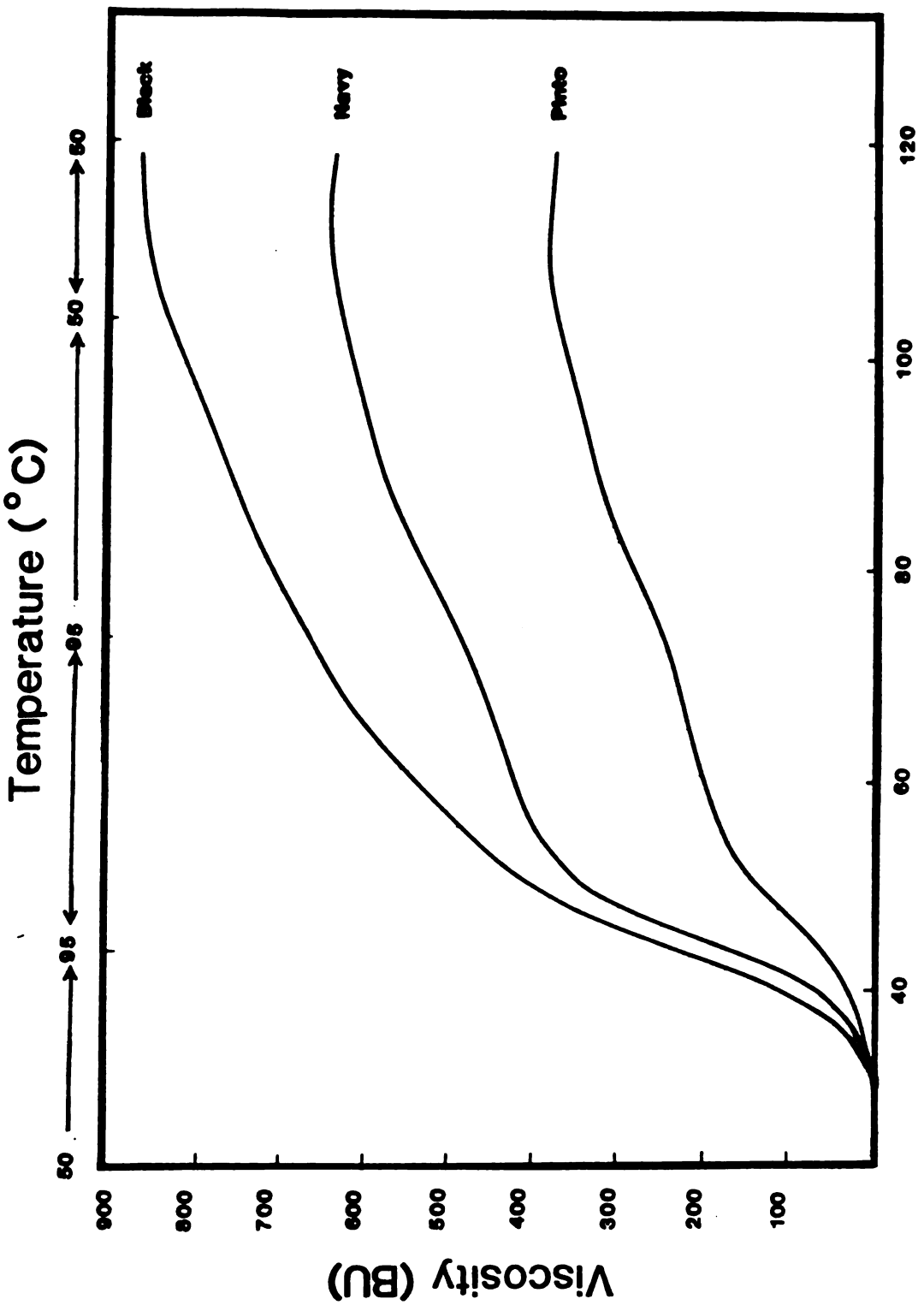

Figure 17. Brabender pasting curves of air-classified high starch flours (10 g/100 ml) at an extended high temperature (95°C) holding period (30 min) in pH 5.3 phosphate buffer solution

Table 7. Pasting characteristics of air-classified high starch flours (10 g/100 ml) and purified starches (8 g/100 ml) from navy, pinto and black beans at different high or low temperature holding periods in pH 5.3 phosphate buffer solution[1]

Bean Type/ Starch Treatment	Viscosity (BU) at 95°C	after 15 min at 95°C	after 30 min at 95°C	at 50°C	after 15 min at 50°C	after 30 min at 50°C
Navy						
air-classified	165	394	-	475	445	-
	164	418	585	628	633	-
	173	405	-	498	460	440
purified	190	425	-	535	550	-
	188	427	500	575	596	-
	182	427	-	538	554	570
Pinto						
air-classified	47	180	-	300	285	-
	57	193	244	377	375	-
	46	176	-	296	280	266
purified	30	157	-	253	274	-
	33	150	200	281	314	-
	34	160	-	254	276	295

Table 7 (cont'd.)

Bean Type/ Starch Treatment	Viscosity (BU) at 95°C	after 15 min at 95°C	after 30 min at 95°C	at 50°C	after 15 min at 50°C	after 30 min at 50°C
Black						
air-classified	230	520	-	737	734	-
	230	515	660	840	860	-
	235	517	-	727	724	720
purified	117	320	-	449	483	-
	125	327	408	534	575	-
	110	326	-	445	478	500

[1]Mean values (n = 2) and standard deviation = ±10.0 BU

conditions (Figure 3). This indicated that prolonged high temperature heating had an effect on the protein structure such that the structures were more resistant to shear force. As discussed earlier, protein denaturation causes physical structure changes from native, folding protein to the unfolding form which results in increased viscosity. Prolonged heating may enhance an increase in hydrophobic hydration and result in a more viscous and higher shear resistant paste.

The Brabender pasting curves of purified starches of three bean types at an extended high temperature (95°C) holding period (30 min) are shown in Figure 18, with specific points presented in Table 7. They all showed a steady increasing viscosity with no shear breakdown. Black and pinto starches show a greater increase in viscosity than did the navy bean starch which was quite stable. This difference in viscosity is not explained by differences in the amylose content of starches determined by this study (Table 3). Differences in molecular structure of starch components may be hypothesized.

The Brabender pasting curves of air-classified flours at an extended low temperature (50°C) holding period (30 min) are illustrated in Figure 19 with specific points recorded in Table 7. While comparing with the curves in Figures 3 and 17, it may be concluded that the stability of the protein viscosity depended on the amount of the heat treatment. This conclusion agrees with a previous study by Kinsella (1982).

Figure 20 shows the Brabender pasting curves of purified starches at an extended low temperature (50°C) holding period (30 min). When compared with Figures 4 and 18, it may be seen that there is no shear breakdown during this longer holding period at a low temperature. These results indicate that bean starches are quite resistant to heat and to shear force.

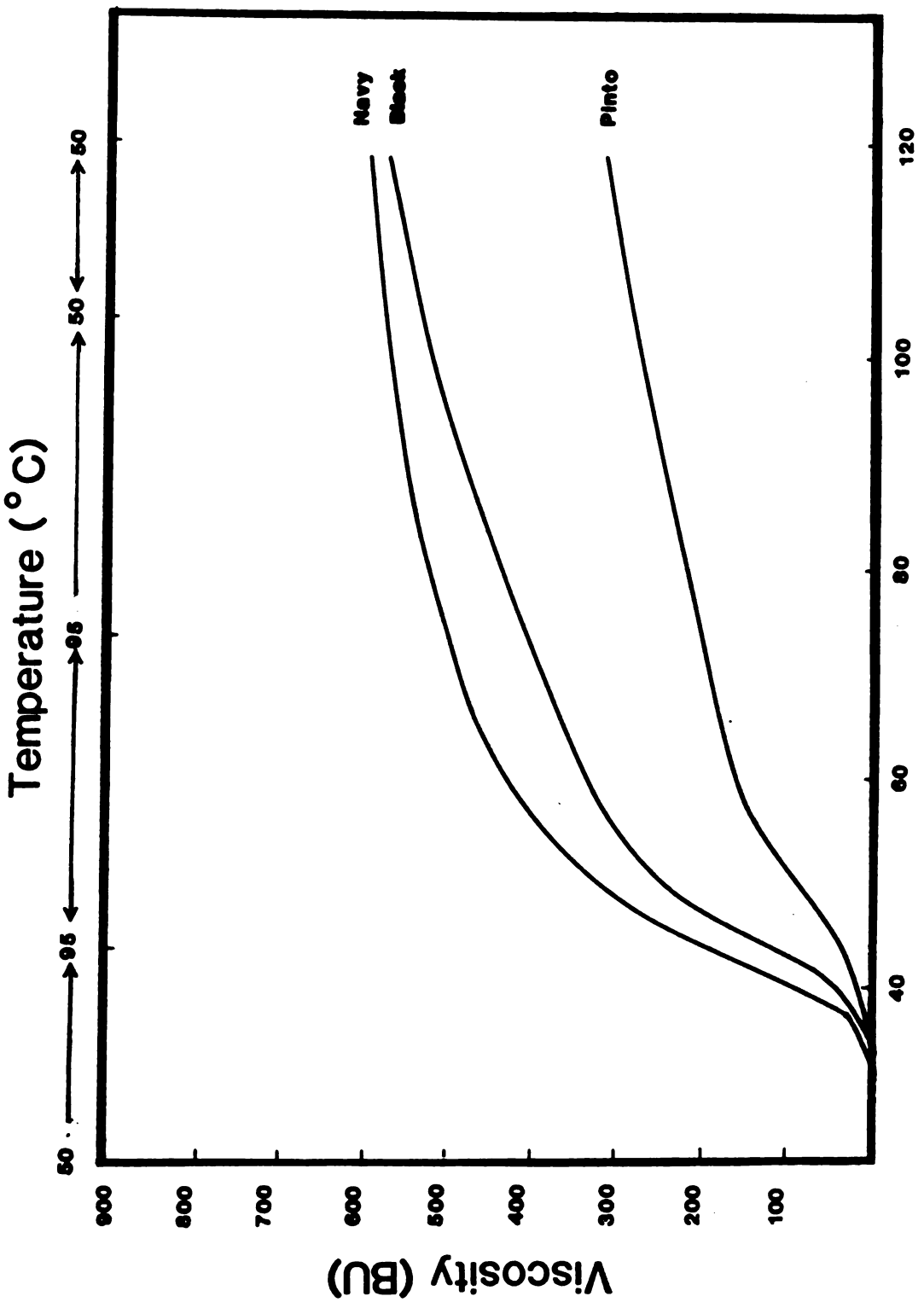

Figure 18. Brabender pasting curves of purified bean starches (8 g/100 ml) at an extended high temperature (95°C) holding period (30 min) in pH 5.3 phosphate buffer solution

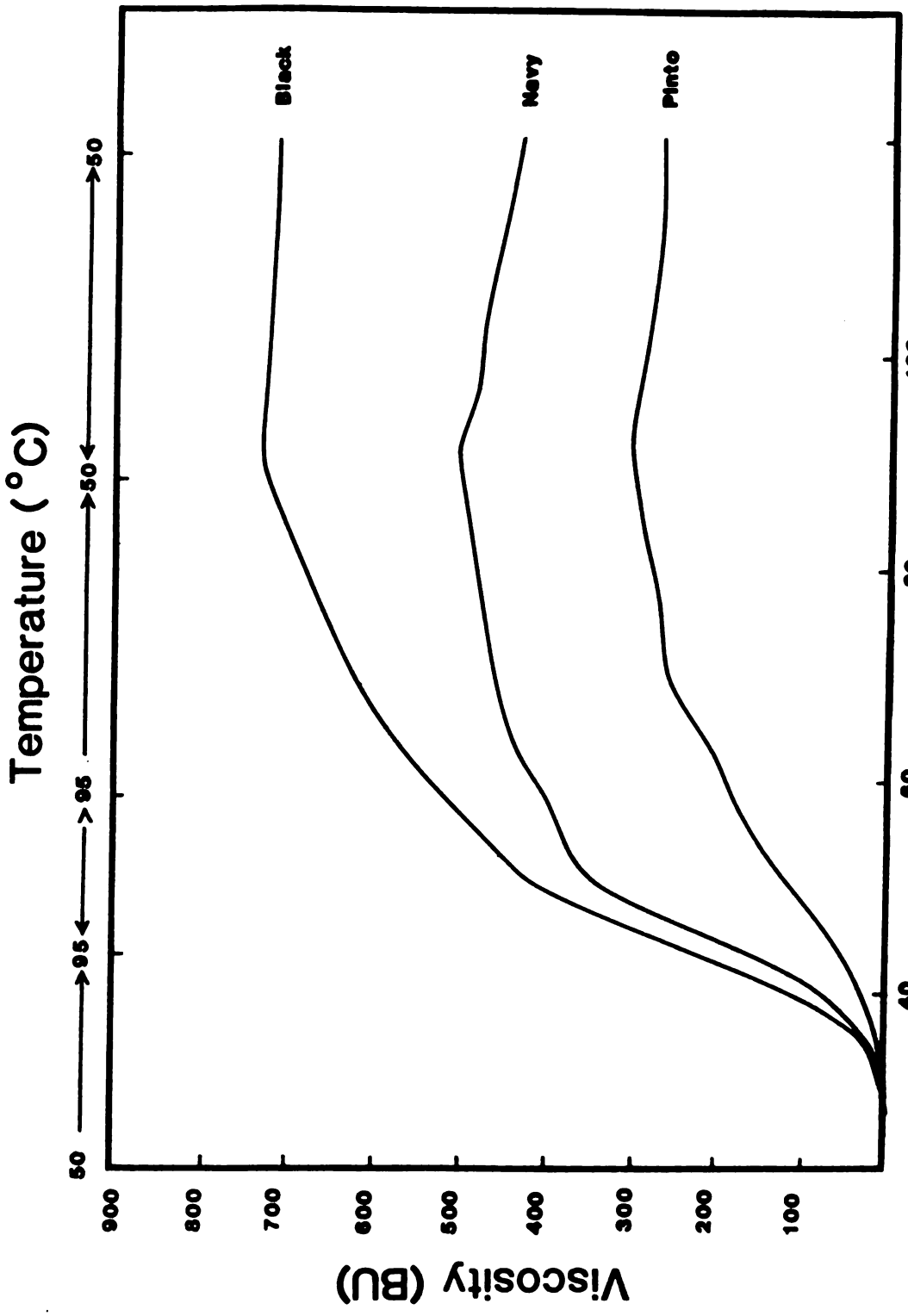

Figure 19. Brabender pasting curves of air-classified high starch flours (10 g/100 ml) at an extended low temperature (50°C) holding period (30 min) in pH 5.3 phosphate buffer solution

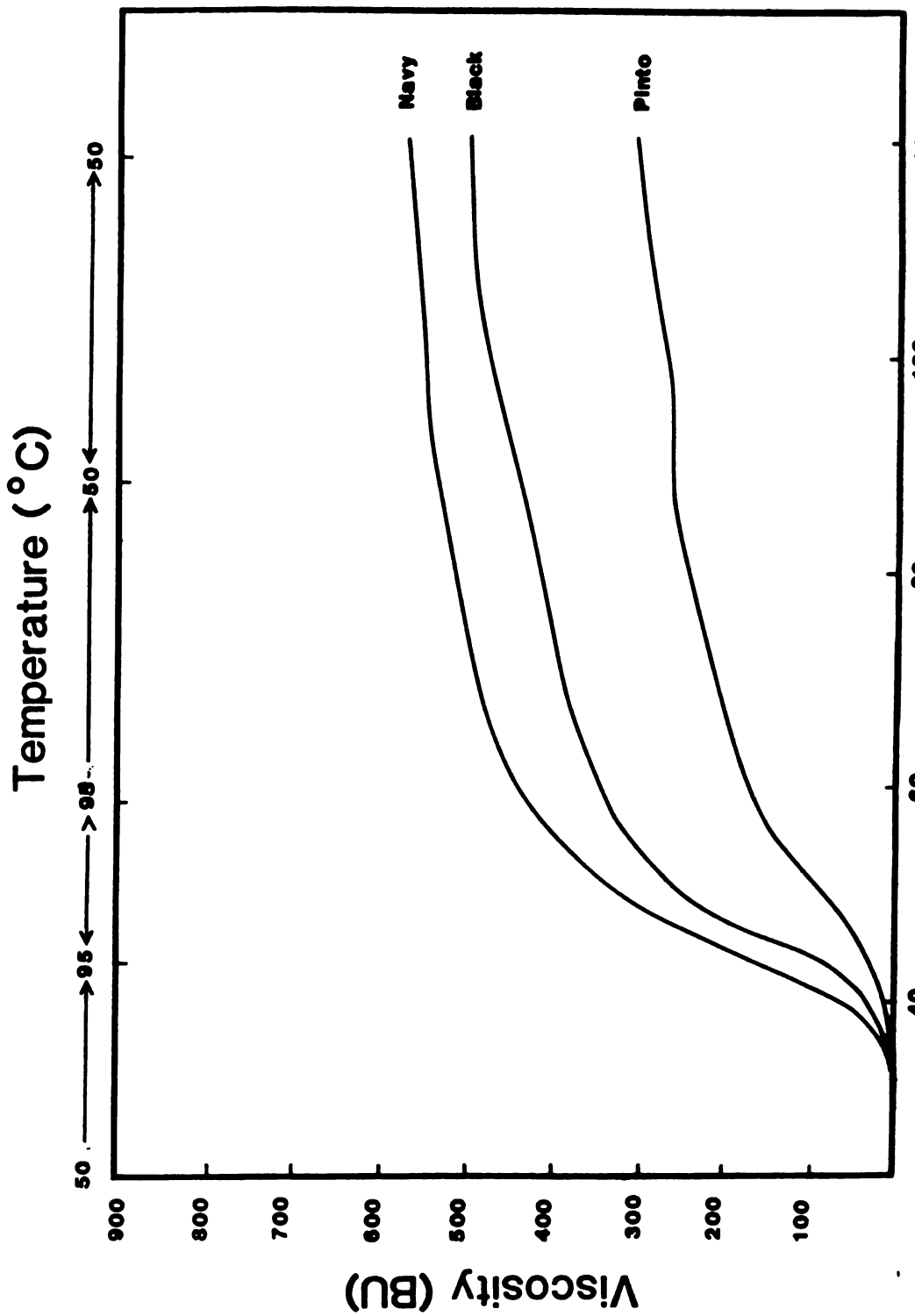

Figure 20. Brabender pasting curves of purified bean starches (8 g/100 ml) at an extended low temperature (50°C) holding period (30 min) in pH 5.3 phosphate buffer solution

Molecular Structure

Because the gelatinization phenomenon and pasting characteristics of the navy, pinto and black bean starches are very complex, differences in their properties cannot be explained by amylose-amylopectin content alone (Table 3). Other factors such as granule size, their molecular structure and organization are important (Banks and Greenwood, 1975).

In recent years, research interest has focused on the molecular structure level which can be determined by combining enzymatic methods with size exclusion chromatography techniques (Mercier, 1973; Juliano and Perdon, 1975; Biliaderis et al., 1979 and 1981; Abdul-Hussain and Varriano-Marston, 1982). Papantonakis (1980) and others have applied the size exclusion methodology with high pressure liquid chromatography to provide molecular weight (size) distribution (MWD) data to characterize the viscosity of paper coating starches. Starches, like polymers, contain molecules with a distribution of different molecular sizes or chain lengths. This study applied the Papantonakis (1980) technique to the bean starches that were degraded with alpha and beta amylase enzymes.

In the process of investigating the molecular structure of bean starches, a number of elution patterns were obtained using pure components: potato amylopectin, potato amylose, maltose and glucose. The elution patterns for the different compounds are presented in Figure 21. Amylopectin elutes faster than amylose, maltose and glucose, respectively. This indicates that the molecular size of amylopectin is larger than that of amylose, maltose or glucose. In addition, the potato amylopectin profile showed a bimodal distribution which indicates that the potato amylopectin may contain two ranges of components with different molecular

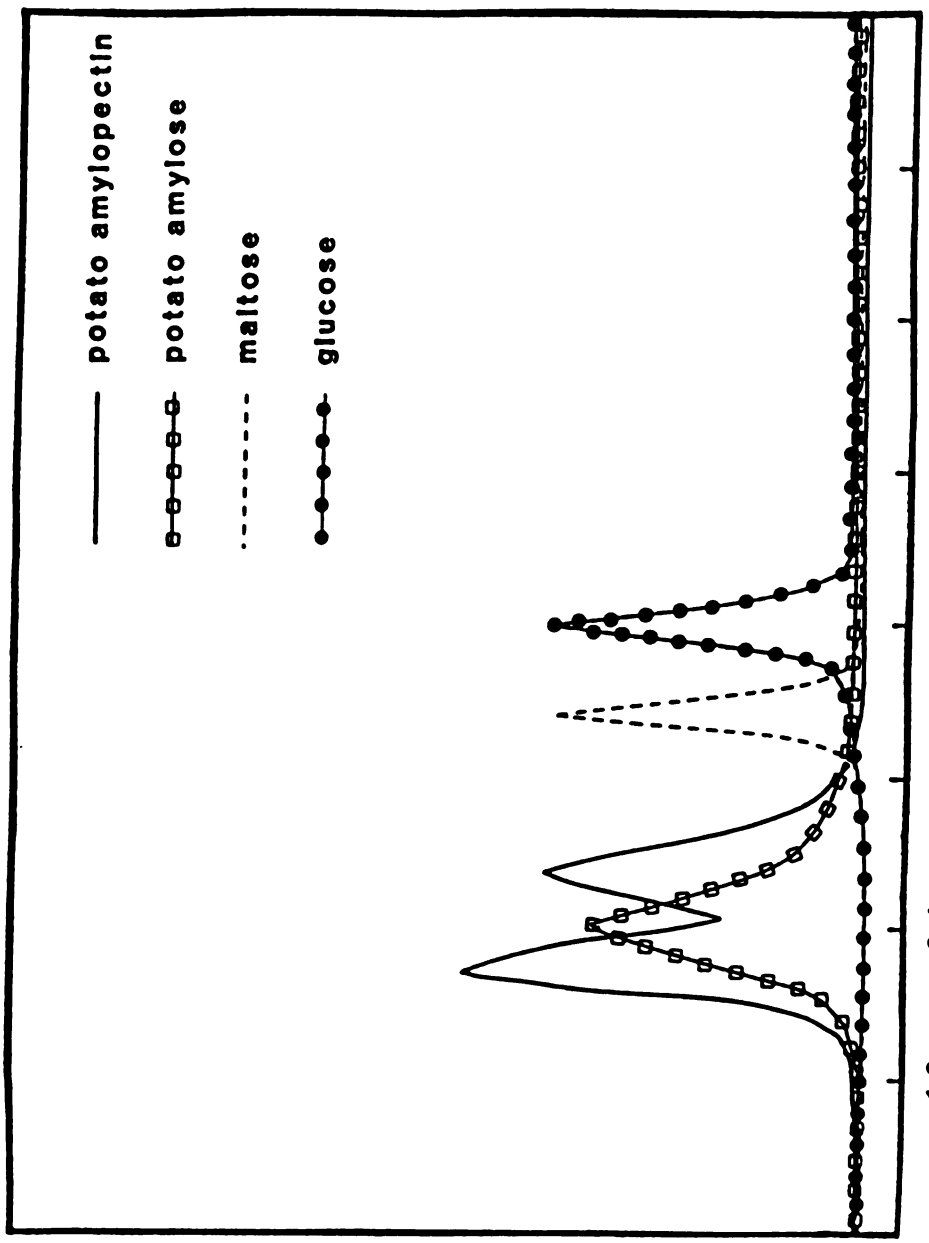

Figure 21. Elution profiles of standard components on micro-BONDAGEL gel permeation columns

sizes. The difference in elution volume between potato amylose and amylopectin was not large enough to show the completely separated peaks when potato starch (amylose and amylopectin) was applied to these columns.

To increase the resolution of the HPLC analysis, many combinations of dimethylsulfoxide (DMSO) and water were tested. The best resolution was obtained using DMSO:H_2O (9:1, v/v). Another factor which contributed to poor separation was the column's lack of efficiency. The efficiency of a column depends on the alignment of the highly porous and delicate silica gel particles. This particle alignment is very sensitive to any physical or thermal shock which may cause the particles to shift to a more dense, but less efficient condition. Due to some conditions during previous use, the column packing was denser than it should have been.

Figures 22, 23 and 24 show elution profiles of purified navy, pinto and black bean starches and of the bean starches after being treated with either alpha or beta amylases. As expected, there was no separation of the amylose and amylopectin peaks; however, the high molecular weight elution peak of the control trailed into the higher elution volumes, which may indicate the presence of amylose.

The same single elution volume peaks were obtained for all three bean types. These elution volumes were close to those exhibited by potato amylose, but larger than those of potato amylopectin. This indicates that the differences in amylose and amylopectin molecular size of the three bean types were not large enough to be shown by these columns, and that their amylose and amylopectin molecular sizes were similar to standard potato amylose, but lower than standard potato amylopectin.

Potato starch exhibits much higher viscosity than bean starches at similar concentrations; however, the viscosity patterns are different.

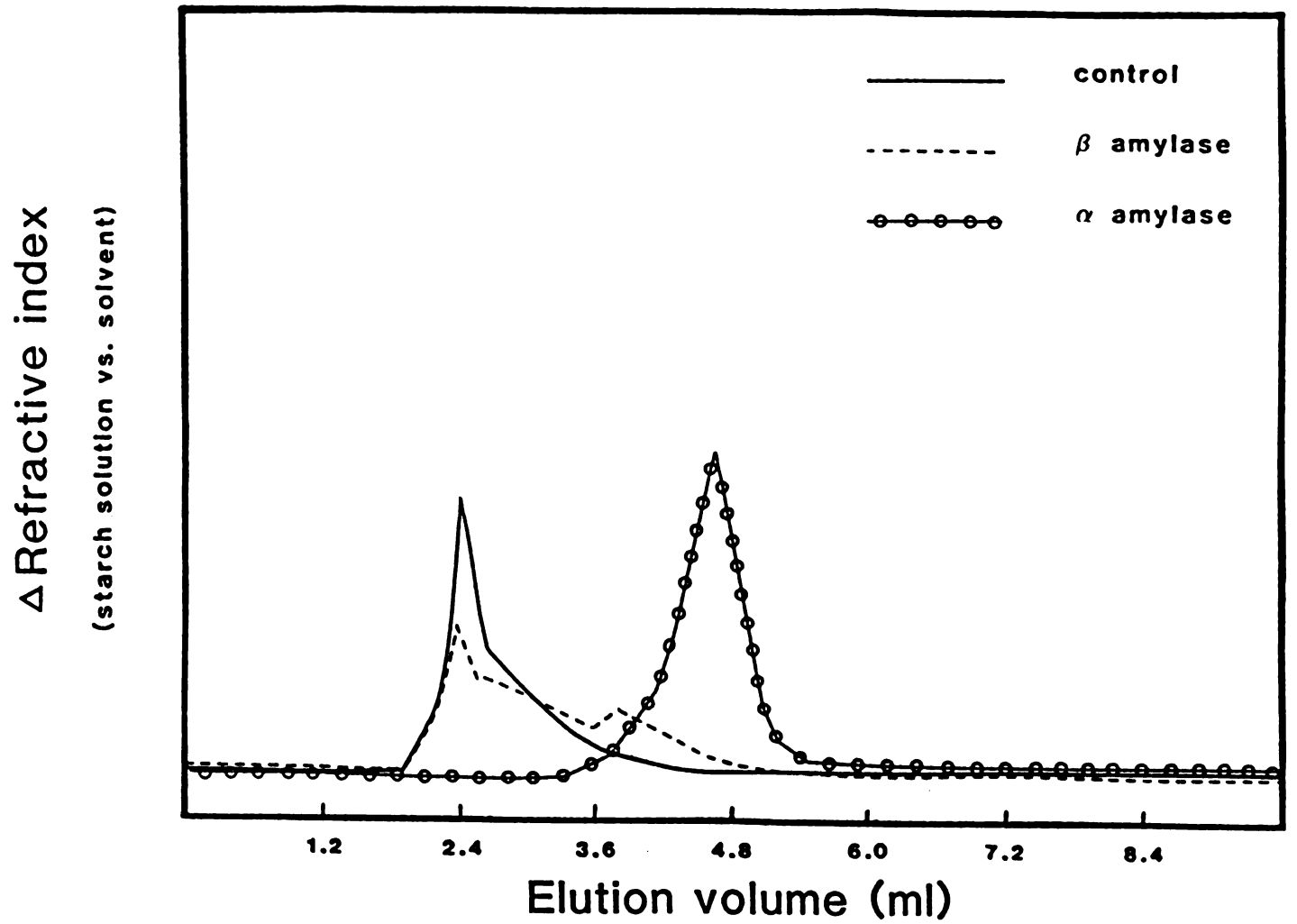

Figure 22. Elution profiles of purified navy bean starch (control) and their products hydrolyzed by alpha and beta amylases on micro-BONDAGEL gel permeation columns

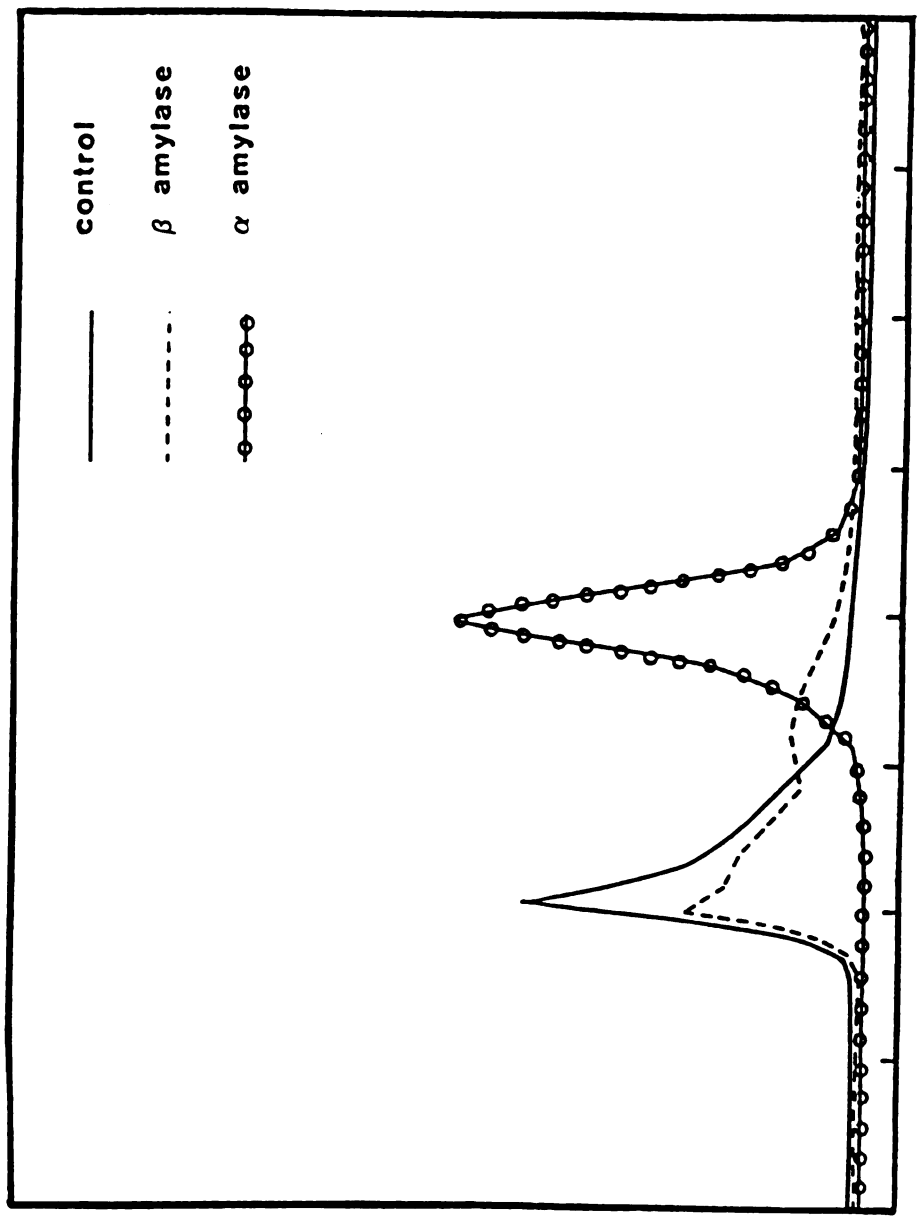

Figure 23. Elution profiles of purified pinto bean starch (control) and their products hydrolyzed by alpha and beta amylases on micro-BONDAGEL gel permeation columns

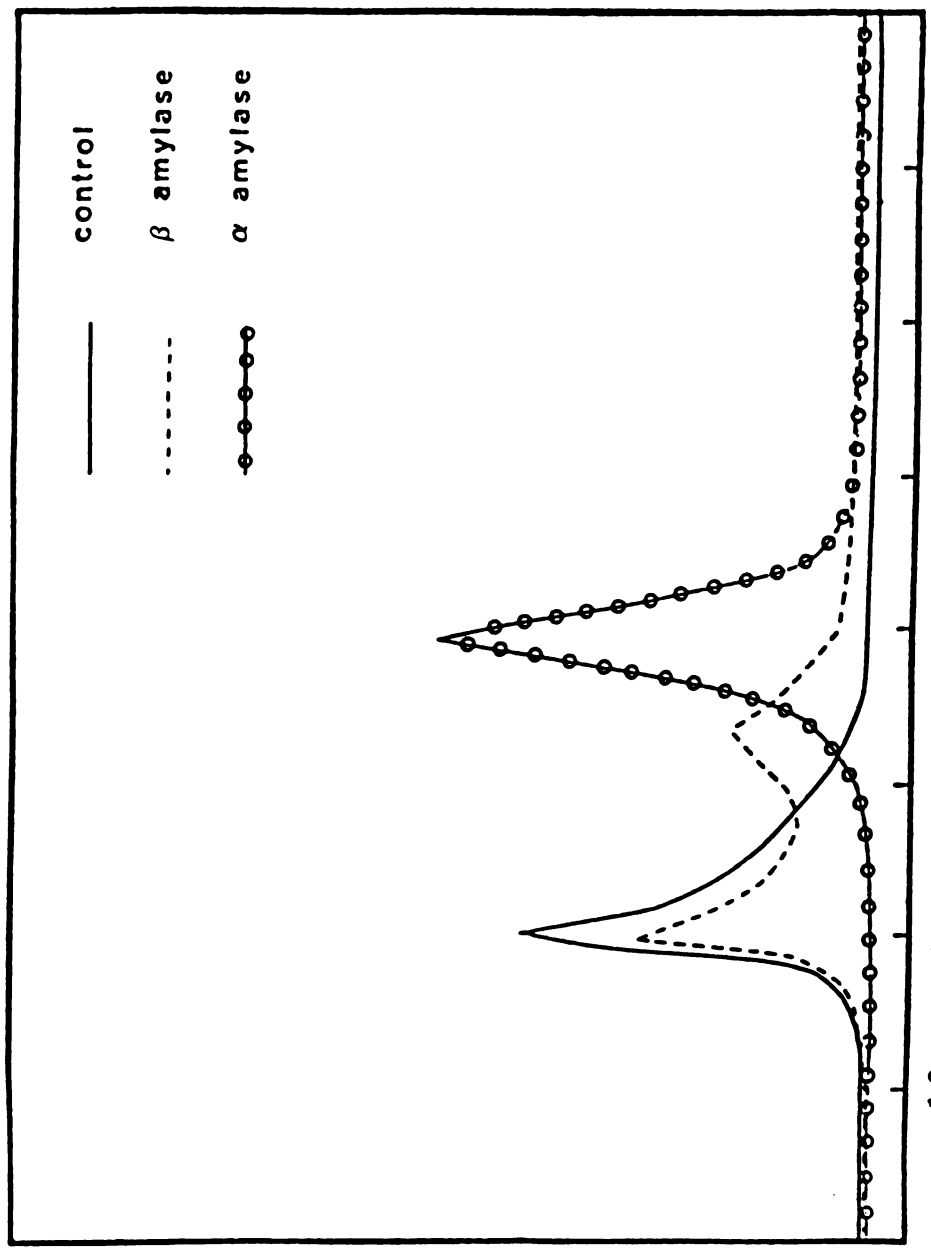

Figure 24. Elution profiles of purified black bean starch (control) and their products hydrolyzed by alpha and beta amylases on micro-BONDAGEL gel permeation columns

Potato starch shows a type A viscosity pattern, characterized by starch granules swelling enormously, internal bonding forces becoming tenuous and fragile toward shear, and Brabender curves showing a high pasting peak followed by rapid and major thinning during cooling. Potato starch has a lower (approximately 20%) amylose content. Therefore, the differences in molecular size may be one important factor to affect pasting characteristics. Unfortunately, there was no difference shown among the three bean types.

When alpha amylase was applied to digest raw bean starches in pH 5.8 acetate buffer and incubated at 35°C for 24 hours, there were no differences among the elution patterns (Figures 22, 23 and 24) of the three bean types. All showed single peaks at the same elution volume of glucose with some tailings. These tailing portions may represent some imcomplete digested oligosaccharide such as isomaltose (two glucose units linked by α 1,6 linkage), because alpha amylase can split only α 1,4 linkages. Generally, different raw starches have different susceptibility to degradation by alpha amylase (Marshall, 1980). The same susceptibility to alpha amylase degradation was obtained from all three bean starches in this study, which indicates a similarity in molecular organization of the starch granules or may implicate the use of excessive incubation periods.

The results from applying beta amylase in a pH 4.8 acetate buffer solution which was incubated at 35°C for 24 hours to navy and black bean starches show a similar peak shape, with broad shouldering with increased elution volume. However, pinto bean starch gave a sharper peak without tailing. These results agree with their pasting characteristics, because navy and black bean starches have similar pasting properties, which differ from those exhibited by pinto starch. Beta amylase

hydrolyzes α1,4 glucose linkages from the non-reducing ends of starch components to form maltose (two glucose units linked by α1,4 glucose linkage) and will cease activity at the branching points of α1,6 glucose linkages. Therefore, these differences may be associated with the different molecular structure of amylopectin. These elution profiles are useful in hypothesis development and not confirmatory for the molecular structure of the starches.

SUMMARY AND CONCLUSIONS

Results of the chemical composition study indicated that air-classified high starch flours contained a relatively high amount of protein (15.51% to 19.06%); however, the starch isolation method modified by Naivikul and D'Appolonia (1979) was efficient enough to prepare the high purified starches which were significantly lower in protein, ash, fat, and ENDF. Among the three bean starches, the amylose content of navy bean starch was not significantly different from that of pinto or black bean starches, but there was a significant difference ($P < 0.05$) between pinto and black bean starches.

As a result of sugar analysis, the same sugar order pattern, from high to low (sucrose, stachyose, glucose and raffinose, with no inositol detection) was shown in all three bean flours. After purification, there were no sugars detected.

The Brabender pasting characteristic study indicated that all flours and starches showed a type C viscosity pattern, which is restricted swelling, increasing in viscosity, with no peak during heating and tending to retrograde during cooling. The pasting curves of flours illustrated higher rates of increasing viscosity during heating and cooling cycles than those of starches. In addition, flours showed shear breakdown during a holding period at 50°C, while starches showed slightly increased viscosity. Variation in pH (range of 4 to 8) showed only minor effects on pasting characteristics for both flours and starches. During an

extended high temperature holding period (95°C, 30 min), both flours and starches showed moderately increased viscosity which indicated their resistance to thermal breakdown. During an extended low temperature holding period (50°C, 30 min), flours showed moderately decreased viscosity; whereas, starches showed slightly increased viscosity. This indicated that starch granules themselves were resistant to shear force; however, the protein in flours was not stable to shear force under these conditions.

From the molecular structure study, using gel permeation HPLC, the beta amylase hydrolysate elution profile of pinto bean starch differed from that of navy and black bean starches, suggesting a difference in pinto bean amylopectin molecular structure.

RECOMMENDATIONS FOR FURTHER RESEARCH

In order to clarify the differences of amylopectin molecular structure, further research should be done on the degree of branching, as well as the length of branches by using pullulanase, debranching enzyme, and isoamylase combined with gel permeation HPLC to characterize the fine structure of amylopectin. Study of amylose molecular structure is also needed because the size of this starch component has affected molecular organization. The relationship between molecular structure and pasting properties should be observed for both starch components, amylose and amylopectin. According to the extremely complex gelatinization phenomenon, not only the structure of a granule and its components, but also other factors such as granule size, phosphorous content, bound lipids, and protein are important (Banks and Greenwood, 1975; Biliaderis et al., 1981).

APPENDIX A

APPENDIX A

Table 8. Analysis of variance of pasting characteristics of air-classified high starch flours and purified starches from navy, pinto and black beans at various concentrations

Source of Variation	df	Viscosity (BU) after 15 min holding at 95°C
		Mean Squares
Main Effects	6	649,096.69***
Bean Type	2	138,613.50***
Starch Treatment	1	586,245.44***
Concentration	3	1,165,963.25***
Two-Way	9	81,552.57***
Bean Type x Starch Treatment	2	6,260.19***
Bean Type x Concentration	5	58,051.46***
Starch Treatment x Concentration	2	209,436.69***
Three-Way		
Bean Type x Starch Treatment x Concentration	4	19,959.57***
Residual	20	76.90
CV (%)		2.73

APPENDIX B

APPENDIX D

Table 11. Analysis of variance of pasting characteristics of air-classified high starch flours from navy, pinto and black beans at different high or low temperature holding periods

Source of Variation	df	Viscosity (BU) extended high temp. holding	Viscosity (BU) extended low temp. holding
		Mean Squares	
Main Effects	3	123,025.94***	142,697.72***
Bean Type	2	157,872.25***	213,505.08***
Time	1	53,333.33***	1,083.00**
Two-Way			
Bean Type x Time	2	4,804.08***	36.75*
Residual	6	27.00	38.00
CV (%)		1.21	1.29

APPENDIX C

APPENDIX E

Table 12. Analysis of variance of pasting characteristics of purified starches from navy, pinto and black beans at different high or low temperature holding periods

Source of Variation	df	Viscosity (BU)	
		extended high temp. holding	extended low temp. holding
		Mean Squares	
Main Effects	3	61,597.16***	58,143.42***
Bean Type	2	84,329.08***	85,360.08***
Time	1	16,133.33***	3,710.08*
Two-Way			
Bean Type x Time	2	473.08**	217.58*
Residual	6	35.83	278.92
CV (%)		1.77	3.80

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