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PROCESSING STRATEGIES TO IMPROVE DRY BEAN (Phaseolus vulgaris) DIGESTIBILITY AND FOOD UTILIZATION

presented by

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has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Food Science

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Date April 29, 1994

O-12771

PROCESSING STRATEGIES TO IMPROVE DRY BEAN (*Phaseolus vulgaris*) DIGESTIBILITY AND FOOD UTILIZATION

By

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1994

ABSTRACT

PROCESSING STRATEGIES TO IMPROVE DRY BEAN (*Phaseolus vulgaris*) DIGESTIBILITY AND FOOD UTILIZATION

By

Lillian Geraldine Occeña

Dry edible beans serve as a major source of protein in many developing countries. However, many constraints, including antinutritional and flatulence factors which interfere with protein digestibility, limit wider utilization of dry edible beans. Processing strategies designed to eliminate these factors and provide a precooked ingredient suitable for food formulations are warranted.

Whole and split dry beans *(Phaseolus vulgaris)* soaked and hydrated for 16 hrs. (25°C) were subjected to hot water (60°C/60 mins.), steam (100°C/15 mins.) and differential enzymatic pretreatments. Cooked CONTROL beans contained all original soak and rinse waters, cook water or condensate (formulation water) prior to milling to a homogeneous slurry whereas leachate of EXTRACT and cooked beans was replaced with fresh formulation water. Bean slurries pre-heated to 95°C were dried on a double drum-dryer. Enzymatic pretreatment involved the use of both indigenous ("sprout slurry") and commercial enzymes (Viscozyme, Alcalase, Neutrase and Celluclast).

The drum-dried bean meals were analyzed for proximate composition (moisture, 5%; protein, 23%; fat, 2%; and ash, 4%), total soluble sugars (stachyose, 0.3% to 0.8%), dietary fiber (soluble, 5% and insoluble, 15-21%), total mineral content (plasma emission spectrometry) and *in-vitro* starch and protein digestibilities. Functional properties of the drum-dried bean meals, including color, water absorption index, water solubility index and degree brix of the soluble fraction were also evaluated.

The resultant bean meals were differentiated by soluble losses and quality characteristics. Hot water extraction provided an approximately 8% increase in protein digestibility *(in-vitro)*. Soluble dietary fiber content was approximately 5%, whereas insoluble dietary fiber averaged 18%. Hot water extraction resulted in significant reduction of minerals (K, Ca, Al, Cu, Fe, Zn and Mg) and soluble sugars (stachyose, 0.3%-0.8%).

The processed product had neutral color and did not possess the frequently objectionable "beany" flavor. The DDBMs were incorporated in a pumpkin quick bread formulation (0%, 20%, 40%, and 60% levels of substitution) and a weaning beverage (5%, 8% and 10%). Acceptable quality breads were produced at the 20% and 40% levels of substitution. Enzyme pre-digested DDBMs possess great potential in weaning food formulations, especially at the 8% concentration level. The use of indigenous enzymes (i.e., sprouting) to modify the functional properties of DDBM used in weaning beverage formulations warrants additional research.

Microwave preconditioning of dry beans demonstrated the potential of microwave energy to improve whole bean seed cooking, ensure rapid processing of beans by disrupting starch granules and facilitating gelatinization. The physical and functional properties (swelling power, solubility, degree of syneresis, appparent viscosity and elasticity) of pure bean starch were significantly affected by microwave heating.

To my parents, who have provided me a strong foundation
to go through life's challenges

ACKNOWLEDGMENTS

My sincere appreciation goes to my Graduate Committee members: Dr. Maurice Bennink, Dr. George Hosfield, Dr. Mary E. Zabik and Dr. Wanda Chenoweth, for their valuable advice and guidance throughout my Ph.d Graduate program:

I am most grateful to my Advisor, Dr. Mark A. Uebersax, who has become a mentor, colleague, teacher and friend; thank you for providing me the support and opportunities for my professional growth.

I am fortunate to have wonderful friends in:

Ahmad Shirazi, who was always encouraging and available to lend a helping hand and listening ear

Sharon Hart, Miriam Nettles, Virginia Vega, Nirmal Sinha, Danny Campos, Leticia Carpio, Cheribeth Tan, who always cheered me up when the going got rough

Danny, Letlet and Muhammad Siddiq, for helping and staying up with me during those last nights before the defense.

My brother, Luis, who is always around when I need help.

I am forever grateful to my family for their love, support and encouragement.

Sincere gratitude is extended to Gerber Products Company in Fremont, MI for the use of their facilities; thank you Jerry Stroven and Duane Dougan, for all your assistance.

I also thank the Bean-Cowpea/CRSP for partial support of this project

To the Aggie family of Xavier University, especially Fr. Ledesma & the Food Tech faculty and staff, for holding the fort while I was on study leave

Above all, I am grateful to the Almighty for keeping me in the palm of His hand.

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INTRODUCTION

Seeds of the annual legume (*Phaseolus vulgaris L.*), commonly termed dry edible beans, serve as the major alternative source of protein and calories in lesser developing countries (LDC's) of Latin America and Africa, and are also frequently considered for applications in world food relief programs in regions experiencing sustained crop failure. Although per capita bean consumption is significantly much less in developed countries compared to LDC's, it is projected to increase due to focused dietary recommendations as well as diet and health positioning currently presented by health authorities. Dry bean fiber is now being promoted as the "Fiber of the 90's" due to potential hypocholesterolemic and hypoglycemic effects .

Major constraints still limit wider dry bean utilization. Biological or nutritional concerns include antinutritional and flatulence factors which interfere with protein digestibility and bioavailability of minerals. The preference for convenient and fast foods in industrialized economies also presents a major drawback to consumer utilization of beans due to the excessive time and expense involved in their preparation. Thus, the technological challenge is to develop highly digestible, pre-processed, prolonged shelf-life food products and ingredients from dry edible beans with minimum process inputs and appropriate technologies. The World Health Organization (WHO) and the United Nations International Children and Educational Fund (UNICEF) which have been promoting and supporting appropriate "complementary" or "weaning" feeding practices in LDC's, have encouraged the use of indigenous food resources such as dry beans. Dry edible beans therefore possess potential in the development of improved, low-cost weaning food

formulations, which could alleviate protein and energy malnutrition in young children.

This dissertation was conducted in conjunction with the Bean/Cowpea Collaborative Research Support Program (CRSP) of coordinated projects in Africa and Latin America focusing on removal of constraints to the production and utilization of beans and cowpeas. The program is funded by a Title XII grant from the United States Agency for International Development (USAID)/BIFAD with the ultimate goal of reducing hunger and malnutrition in developing countries. In many of the countries where beans and cowpeas are staple foods, responsibility of production and preparation for family consumption rests with women and children. It is envisioned that village-level food centers, possibly managed by cooperatives, could centrally process locally produced raw materials such as dry edible beans into nutritious foods which can be distributed through day-care centers, school lunch programs and nutrition intervention clinics. Such a strategy will not only benefit infants, young children, pregnant and lactating women, but will also serve as an activity which will directly involve women in management and employment enterprises.

The purpose of this research was to investigate possible processing strategies which will reduce antinutritional and digestibility factors, as well as decrease the time involved in the preparation of dry beans. Individual studies were conducted to address specific hypotheses associated with the objectives, and are discussed in three Chapters. Chapter I (Studies I-1 and I-2) involved the development of shelf-stable drum-dried bean meals which possess potential in preparation of improved weaning food formulations. The effects of different extractive pretreatments (hot water, steam and enzymatic) on drum-dried bean meal composition and digestibility were evaluated *in-vitro*. Further, the feasibility of the use of germinated sprouted dry beans to serve as an indigenous source of enzymes was investigated. Chapter II (Studies II-1, II-2 and II-3) was undertaken to characterize the functional properties of the drum-dried bean meals and to evaluate their performance in selected food systems (quick bread and weaning food beverage). Chapter

III (Studies III-1 and III-2) was conducted to assess the feasibility of using microwave energy as a preconditioning treatment to reduce cooking time of dry beans. The physical properties of isolated bean starch during microwave heating and conventional cooking were further compared in this study.

REVIEW OF LITERATURE

CHEMICAL COMPOSITION OF DRY EDIBLE BEANS (*Phaseolus vulgaris*)

Although dry edible beans clearly differ in size and seedcoat color, there is a very basic commonality among commercial classes (navy, pinto, dark and light red kidney, Great Northern, pink, cranberry and black turtle soup beans). The compositional components of dry edible beans including protein, starch, fiber and minerals are generally very similar among these classes of dry beans (Figures 1, 2, 3 and 4). Further, the total dietary caloric energy derived from different bean classes is identical (Figure 5). Dry beans are of lower caloric value than soybeans (*Glycine max L. Merr.)* and peanuts (*Arachis hypgea L.*), both of which contain significantly higher amounts of lipids. These data (Table 1) are compiled from a major USDA funded analytical screening project and represent numerous sampling points of raw edible beans. It is most evident that only minor variations in each component exists among the diverse bean types presented. Thus, the interchange or substitution of different bean types within a major seedcoat class in recipes which require milling, mashing or mixing can be readily accomplished without dramatic functional or nutritional consequences (Uebersax and Occeña, 1991).

An outlined overview of the major constituents is provided to specifically delineate and to further demonstrate the similarity in composition among different commercial classes of dry beans.

Carbohydrates

The total carbohydrates of dry legumes range from 24.0% in winged beans to 68.0% in cowpeas (Reddy et al., 1984). Starch constitutes the most abundant legume

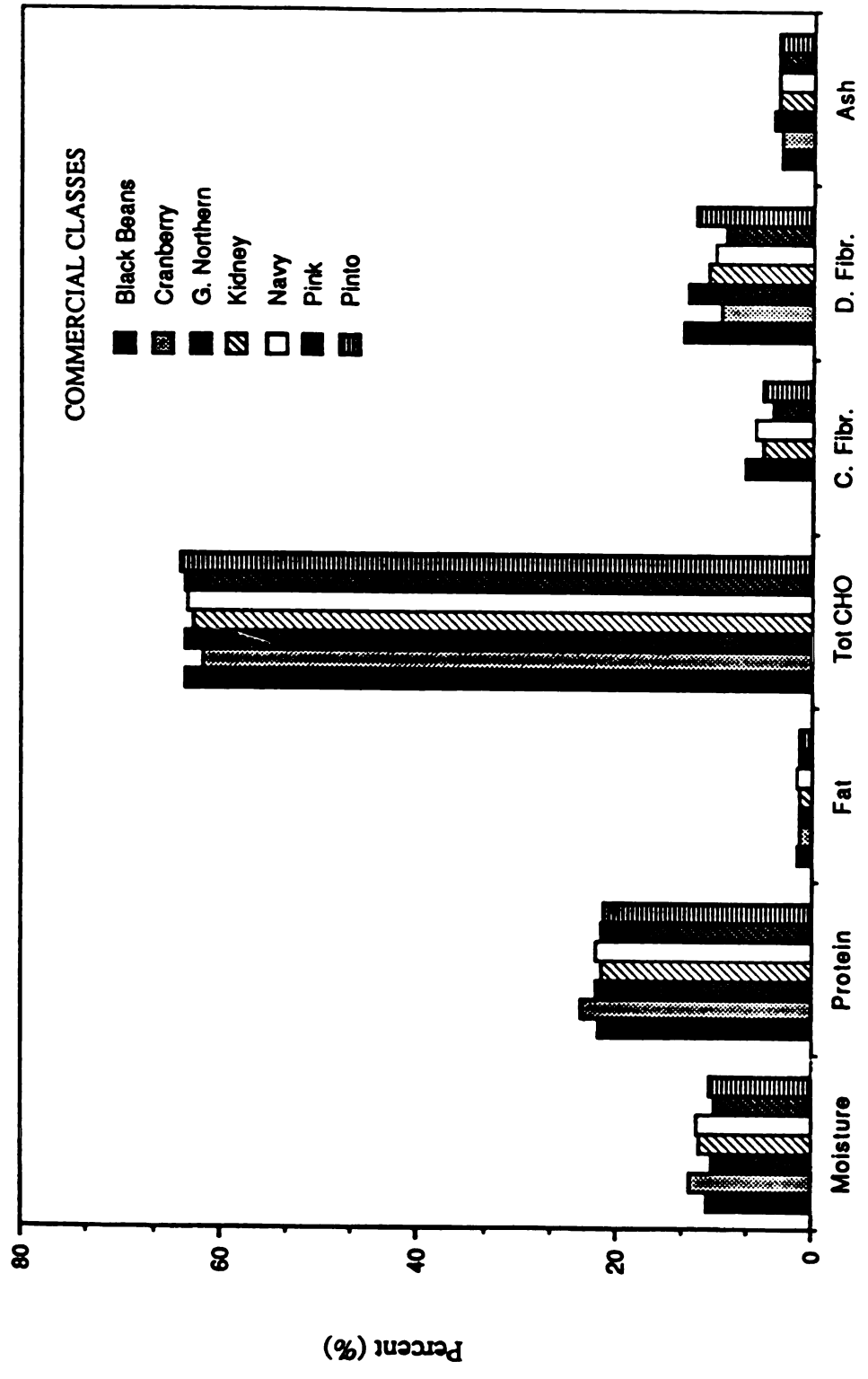


Figure 1. Proximate composition of selected raw legumes (Phaseolus vulgaris)

Source: Uebersax and Occeña, 1991

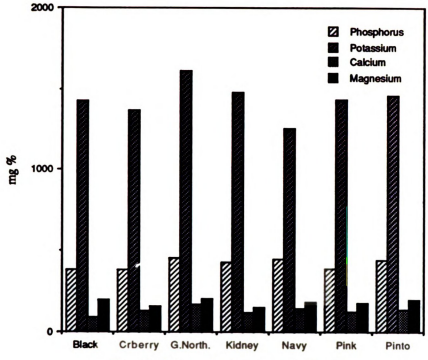


Figure 2. Macro-mineral content of selected raw legumes (Phaseolus vulgaris)

(Source: Uebersax and Occena, 1991)

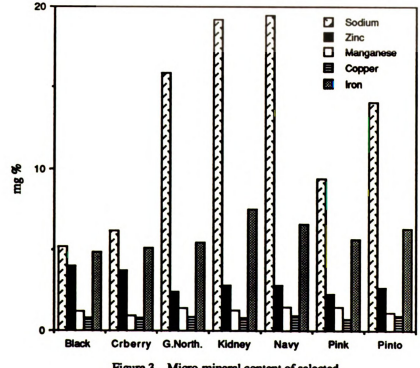


Figure 3. Micro-mineral content of selected raw legumes (Phaseolus vulgaris)

(Source: Uebersax and Occena, 1991)

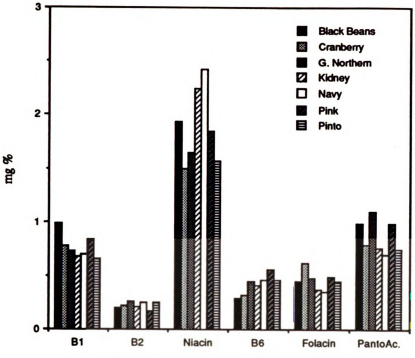


Figure 4. Vitamin content of selected raw legumes (Phaseolus vulgaris)

(Source: Uebersax and Occena, 1991)

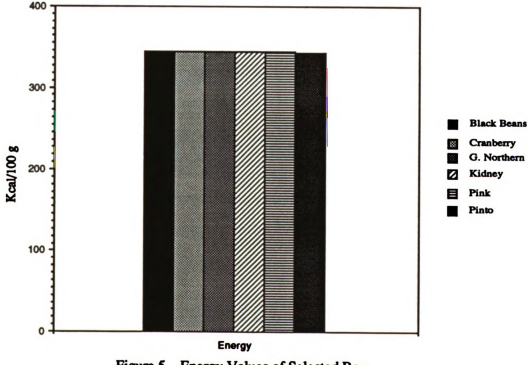


Figure 5. Energy Values of Selected Raw Legumes (Phaseolus vulgaris)

(Source: Uebersax and Occena, 1991)

Table 1. Nutritional composition of selected raw dry edible beans (Phaseolus vulgaris).

Composition	Navy	Kidney	Pinto	Black beans	Cranberry	Great Northern	Pink
A. Proximate composition (%, dwb)							
Moisture	11.80	11.40	10.30	10.60	12.40	10.10	9.90
Protein	22.00	21.50	21.40	21.80	23.70	22.00	21.60
Fat	1.50	1.30	1.20	1.40	1.30	1.20	1.20
Total Carbohydrates	63.20	61.70	64.10	63.50	61.70	63.40	63.60
Crude fiber	5.80	5.20	5.00	0.00	0.00	6.90	4.10
Dietary fiber (NDF)	9.80	10.60	12.00	13.30	9.50	12.60	8.90
Ash	3.50	3.50	3.60	3.40	3.40	4.00	3.50
B. Macro and micro-mineral content (ppm)							
P	449.30	427.20	443.00	380.30	382.50	452.70	387.00
K	1257.40	1475.70	1458.60	1424.30	1368.00	1611.90	1430.50
Na	19.50	19.20	14.10	5.20	6.20	15.90	9.40
Ca	144.60	117.30	138.10	92.30	130.50	170.90	124.90
Mg	182.60	152.30	196.40	195.60	159.80	204.10	181.20
Zn	2.79	2.80	2.63	3.96	3.74	2.38	2.28
Mn	1.43	1.26	1.13	1.17	0.95	1.37	1.13
Cu	0.90	0.79	0.91	0.77	0.82	0.84	0.75
Fe	6.57	7.52	6.29	4.82	5.12	5.44	5.63
C. Vitamin content							
B1	0.70	0.68	0.66	0.99	0.78	0.73	0.84
B2	0.25	0.21	0.25	0.20	0.22	0.26	0.17
Niacin	2.42	2.24	1.56	1.93	1.50	1.64	1.84
B6	0.46	0.41	0.46	0.29	0.32	0.45	0.55
Folacin	0.35	0.37	0.45	0.45	0.62	0.48	0.49
Pantothenic acid	0.70	0.76	0.75	0.99	0.79	1.10	0.99
D. Energy	346.00	345.00	343.00	345.00	344.00	344.00	344.00

Source: Adapted from Matthews, R. 1989

carbohydrate ranging between 24.0% to 56.6%. Total sugars represent only a small portion of the total carbohydrate content.

Sugars. Among the sugars, oligosaccharides of the raffinose family (raffinose, stachyose, verbascose, and ajugose) predominate in most legumes and account for 31.1% to 76.0% of the total sugars (Akpapunam and Markakis, 1979; Kon, 1979; Rockland et al., 1979; Ekpenjong and Borchers, 1980; Reddy and Salunkhe, 1980; Fleming, 1981 and Sathe and Salunkhe, 1981). Walker and Hymowitz (1972) found that the sugar content ranged from 4.4% to 9.2% in 28 varieties of *Phaseolus vulgaris*. Sucrose accounted for 46.4% of total sugars present, while raffinose and stachyose made up 10.4% and 43%, respectively. The predominance of a particular oligosaccharide depends on the type of legume. For example, stachyose represents the major oligosaccharide in most varieties of *Phaseolus vulgaris* while verbascose is the major oligosaccharide in black gram, red gram, mung bean and broad beans (faba beans); raffinose is present in moderate to low amounts.

Starch. Variations in reported starch contents (24-56%) are partially due to differences in cultivars and analytical procedures. The physico-chemical properties and internal molecular structures of bean starches differ depending on the original source, maturation and environmental factors. Most bean starch granules have wide variability in size (1-80 *u*) and shape which could be due to genetic control and seed maturity. For dry beans, a wide variability in shape (oval, oblong, round) is found in starch granules from the same source (Reddy et al., 1984). Biliaderis et al. (1979) reported that the major portion of legume starches have molecular weights higher than 2×10^6 kd , and over 90% of them have molecular weights above 4×10^4 kd.

Amylose contents ranging from 10% to 66%, may influence starch solubility, lipid binding, and other functional properties. Biliaderis et al. (1981) fractionated legume starches and 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 storage history. A high degree of amylose polymerization may confer structural stability to the granule but may also be partially responsible for its resistance toward *in-vitro* alpha-amylolysis. Most legume starches have gelatinization temperatures of 60°C to 90°C and are characterized by no distinctive pasting peak, but rather, a very high viscosity which remains constant or increases during cooling. The crystalline nature of the starch granule manifests birefringence (exhibited by the appearance of a "Maltese cross" under polarizing light) which is lost when starch is completely gelatinized. Indigestible or resistant bean starch and its possible role in flatulence is an area of interest in bean fiber research (Thompson et al., 1987).

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; however, above 75°C, bean starch surpassed wheat starch in swelling power. The restricted swelling, plus the single-stage swelling pattern, have been interpreted to explain the crystalline (ordered) and amorphous (unordered) regions of starch granules and the presence of strong binding forces which relax within a temperature range. The restricted swelling characteristics are similar to those shown by chemically cross-linked starches (Schoch and Maywald, 1968; Lineback and Ke, 1975; Vose, 1977; Lai and Varriano-Marston, 1979; Naivikul and D'Appplonia, 1979; Li and Chang, 1981; Sathe and Salunkhe, 1981). According to Schoch and Maywald (1968), these restricted swelling pastes are classified as Type C starches which show no pasting peak, but rather possess a very high viscosity which remains constant or increases during cooling and have relatively constant cold-paste viscosity during a holding period at 50°C.

An important feature of dry beans is their relatively high content of non-starch polysaccharides (NSP), and their reported hypocholesterolemic and hypoglycemic effects. Although in general beans contain slightly more insoluble than soluble fiber, they are rich sources of soluble NSP, particularly in comparison with cereals grains. Cellulose is the major component of fiber in smooth and wrinkled peas, red kidney and navy beans, while

in other legumes (lupins, lentils and broad beans), hemicellulose is the major component. Cotyledon cell walls contain higher levels of pectin than cellulose. The seed coats are primarily composed of cellulose (29 - 41%) with small amounts of lignin (1.2 - 1.7%). Total dietary fiber is higher in cooked beans than in raw beans. Water-soluble fractions can be lost with cooking water even though the beans are intact, while long boiling may cause modifications in certain constituents of other fiber fractions.

Proteins

Dry bean (*Phaseolus vulgaris*) protein content ranges from 18.8 to 29.3% (Meiners et al., 1976; Varriano-Marston and De Omana, 1979; Hosfield and Uebersax, 1980 and Chang and Satterlee, 1982). Koehler et al. (1987) investigated thirty-six varieties of eight types of dry beans for protein content (kjeldahl nitrogen) and protein quality (digestibility) as determined by *Tetrahymena pyriformis* on the raw bean flour. There was a great deal of variability among different commercial classes of *Phaseolus vulgaris* and also among cultivars within a class. The amount of protein ranged from 17.5% for the Pinto (cultivar NW-590) to 28.7% for the red kidney (cultivar Royal Red). Except for kidney beans, there was little varietal difference in protein content. Pinto beans had the highest protein quality with six varieties having values of 90 % or greater and the remaining having values of 80 % or greater. Red kidney beans had the lowest protein quality with a mean value of 59%. The proteins in beans can be classified into two categories: metabolic and storage proteins. The storage proteins tend to be found in the globulin fractions (salt soluble G1) while the metabolic (enzymatic or non-storage) proteins are primarily found in the (water soluble G2) albumin fraction (Deshpande and Nielsen, 1987; Nielsen, 1991). The storage proteins have high affinity for water and contribute dramatically to the nutritional and functional properties of the cooked seed (Boulter, 1981). They also serve as a source of nitrogen and carbon compounds for the germinated seedling.

Total biological utilization of the legume protein is relatively low. Protein digestibility may be impaired possibly by the presence of numerous anti-nutritional compounds which must be removed or destroyed during processing (Bressani et al., 1982 and Aw and Swanson, 1985). Raw legumes are poorly digested, but adequate heat treatment improves the digestibility significantly. However, in many parts of the world, the thermal treatment provided for bean preparation in the home setting is not sufficient to inactivate toxic phytohemagglutinins (lectins), but is often just sufficient to heat and hydrate the beans (Coffey et al., 1985). Gomez-Brenes et al. (1975) reported that peak digestibility and Protein Efficiency Ratios (PER) of dry *P. vulgaris* were obtained after soaking for 8 or 16 hours and cooking at 121°C for 10 to 30 minutes. Heating for longer than this resulted in lowered protein quality and decreased available lysine. Bean proteins have been reported to be resistant to *in-vitro* enzymatic digestion (Sathe et al., 1981; Bressani, 1975); however, heating improved *in-vitro* susceptibility to enzymatic hydrolysis (Sathe et al., 1982).

Bean proteins are relatively rich in essential amino acids, particularly lysine, threonine, isoleucine, leucine, phenylalanine and valine (Sathe et al. 1981; Bressani, 1975). However, they are deficient in sulfur-containing amino acids, particularly methionine and cystine. This explains their complementation with cereal grains (deficient in lysine) in plant-based diets. Besides their low content, the bioavailability of the sulfur-containing amino acids is also quite low even after cooking (Liener and Thompson, 1980).

Minerals and Vitamins

The total ash content of *P. vulgaris* ranges from 3.5% to 4.1% (db) (Table 1). Beans are generally considered to be substantial sources of calcium and iron. They also contain significant amounts of phosphorus, potassium, zinc and magnesium (Augustin et al., 1981, Koehler et al., 1987, Meiners et al., 1976, Watt and Merrill, 1963 and Sgarbieri et al., 1978). Differences among bean classes (Figures 2 and 3) and environmental factors

influence variability (Augustin et al., 1981). Ash content decreases after cooking due to leaching, with losses ranging from 10 to 70%. The wide range of reported losses could be due to different soaking and cooking methods. Although dry beans are relatively rich sources of some minerals, high mineral values cannot be equated with high bioavailability of some of these minerals because of the presence of significant amounts of phytic acid (myoinositol hexaphosphoric acid) which impedes the absorption of multivalent cations, e.g. calcium, magnesium, zince and iron (Augustin and Klein, 1989).

Dry edible beans provide several water soluble vitamins (thiamine, riboflavin, niacin and folic acid), but very little ascorbic acid. However, variability of vitamin content is high. Both genetic and environmental factors, e.g., storage time and conditions as well as differences in analytical methodologies could attribute to the variability among these reported values (Augustin and Klein, 1989). Commercial methods of preparation of canned beans cause a significant loss of water soluble vitamins.

The mineral and vitamin composition of dry beans are summarized in Figures 2,3 and 4 and Table 1. These data further demonstrate the compositional similarity among various dry bean classes.

Lipids

Dry beans possess relatively low total fat content, generally 1-2% (Korytnyk and Metzler, 1963 ; Koehler and Burke, 1981; Sathe and Salunkhe, 1981; Drumm et al., 1990). Neutral lipids are the predominant class and account for 60% of the total lipid content. Phospholipids make up 24-35%, while glycolipids account for up to 10% of the total lipid content of legume seeds. The fatty acid composition of legumes shows a significant amount of variability. However, legume lipids are generally highly unsaturated (1% to 2%), with *Phaseolus vulgaris* containing 63.3% (Watt and Merrill,1963; Takayama et al., 1965), hence the storage of legumes can result in a loss of quality,

nutritional value and functionality. Unsaturated linolenic acid is present in the highest concentration, while palmitic acid is the predominant saturated fatty acid.

Tannins and Polyphenols

The procyanidin and condensed tannin content of dry beans ranges from 0.4 to 1.0% (Sgarbieri and Garruti, 1986). These compounds are localized in the bean seed coat with low or negligible amounts present in the cotyledon (Ma and Bliss, 1978). The hydroxyl groups of the phenol ring enable the tannins to form crosslinks with proteins which may be implicated in post-harvest seed hardening or decreased digestibility (Ma and Bliss, 1978). During cooking, polyphenolic compounds are partially leached into the cooking medium and may interact with cotyledonary constituents, yet complete destruction of the tannins does not occur during heat processing (Elias et al., 1979; Bressani and Elias, 1980; Bressani et al., 1982). According to Leakey (1988), some colored seeded beans also produce particular flavonoids, proanthocyanidins, which polymerize to form phlobaphenes implicated in the afterdarkening process with seed storage (aging). Afterdarkened beans resist water inhibition, become hard-to-cook and are less digestible than freshly harvested seeds.

Bean Flavor Constituents

Native Flavor Precursors. The development of the characteristic flavors of dry beans accompanies heat treatment, with non-enzymatic browning (Maillard) reactions contributing significantly to the formation of numerous flavor compounds. The formation of Maillard reaction products has been shown to be influenced by the temperature of the reaction and the amine and carbonyl source (Hodge, J.E. and Hofreiter, B.T., 1972).

Oxidative degradation, catalyzed by heat, enzymes, light or metals, leads to the formation of hydroperoxides which decompose to react with other components in the system to produce off-flavors.

Off-flavor. Phenolic acids are localized in the cotyledon of the bean. These compounds are characterized by sour, bitter, phenol-like, and astringent flavor notes, and contribute to the formation of adverse flavors and colors, and changes in nutritional quality during processing (Sosulski, 1979).

Saponins are present in dry beans and are present following processing. These compounds are suspected of causing bitter off-flavors and may be associated with frost damaged seed. Further off-flavor development during post-harvest storage has been associated with mold growth (Uebersax, M.A. and Bedford, 1980; Maddex, 1978).

All dry beans posess remarkably similar compositional and nutritional characteristics and serve well to supply energy and nutrients in diverse dietary forms. The associated common traits of dry bean have practical applications commercially for comminuted or formulated foods, and in developmental world food relief programs.

NUTRITIONAL DIGESTIBILITY AND PHYSIOLOGY OF DRY EDIBLE BEANS

Dry legumes are important dietary sources of protein, complex carbohydrates, essential nutrients and energy. However, legume protein is generally less digestible than protein in grains and animal products (Tobin and Carpenter, 1978; Wolzak, Elias and Bressani, 1981; Wolzak, Bressani and Gomez Brenes, 1981). Table 2 and Figure 6 summarize the major constraints to utilization of dry edible beans (Uebersax et al., 1991). The relatively poor digestion of legume protein limits the biological value of the protein and reduces the overall contribution of legumes towards providing a nutritionally well balanced diet. Bean starch, the main component of dry legumes, is digested at a slower rate than starch from grains and root crops (Thompson et al., 1987).

Protein digestibility is expressed in terms of calculated responses related to body nitrogen balance. "True" digestibility gives consideration to the component of the fecal nitrogen originating from the body (mucosal cells of the gastrointestinal tract and digestive enzymes) or endogenous N, while "apparent" digestibility considers only the relationship between total fecal N and total dietary N. The relationships of absorption, retention and digestibility are shown diagrammatically in Figure 7.

Factors Affecting Digestibility of Dry Legume Starch and Protein.

A factor that affects digestion of legume protein and starch is the physical form of the protein and starch when it enters the stomach and small intestine. Grains and root vegetables are usually ground and/or cooked prior to consumption. Grinding and cooking serve to break the cell structures and to release the protein and starch so that proteolysis and amylolysis can occur. Legumes are generally consumed as whole seeds that have been cooked. Cell wall structures in cooked beans are intact and surround the protein bodies and starch granules. Chewing macerates some of the cells, but most of the cells remain whole

Table 2 - Constraints Limiting Utilization of Dry Beans

Constraints	Reference
PHYSICAL	
• **Preparation** (*time, convenience and expense*)	Uebersax and Ruengsakulrach, (1989)
• **Post Harvest Storage Changes** (*seed hardening*)	Sefa-Dedeh et al. (1979); Varriano-Marston and Jackson (1981); Stanley and Aguilera (1985); Srisuma et al.(1989)
BIOLOGICAL	
• **Anti-nutrients**	
- *protease inhibitors and lectins*	Liener, I. E.(1978); Thompson et al.(1983, 1986) ;King et al.(1980) Coffey et al. (1985)
- *amylase inhibitors*	Jaffe et al.(1973); Powers, J. R. and Whitaker, J.R.(1977); Pick, K.H. and Weber, G. (1979)
- *procyanidin interactions*	Bressani et al. (1982)
• **Digestibility**	
- *phytic acid*	Maga (1982); Yoon et al. (1983)
- *protein*	Wolzak et al. (1981); Chang, K.C. and L. D. Satterlee (1981); Bressani et al. (1982) Coffey et al. (1985) Koehler et al. (1987)
- *starch*	Hoover, R. and F. Sosulski (1985); Thompson et al. (1987); Bennink and Srisuma (1989);Tovar et al.(1990); Englyst and Cummings (1990)
• **Flatulence Factors**	
- *oligosaccharides*	Murphy et al. (1972); Fleming (1981a,b); Olson et al. (1981)
- *proteins*	Becker (1971)
- *retrograded starch*	Englyst, H.N. and J.H. Cummings (1990)
- *fiber*	Hellendoorn (1978); Fleming (1981a,b); Reddy et al., 1984

CONSTRAINTS to UTILIZATION
BIOAVAILABILITY and ANTINUTRITIONAL FACTORS

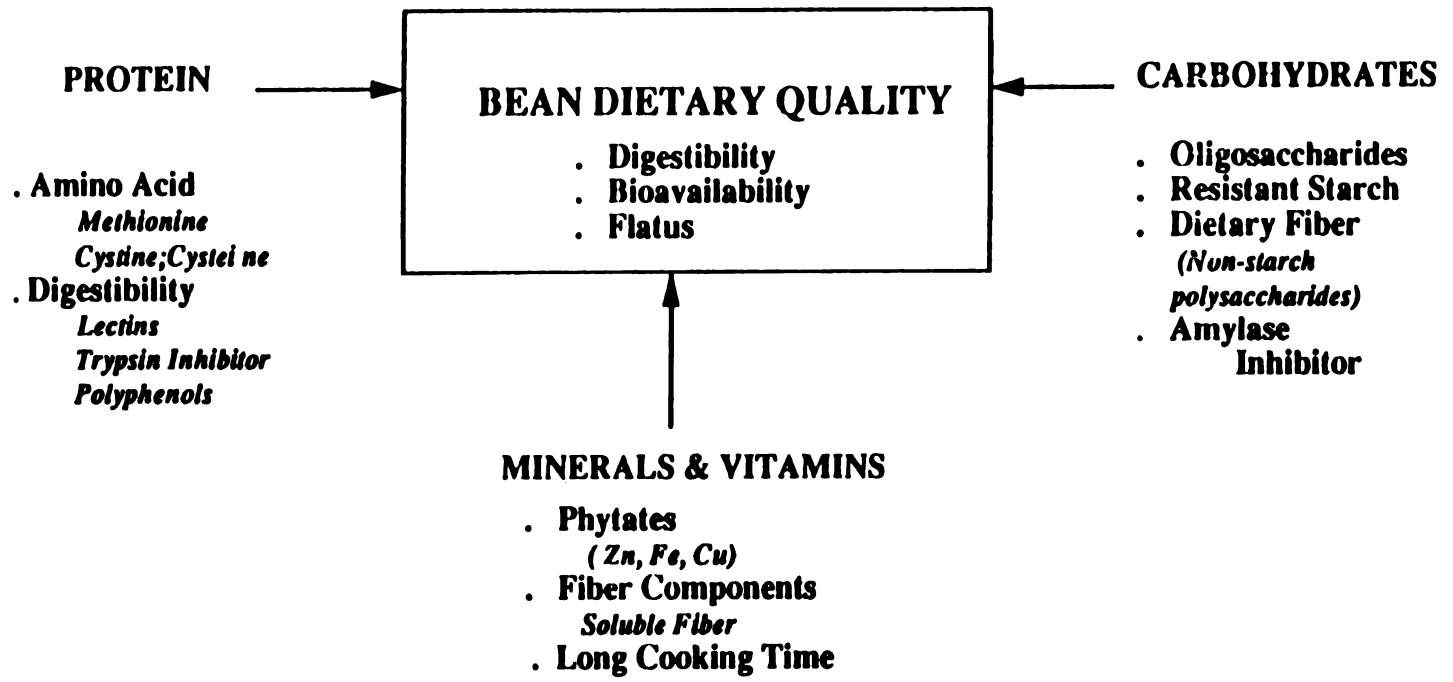
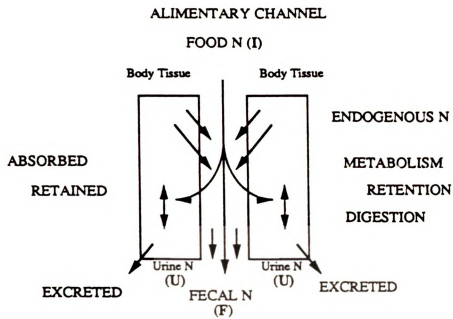


Figure 6. Outline summarizing factors influencing digestibility and bioavailability of nutrients in dry beans



CLASSIFICATION	DEFINITION	CALCULATION	
		True	Apparent
Digestibility (D)	$\frac{\text{N Absorbed}}{\text{N Intake}}$	$\frac{I - (F - Fk)}{I}$	$\frac{I - F}{I}$
Biological Value (BV)	$\frac{\text{N Retained}}{\text{N Absorbed}}$	$\frac{I - (F-Fk) - (U - Uk)}{I - (F - Fk)}$	$\frac{I - F - U}{I - F}$
Net Protein Utilization (NPU)	$\frac{\text{N Retained}}{\text{N Intake}}$	$\frac{I - (F - Fk) - (U - Uk)}{I}$	$\frac{I - F - U}{I}$

where I, F and U are intake, fecal and urinary nitrogen values, respectively, on the test diet, and Fk and Uk are the corresponding values on a non-protein diet. NPU can also be defined in terms of body N.

Figure 7. Nitrogen digestion, absorption and retention (Pellett, 1978)

and enter the digestive tract intact. Thus, the cell structures render legume protein and starch less available to the digestive enzymes except for the small amounts of starch that leaches from the cells (Wursch et al.,1986; Golay et al., 1986).

Another factor affecting digestion of both protein and starch is the amount of total dietary fiber in the diet. *Phaseolus vulgaris* beans typically contain 17.5 - 20% dietary fiber and are regarded as an excellent source of dietary fiber (Prosky et al., 1985; Anderson and Bridges, 1988). As an individual increases dietary fiber intake, nutrient digestibility and/or absorption tends to decrease (Kies, 1981). When dietary fiber was increased from 16.4 g/day to 37.4 g/day, protein digestion decreased 6%, fat digestion decreased 2% and total energy digestibility decreased by 1.8% Decreased digestibility of protein and starch is presumably due to physical entrapment and/or premature release of food particles from the stomach, although direct inhibition of digestive enzymes by fiber cannot be excluded (Schneeman, 1982). Dietary fiber can encase nutrients in a fibrous matrix which impedes digestive enzymes from gaining access to protein and starch or fiber can reduce absorption of digested protein and starch by slowing or preventing diffusion of the digestive products to the mucosal cells (Johnson and Gee, 1981; Elsenhaus et al., 1981). Food is normally held in the stomach until it is reduced to particles less than 1 mm, but fiber can cause the premature release of large particles from the stomach. Large particles represent another type of physical entrapment which limits digestion.

A potential factor which could also limit protein and starch digestion is the presence of naturally occurring protease and amylase inhibitors. During processing and cooking, most of these inhibitors are inactivated; however, residual levels of active inhibitors may remain, particularly in improperly cooked beans. Rayas-Duarte et al.(1992) reported that trypsin inhibitory activity (TIA) in boiled (30 mins.) Great Northern beans exhibited minimum heat stability (2.5-5% TIA retention) in the whole intact bean matrix, medium heat stability in the dry bean flour, and maximum heat stability (97-100% TIA retention) in the extracted bean albumins compared to unheated samples. Individuals accustomed to

consuming beans with residual levels of inhibitors possess an enlarged pancreas which secretes more digestive enzymes to compensate for the enzymes which have been inactivated by the inhibitors (Schneeman, 1982; Madar et al., 1976). As a result of pancreatic hyperplasia and hypertrophy, adequate amounts of digestive enzymes are secreted and digestion is not limited. The best evidence to support this conclusion is based on the experience with exogenous amylase inhibitors ("starch blockers"). It has been demonstrated that chronic consumption of amylase inhibitors was ineffective in reducing starch digestion and caloric utilization. Essentially the same results are found with continued consumption of protease inhibitors (Madar et al., 1976).

Legumes also contain lectins which are toxic factors that interact with glycoprotein on the surface of red blood cells and cause them to agglutinate (Singh, 1988). Einhoff et al. (1985) have shown that *Leguminosa* lectins will bind both the storage protein and the glycosidase enzymes and that both the lectins and the lectin-bound proteins are found in the protein bodies. They suggested that lectins act during maturation of the plant to contribute to an orderly arrangement of the storage proteins in the protein bodies. Phytohemagglutinin (PHA), the lectin of dry beans, is a 6.4S protein with two subunits of molecular weight 34 kd and 36 kd. Estimates of PHA's molecular weight range from a low of 115 kd to a high of 150 kd (Coffey, 1985). Cooking beans will inactivate much of the lectin activity in raw beans; however, fully cooked beans can still contain a significant amount of the original lectin activity (Thompson et al., 1983; Rea et al., 1985). Reaidi et al.(1981) reported one cultivar of *P. vulgaris* contained appreciable amounts even after 20 min. boiling. For complete elimination of the toxic effects, pre-soaked (minimum 4-5 h) kidney beans should be heated for either 4 hr at 90°C, 90 min. at 95°C or 10 min at 100°C (Annual Report, 1982, Rowett Research Institute). It is difficult to measure the effect of residual lectins on digestion and absorption. In rats, plasma protein losses into the intestine is one of the main signs of lectin toxicity. Ingested kidney bean lectins bind to membrane-glycocalyx receptors on microvilli in the proximal part of the small intestine, and

is associated with considerable disruption of intestinal microvilli (Annual Report, 1982, Rowett Research Institute). Data provided by Donatucci et al. (1987) strongly suggest that small quantities of lectins are sufficient to reduce absorption of glucose and presumably amino acids and small peptides.

Phytic acid, another antinutritional factor, has been reported to reduce the biological availability of minerals. The study of Tecklenburg et al. (1984) indicated phytic acid to be partitioned with the protein fraction during air classification processing. The high degree of correlation between protein content and Zn, Fe, K and Mg, and between phytic acid and these minerals suggested that these elements are present as metallic phytates. Sandberg et al. (1989) reported that addition of 10 umol inositol hexa- and penta-phosphate reduced iron solubility from the reference 39% to 0.2% and 6%, respectively, while the same amount of inositol tetra- and tri-phosphate slightly increased iron solubility. This indicates that degradation of inositol hexa- and pentaphosphates seemed to significantly reduce the inhibiting effect on iron bioavailability.

Huisman et al. (1990) made a comparison of the effects of antinutritional factors present in *Phaseolus vulgaris* on piglets, rats and chickens. They found that live-weight gain was markedly reduced during feeding 200 g raw beans in piglets, but this response was not shown in the rats and chickens. When the supply of dietary protein is adequate, there is no reduction in pancreas weight, but weight of the intestine was increased in all three species due to feeding raw beans.

Digestibility of Legume protein.

There have been two prevalent concepts to explain poor digestion of legume protein: (a) legumes contain protease inhibitors and lectins which reduce legume protein digestion (Jaffe and Vega Lette, 1968; Liener, 1983) and (b) the inherent structure of legume protein is such that it resists proteolysis. Deshpande and Nielsen (1987) provided evidence that heated legume protein is not resistant to trypsin proteolysis *in-vitro*; however,

their research does not preclude the possibility that a portion of the trypsin digestion products are resistant to other proteolytic enzymes which are necessary to hydrolyze the protein to absorbable end products (free amino acids, di- and tripeptides).

Globulin GI is the major protein fraction of *Phaseolus* beans (50 to 75% of the total bean protein). Marquez and Lajolo (1981) found that the globulin GII fraction (phytohemagglutinin or lectin) was 60% digestible, whereas the glutelin fraction was only 40% digestible. Trypsin inhibitor is also poorly digested, only 38% digestible (Boulter, 1984). Heated albumin was only 53% digestible, but unheated albumin was 100% digestible. It was noted that the heated albumin formed large aggregates which were probably inaccessible to proteolytic enzymes (Marquez and Lajolo, 1981). If the albumins were in the bean rather than isolated, it is likely that its digestibility would be approximately 100%. Nonextractable proteins are probably located in the cell walls and hulls and these nonextractable proteins comprise about 10 - 12% of the total protein. The digestibility of the nonextractable proteins in *Phaseolus* beans is not known; but, based on the digestibility of proteins in brans and hulls of other plant products, one would expect relatively poor digestion of the nonextractable proteins. Additional factors such as tannins (Elias et al., 1979; Bressani et al., 1982; Aw and Swanson, 1985), dietary fiber, residual lectin activity and protein encasement within cell walls will decrease total protein digestibility to the relatively low levels commonly reported for *Phaseolus vulgaris*. Several hypotheses have been presented to explain decreased protein digestibility of the various fractions. Steric hinderance of proteolysis by the carbohydrate moieties of the glycoproteins (globulin GII and protease inhibitor fractions) is one possibility for the measured digestibility of these two fractions (Chang and Satterlee, 1981). They have hypothesized that poor legume protein digestibility is due to the compact, dense nature of bean protein which prevents proteolytic enzymes from reaching the internal catalytic sites. Dissociation of protein fractions by urea and/or sulfhydryl agents resulted in a significant improvement in protein digestibility in soy protein (Rothenbuhler and Kinsella, 1986) and

sorghum protein (Hamaker, 1987). The presence of carbohydrate-protein bonds and protein-protein bonds have been proposed to reduce protein digestibility in legumes. Carbohydrate-protein and protein-protein bonds are not susceptible to mammalian proteases and reduce overall protein digestibilities. Formation of carbohydrate-protein bonds is thought to occur via oxidative coupling of two activated benzene rings, one from cell wall phenolic compounds and the other from tyrosine. Similarly, protein crosslinking is thought to occur via peroxidase-catalyzed coupling of two tyrosine residues. If the nonextractable protein is in cell walls and hulls and if it is poorly digested, at least part of the poor digestibility would likely be due to crosslinking of protein with carbohydrate or other protein.

The high level of bean protein (about 25% db) is about double many common cereal grains; however, this factor of quantity cannot exclude the concept of protein quality which is most important to nutritional comparisons among protein sources. The nutritional value of a protein is a direct consequence of the qualitative and quantitative distribution of amino acids comprising the primary structure of the protein. Overall quality of the protein is determined by the essential amino acids. Methionine, a sulfur containing amino acid, has consistently been shown to be first limiting in dry bean. Cereal grains (wheat, corn) are limiting in lysine. Thus, these two protein sources are "complementary" and when blended in a diet provide for each other's limitations. Batterham et al. (1990) determined the utilization of ileal digestible lysine in soya-bean meal by pigs. They reported that values for the ileal digestibility of lysine in protein concentrate are unsuitable in dietary formulations as the assay does not reflect the proportion of lysine that can utilized by the pig. Their study indicated that with heat-processed meals, a considerable proportion of the lysine is absorbed in a form(s) that is(are) inefficently utilized.

A comprehensive review focusing on molecular strategies to improve protein quality has been conducted by de Lumen (1992). Techniques used to increase the methionine content of tobacco seeds using heterologous seed protein genes from peas,

common bean and soybean have long term potential utility in modifying lysine, tryptophan and threonine contents in legumes.

Digestibility of Legume Starch.

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

The "glycemic index" has been developed to aid dietary treatment of diabetics (Jenkins et al., 1982) and is defined as :

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

Canned beans have been reported to have a low glycemic index indicating that bean starch is digested more slowly than starch from most other foods. Elevated blood glucose promotes triglyceride and cholesterol synthesis by the liver which results in more very low density lipoproteins (triglycerides and cholesterol) being secreted into the blood (Anderson et al., 1984a & b; Jenkins et al., 1984; Thompson et al., 1984). Furthermore, elevated blood glucose promotes glycosylation of proteins in the vascular system (Jenkins et al., 1988). The net effect of elevated blood lipids and unwanted protein glycosylation is an increased risk of cardiovascular disease. Thus, consumption of legume starch reduces the risk of premature cardiovascular disease compared to consuming starch with a high glycemic index. While slow starch digestion is desirable, it is undesirable for the starch to be digested too slowly since there will be incomplete digestion and absorption in the small

intestine. Undigested starch will pass to the colon where rapid fermentation will take place and flatulence will result.

Two major factors affect the rate and perhaps the extent of starch digestion: 1) gastric emptying time and 2) accessibility of the glycosidic bonds in starch to pancreatic amylase and other digestive enzymes. If the rate of starch digestion is constant, starch which is released from the stomach slowly and over an extended time period will be digested and absorbed more slowly than when gastric emptying is rapid and over a short time period. There is a strong negative correlation between postprandial changes in blood glucose and gastric emptying (Mourot et al., 1988). Likewise, if gastric emptying is constant, starch digestion rate determines how high the blood glucose rises in response to starch ingestion. The digestion rate of starch strongly influences the glycemic response to different foods (Thompson, 1988; Jenkins et al., 1982). The glycemic index of a food is obviously a function of both gastric emptying and starch digestion rate.

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

A significant but less recognized factor which limits starch digestibility is the tendency for some starches to retrograde. Retrogradation is a phenomenon initiated when gelatinized starch cools slowly, hydrogen bonds within or between starch chains reform, and water between the starch chains is squeezed out resulting in a compacted dense molecular structure with differentiated functional properties. Amyloses with degree of polymerization (DP) between 200 and 1200, have a high tendency to retrograde to crystalline "beta sheet" type configurations. The average DP for legume amylose is DP 1000 - 1400 (Biliaderis et al., 1981), which is highly conducive to formation of

retrograded crystalline starch structures. Retrograded amylose is indigestible within the small intestine (Englyst and Cummings, 1987). Legume starch has a higher percentage of amylose than most starches which increases the potential for formation of indigestible retrograded starch. During cooking some of the amylose leaches from the starch granule. Since the cell structure in cooked beans remain intact, much of the leached amylose remains within the cell and fills spaces between protein bodies and starch granules. If amylose retrogradation occurs, another indigestible matrix (similar to fiber in cell walls) in and around the protein and starch granules is formed (Bennink and Srisuma, 1989).

Flatulence Associated with Legume Consumption

Flatulence refers to the passage of gas through the rectum. Any material that is not digested and absorbed in the small intestine is a susceptible substrate for microbial catabolism within the colon with the subsequent production of variable levels of flatus.

Suspected Causes of Flatulence. Establishing a cause and effect relationship between specific factors and flatulence has been a long and active area of research. Alvarez (1942) suggested that swallowed air or the diffusion of gasses from the blood into the intestinal lumen were responsible. Danhof et al. (1953) proposed that because of the high carbon dioxide found in flatus, secretions in the intestine (especially the pancreatic secretion) may be the causal factor. As early as 1929, Kantor (1929) linked bean diets and gas production to bacterial fermentation within the lumen to the intestine. An early suggestion that carbohydrates in dry beans may in part be responsible was made by Anderson (1924). He was able to demonstrate that the primary gases evolved in anaerobic cultivation of various carbohydrate media were high percentages of carbon dioxide and hydrogen and low percentages of nitrogen and oxygen. Olson et al. (1975) reported that protein rich fractions from beans and soybeans did not significantly contribute to flatulence in rats and humans. Tomlin et al. (1991) reported that fermentation gases make the highest contribution to normal flatus volume, and that a "fiber free" diet eliminates these without

changing residual gas release of around 200 ml/24 h. Figure 8 illustrates the proposed mechanism for gas production involving oligosaccharides, indigestible starch and dietary fiber.

Raffinose, Stachyose and Verbascose. The oligosaccharides of the raffinose family have been implicated in flatus formation (Fleming, 1981a): raffinose (a trisaccharide containing galactose, glucose and fructose, with the galactose moiety having a 1-6 bond); stachyose (a tetrasaccharide made up of two adjacent galactose units, glucose and fructose with the 1-6 bond) and verbascose (a pentasaccharide made up of three adjacent galactose units, glucose and fructose with the 1-6 bond). These sugars cannot be digested by humans because the human intestinal mucosa and pancreatic secretions do not contain the enzyme (*a* -1, 6 galactosidase) necessary to split these oligosaccharides into simple sugars. Figure 8 illustrates the proposed mechanism for gas production involving oligosaccharides, indigestible starch and indigestible starch. Ruttloff et al. (1967) found no enzymatic hydrolysis of raffinose in the intestinal mucosa of rats, pigs, and humans. Other studies on the absorption and degradation of oligosaccharides containing ß-galactosyl groups show that less than 1% of the administered dose was able to pass through the intestinal wall of man and animals (Taeufel et al., 1960). The indigestible oligosaccharides pass through the small bowel and reach the colon where bacterial fermentation produces large amounts of methane (Olson et al., 1981). Oral alpha-galactosidase (Novozyme) therapy was found feasible to promote oligosaccharide hydrolysis and reduce intolerance symptoms in healthy adult subjects after ingestion of refried black beans (Solomons et al., 1991). This *in situ*, intraintestinal assisted digestion had been previously proven succcessful for lactose and sucrose intolerance.

Steggerda et al. (1966) reported that low molecular weight carbohydrate fractions were especially potent in gas production compared with fat, protein, and complex polysaccharide fractions when human subjects consumed various fractions of soybean meal. Steggerda and Dimmick (1966) showed that differences in human flatus production

Proposed Mechanism of Gas Production Involving Oligosaccharides and Resistant Starch

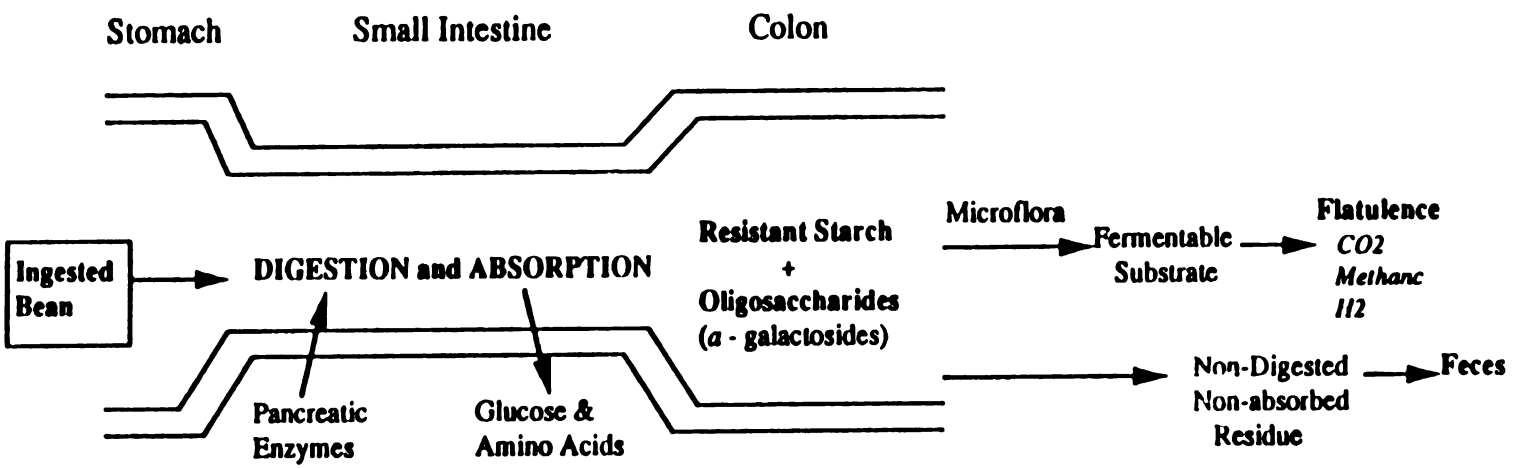


Figure 8. Proposed mechanism of gas production involving oligosaccharides and resistant starch

and gas composition occurred with different kinds and quantities of bean diets. They observed the average concentration of carbon dioxide in the collected flatus changed from 11% on a controlled non-gas producing diet to 51% when large quantities of pork and beans were consumed. Calloway (1966) subjected pure carbohydrates to fermentation with colonic bacteria and found they had the capability to hydrolyze the *a*-1,6 galactoside of stachyose. Up to this time no *a*-(1,6) galactosidase could be found in mammals (Gitzelmann & Ajurricchio, 1965) leading to speculation that sugars of raffinose family may cause much of the intolerance associated with beans.

Cristofaro et al., (1973) found that diets containing stachyose and verbacose exhibited the highest flatus activity. Rackis et al. (1970), using an *in-vitro* assay, showed that toasted, dehulled, and defatted soybean meal had gas producing factors of sucrose, raffinose and stachyose and gas-inhibiting factors of phenolic (syrigic and ferrulic) acids. The lipids, proteins and water-insoluble polysaccharides of soybean meal had no gas producing activity.

Murphy et al. (1972) using human subjects and the California Small White bean (CSW), showed that the flatulent activity was associated in the 60% and 85% aqueous extracted ethanol phase which contained raffinose and stachyose. But purified raffinose and stachyose fed alone at levels found in CSW did not increase the carbon dioxide level of the flatus. The sugars, however, made a major contribution to the hydrogen component of the breath and flatus during the period of high flatus volume; but the major factors responsible for the increase in carbon dioxide volume remained unidentified.

Raffinose and stachyose were reduced by high temperature extrusion of pinto bean high starch fractions (Borejszo and Khan, 1992). Loss of these sugars could be possibly due to Maillard reaction between charged protein groups and reducing sugars.

Fleming (1981), using rats, showed a significant positive correlation between hydrogen production and raffinose plus stachyose, and glucans and pentosans hydrolyzable in dilute acid. Studies by Olson et al. (1975;1982) revealed that flatus-

producing capacity was not totally eliminated by removal of oligosaccharides, suggesting that there are other substances that contribute to flatulence. The role of oligosaccharides in plant biology can be elucidated through molecular techniques, and knowledge of biosynthetic pathways for raffinose oligosaccharides enables control and modification of these sugars to alleviate the problem of flatulence associated with bean consumption (de Lumen, 1992).

Dietary fiber. Studies indicated that even after the removal of oligosaccharides, dry beans could still induce appreciable flatus (Olson et al., 1975; Fleming, 1981 & 1982; Hellendoorn, 1976, 1978; Kamat & Kulkarni, 1981). Wagner et al. (1977) then compared a cooked California Small White (CSW) bean which contained 4% oligosaccharides, to oligosaccharide free CSW solids (residue from hexane and 70% ethanol extraction of CSW). The oligosaccharides served as a source of hydrogen when ingested by rats in life support systems. His results indicated that if the oligosaccharide content was the only hydrogen source in CSW, it would have to be 25 times more potent; the stachyose would have to be seven times as potent as free the CSW solids. Thus, CSW must contain at least one 70% alcohol-insoluble substance which, in addition to the oligosaccharides, was essential to bring about the observed quantitative physiological response in rats to whole beans.

Dietary fiber, being the indigestible component for man, has been shown to be fermented by microorganisms in the colon. Reddy et al. (1984) mentioned that fiber may be involved in the fermentation by microorganisms with subsequent flatulence production. Analysis of feces for the presence of fiber showed that the greater part of cellulose and lignin can be recovered in the stool. Calloway et al. (1980, 1983) measured the digestibility of pectin and hemicellulose in humans on an input/output basis. Of the subjects fed pectin diets, 15.3% of the pectin was digested by women ileostomy subjects, 46.5% was digested by men ileostomy subjects, respectively, while 96.5% and 95% of the pectin was digested by men and women subjects with intact colons. When subjects

were fed hemicellulose diets, 65% of the hemicelluloses were digested by women ileostomy subjects and 63% by male ileostomy subjects. However, 95 and 97% of the hemicelluloses were digested by men and women subjects with intact colons.

Apparent digestibility of total fiber ranged from 47 to 82% during the experimental period using human subjects fed a strictly controlled diet of a fiber supplement. Prynne and Southgate (1979) reported values of 70% to 80% for the apparent digestibility of the fiber in a control segment during the experimental period.

Farrell et al. (1978) fed human subjects neutral detergent fiber and observed a complete disappearance of hemicellulose from diets with decreased transit time, while 80% and 55% of the neutral detergent fiber in the diet disappeared from low and high fiber diets, respectively.

Fleming (1981) showed a significant positive correlation between hydrogen production and glucans and pentosans hydrolyzable in dilute acid. Significant negative correlations were found between hydrogen production and starch or lignin contents. Marthinsen and Fleming (1982) measured the breath and flatus gasses of humans consuming high-fiber diets and found xylan and pectin diets resulted in a production of high flatus volume, hydrogen and carbon dioxide excretion. Cellulose and corn bran generally produced breath and flatus gas excretion at levels equivalent to a fiber-free diet.

Hellendoorn (1978) found that while most of the hemicelluloses and soluble pectins were fermented in the large intestine, some could be recovered from the stool. Tadesse and Eastwood (1978) reported a hemicellulose preparation increased hydrogen production in man, but cellulose, lignin, and pectin did not. Fleming (1981), using the smooth seeded field pea, found hydrogen production in the rat to be closely associated with the quantity of oligosaccharides remaining in the seed meal. However, the flatulent activity of the whole seed appeared to be due in equal parts to the indigestible oligosaccharides and components of the cell-wall fiber.

Fleming (1983) compared the effects of selected purified fibers to those derived from cereals or legume seeds. Rats were fed diets containing 10% dietary fiber from selected sources and 10% protein. Pectin reduced rat weight, while Feed Efficiency Ratio (FER), Protein Efficiency Ratio (PER) and apparent protein digestibility values compared to a fiber-free diet. Cellulose, xylan and raffinose had no influence on feed intake, weight gains or FER. Cellulose and xylan increased PER values and the rates of food passage through the gastrointestinal tract, but decreased the apparent protein digestibility values. Finally, the cell-wall-fiber fraction of beans had little effect on feed consumption, growth, FER and PER. The cell-wall- fiber fraction reduced apparent protein digestibility and the hull fraction accelerated food passage relative to the fiber-free diet.

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

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

Starch digestibility may also be dependent on the type of native starch, cooking time, and procedure used for preparation of dry beans. Accordingly, 5 to 15% of bean starch can remain indigestible even after prolonged cooking (Hellendoorn, 1969). Faki and Bhavanishangar (1983), using *in-vitro* and *in-vivo* studies, showed that apart from

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

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

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

The actual crystalline structure of the starch granule is suggested to depend on the chain length of amylopectin and has been classified using x-ray diffraction techniques into groups termed A, B and C. A-type starch is the normal pattern for cereal starch granules, B-type is typical of potato, amylomaize and retrograded starch, and C-type (combination of A and B patterns) is characteristic of certain pea and bean starches. In general, starch granules showing X-ray diffraction patterns B or C tend to be the most resistant to pancreatic amylase, though the degree of resistance is dependent on the plant source (Fuwa,Takay and Sugimoto,1989, as cited by Englyst and Cummings, 1990). Cooking

disrupts the granules and facilitates the hydrolysis of the starch contained within them. Crystallization at higher temperature and lower water content will favor the A pattern, and lower temperature and high water content, the B pattern. On cooling, gelatinized starchy foods will retrograde, solubility of the starch molecule decreases and so does its susceptibility to hydrolysis by acid and enzymes.

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

Socorro et al (1989) reported that dietary fiber decreased digestibility of black bean, corn, rice and wheat starches by pancreatic porcine enzyme, especially when whole grain fiber was used. However, black bean and rice starch digestibility by human pancreatic a-amylase was not affected by fiber, while corn and wheat starch was slightly inhibited. The use of the human enzyme is therefore recommended for amylolysis assays, although the difficulties of extrapolating results obtained with animal enzymes to humans should be carefully considered.

Studies by Fernandez and Berry (1989) reported that germination sharply increased the susceptibility of chickpea starch to digestibility by *a*-amylase, but no change in the appearance of SEM could be attributed to germination. *In-vivo* and *in-vitro* methods with experimental rats and commercial digestive enzymes, respectively, were used by Nnanna and Phillips (1990) in assessing the protein and starch digestibility and flatulence potential

of germinated cowpeas. Germination reduced the flatulence potential of seeds. *In-vivo* digestibility of starch and protein was also significantly increased by germination. Germination did not affect *in-vitro* protein digestibility, but reduced in vitro digestibility of freeze-dried and 70°C-dried starch. Cooking in boiling water significantly increased *in-vivo* protein and starch digestibility of both ungerminated and germinated seed.

Tovar et. al. (1990) found the starch content of a raw red kidney bean (*Phaseolus vulgaris*) flour (RBE) was higher than that of a cooked and blended (CBB) and of a cooked, freeze-dried, and milled (CBF) preparation. However, wet homogenization as well as pepsin pretreatment of CBF increased the starch yield, indicating that starch in the cooked samples is not completely available to enzymic degradation unless cell wall entrapped granules are released by mechanical or enzymatic disruption of the fibrous walls. This could partly explain a number of inconsistencies reported in literature (Fleming and Vose, 1979; Jenkins et. al., 1982a and b; Wursh et. al., 1988; Socorro et. al., 1989 and O'Dea and Wong, 1983). Influence of encapsulated and resistant starch fractions on dietary fiber values was also observed. CBF showed remarkable low values of *in-vitro* amylolysis rate and starch digestibility index in a digestion/dialysis system, features that seemed to depend also on the integrity of cell walls. In a related study, Tovar et al. (1991) suggested the persistence of starch granules (resistant starch i.e., retrograded amylose, 3-9% dwb) enclosed in cotyledon cells as the primary reason for the limited enzymatic availability of starch in precooked flours (PCF) as evidenced by SEM of PCFs of milled boiled and freeze-dried red kidney beans, white beans and lentils demonstrating relatively large particles which contained cell structures filled with starch. The susceptibility to enzymatic hydrolysis of PCF's was enhanced with pepsin preincubation or with additional boiling, resulting in an apparently thinner surface. Homogenization of PCF's resulted in the largest increase in *in-vitro* α-amyloysis rate, with almost complete disruption of the cotyledon cell walls. When these PCF's were subjected to *in - vivo* feeding studies with rats, between 8% (beans) and 11% (lentils) of the total starch ingested appeared in the

feces, indicating a relatively low starch digestibility; 60% of the fecal starch in the bean-fed animals and 70% in the lentil-fed group was retrograded amylose (Tovar et al., 1992).

The rate of wheat starch digestibility in the presence or absence of polyphenols (catechin or tannic acid) and/or phytic acid at concentrations found in legumes was determined in an *in-vitro* dialysis system. Addition of tannic acid and phytic acid reduced the starch digestibility 13 and 60% respectively, at 5 hr. Combined tannic and phytic acid reduced the digestibility at a level (63%) which did not differ significantly from that with only phytic acid. These results agree with those observed in an earlier study by Thompson et. al. (1983). There was no additive or synergistic effect between these two antinutrients. Catechin had no significant effect on rate of starch digestibility, or it may have been obscurred by the large reduction by phytic acid. A future dose-response study of these antinutrients and starch digestibility may help understand the interaction between phytate and polyphenols.

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

Thompson et. al. (1987) determined digestion of wheat starch (WS) and red kidney bean (RKB) starch by pancreatic (PA) and salivary (SA) amylose in the presence

or absence of lectins . Compared with WS, digestion of RKB starch by PA and SA was 70.0% and 66.6% lower, respectively. RKB lectin added to WS at the hemagglutinin activity level in RKB starch resulted in significantly decreased digestion with PA (63.9%) and SA (43.8%) as did heated RKB lectin with insignificant hemagglutinin activity (41.1% with PA, 35.8% with SA). Jack bean lectin (concanavalin A) also resulted in reduced rate of starch digestibility. Kinetic analyses revealed noncompetitive inhibition by RKB lectins on both amylases. Results confirmed the role of lectins in reducing the rate of starch digestion and its possible health benefit.

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

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

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

Hypocholesterolemic and Hypoglycemic Responses of Bean Dietary Fiber

Hypocholesterolemic Effect. There is considerable literature evidence that foods which contain high levels of water-soluble dietary fiber such as oats or bean products, and purified forms of water-soluble dietary fiber reduce blood cholesterol (Kritchevsky, 1987 as cited by Gatenby, 1990; Anderson, 1985; Anderson et al., 1984 ; Kirby et al., 1984; Storch et al., 1984). Soluble fiber may bind bile acids and cholesterol in the intestine, decreasing their absorption. Bacteria in the colon convert primary bile acids into secondary bile acids, which are less well absorbed. Thus, less bile acid is returned to the enterohepatic circulation which drives *de novo* hepatic synthesis of cholesterol to serve as a precursor for bile acid synthesis, with resultant decreases in the amount of cholesterol available for lipoprotein synthesis (Anderson and Tietyen - Clark, 1986; Kirby et al., 1981; Kay, 1982). However, not all sources of soluble fibers increase fecal bile acid excretion, and the magnitude of increased excretion may be small (Anderson, et al, 1984; Chen and Anderson, 1986).

Short-chain fatty acids (SCFA) may also mediate the hypocholesterolemic effects of soluble fiber. Soluble fibers are almost completely fermented in the colon to SCFA, primarily acetate, propionate and butyrate. These fatty acids are absorbed into the portal vein and appear to inhibit hepatic cholesterol synthesis. The increased fecal loss of bile acids coupled with reduced hepatic cholesterol synthesis may decrease hepatic secretion of very-low-density lipoprotein (VLDL) cholesterol, a precursor to LDL cholesterol (Chen, et

al., 1981). SCFA may also inhibit cholesterol synthesis in peripheral tissues, resulting in an increase in peripheral LDL receptors and an increase in LDL clearance (Chen and Anderson, 1986; Kirby et al., 1981).

Effects of oat-bran or bean supplemented diets were assessed for 10 hypocholesterolemic men who were observed for 3 weeks on a metabolic ward. Oat-bran or bean (100 g db) supplements decreased serum cholesterol and LDL - cholesterol concentrations by 23% below initial values without alterations in cholesterol or fat intakes (Anderson et al., 1984). However, in an earlier study, Jenkins et al., (1983) reported that low- and- high density lipoprotein cholesterol levels remained unaltered when dried beans where fed to 7 male hyperlipidemic patients to substitute for other sources of starch. Mean fasting serum triglyceride levels were reduced by 25% while total serum cholesterol levels were 75% lower than the non-bean supplemented control group.

Anderson and Chen (1982) found bean supplemented diets (115g dried beans/day; 50 g total and 20 g soluble fiber) to selectively lower atherogenic LDL cholesterol fractions while preserving anti-atherogenic HDL cholesterol levels. This effect probably relates to the high soluble storage polysaccharide content of legumes. Short chain fatty acid fermentation products of soluble fibers may attenuate hepatic cholesterol synthesis.

The hypolipidemic effects of canned beans were also studied by Anderson (1985) in 10 hypercholesterolemic men. After 3 weeks on the canned-bean diet (122g daily; 24 g total and 7 g soluble fiber), serum cholesterol values averaged 13% lower and triglycerides values 12% lower than control values.

Daily consumption of 100-135g dried beans (dry measure) reduces serum cholesterol levels ~ 20% short-term, hypothetically reducing risk for CHD by 40% (Anderson et al., 1984). Smaller portions of 100-200g cooked or canned beans lower serum cholesterol ~12% short-term and 20-25% long-term (Anderson et al., 1984b; Anderson, 1985). Nutritional therapy combining "High Carbohydrate Fiber" (HCF) diets

with bean supplementation is well tolerated and associated with no major side effects, except for reported increased flatulence and eructations.

Swain et al. (1990) concluded that oat-bran had little cholesterol-lowering effect and that high-fiber and low-fiber dietary grain supplements about equally reduce serum cholesterol levels probably because they replace dietary fats. This confirms earlier studies by Anderson et al.(1984) where serum cholesterol levels decreased by 19% and low-density lipoprotein levels decreased by 23-24% with both the oat-bran and bean diets. Since there was no randomized low-fiber control group, this suggests only that oat bran and beans are equivalent, not that either one have direct - cholesterol - lowering properties.

Hypoglycemic Effect. Jenkins et al.(1985) indicated that selection of low glycemic index carbohydrate foods may be a useful adjunct to the management of hyperlipidimia. They found that reduction in the mean glycemic index (GI) of diets of 12 hyperlipidemic patients resulted in a significant reduction in total and LDL serum cholesterol and serum triglyceride by comparison with the mean lipid values for the preceding and subsequent months on the control diets. Low glycemic responses have been observed for legumes compared with other starchy foods such as bread, potato and certain breakfast cereals (Potter et al., 1981; Jenkins et al., 1981, 1982b; Walker and Walker, 1984; Tappy et al., 1986).

Jenkins et al., (1980) incubated carbohydrate portions of lentils, soya beans and whole meal bread with human digestive juices. Results suggested that the rate of digestion might be an important factor determining the rise in blood glucose concentration after a meal and that supplementing chemical analysis with *in-vitro* and *in-vivo* food testing might permit identification of useful foods for diabetics.

To test the effect of processing on digestibility and the glycemic response to a leguminous seed, a group of eight healthy volunteers took a series of breakfast test meals containing either lentils which had been processed in four different fashions or the same amount of carbohydrate as white bread. Processing variables included : 1) boiling 20

minutes, consumed whole; 2) boiled 20 minutes blended to paste; 3) Boiled 60 minutes and 4) Boiled and drying 250°F/12 hours. Lentils boiled for 20 min. resulted in a flattened blood glucose response in comparison to bread. This was unaltered by blending the lentils to a paste or boiling them for an additional 40 minutes. However the blood glucose response was significantly enhanced by drying the boiled blended lentils for 12 h at 250°F. In vitro digestion with human saliva showed the rate of sugars released from the food related positively to the blood glucose rise. Breath hydrogen studies indicated that carbohydrate malabsorption was too small to account for differences in the blood glucose response. These results emphasize the importance of processing in determining digestibility and hence the glycemic response to a food (Jenkins et al.,1982).

Wolever et al. (1986) fed 50 g carbohydrate portions of five varieties of beans, both cooked and canned, to groups of diabetic patients, and found their glycemic indices to be lower than that of white bread ascribed a GI of 100. They concluded that the glycemic effect of dried legumes was increased by the canning process. This observation may be of significant dietary influence because canned beans are also more convenient to use then dried beans.

The effect of a 50 g starch meal prepared with pre-cooked instant bean flakes on glucose and insulin plasma levels and on glucose oxidation rate as measured by continuous indirect calorimetry was assessed in six healthy human volunteers during 4 h following the meal. Comparison was done with the same load of starch as potato flakes to which the fiber and protein content of the bean meal had been added. Thirty minutes after ingestion, plasma glucose and insulin rise was less after beans than after potatoes (0.4 vs 2.0 mol $^{-1}$ and 8 vs 38 U·ml^{-1}, respectively). The elevation of glucose oxidation was markedly less during the 2 h following the ingestion of beans when compared to potatoes. The bean flake meal gave rise to only moderate elevation of plasma glucose and insulin levels and of glucose oxidation rate when given to four non-insulin-dependent-diabetics mellitus

patients. The slow carbohydrate property of legumes was related to the histological structure of the seed rather than due to their fiber content (Tappy et. al., 1986).

Jenkins et al. (1980) conducted a study where normal volunteers took 50-g carbohydrate portions of eight types of dried legumes and 24 common foods drawn from among grains, cereals and pasta, breakfast cereals, biscuits, and tuberous vegetables. Both the mean peak rise in blood glucose concentration and mean area under the glucose curve of the subjects who ate beans were at least 45% lower than those of subjects who ate the other foods. These results suggest a potentially valuable role for dried leguminous seeds in carbohydrate exchanges for individuals with impaired carbohydrate tolerance.

Leathwood and Pollet (1988) reported the effects of slow release carbohydrates in the form of bean flakes on plasma glucose and satiety in man. After a meal containing potato, plasma glucose levels rose sharply, but fell below initial levels 2 to 3 hours later. In contrast, there was a low, sustained increase in blood glucose after consumption of bean puree. In a related study, consumption of bean puree delayed the return of hunger (increased satiety) and decreased ratings for propensity to eat a tasty snack between meals. Jenkins et al., (1982) also found slow release dietary carbohydrate to improve second meal tolerance. A breakfast of lentils produced a significant reduction (71%) in the blood glucose area and flattened the plasma insulin and gastric inhibitory polypeptide responses in comparison to whole meal bread of identical carbohydrate content. The lentil breakfast was followed by a significantly flatter blood glucose response to the standard bread lunch which followed 4 hours later. These results, together with breath hydrogen studies, indicate that the flattened response to lentils is not due to carbohydrate malabsorption.

PROCESSING AND UTILIZATION OF DRY EDIBLE BEANS AS SPECIALIZED FOODS

Conventional processing techniques for dry edible beans include simmering or "crock-pot" low temperature cooking, commercial canning, roasting and germination or sprouting. Figure 9 summarizes in a flowchart processing strategies and edible by-products of common dry beans. Dry beans are handled, stored and processed in diverse manners in Lesser Developed Countries (LDC) and in industrialized systems appropriate for developed economies.

Greater utilization of prepared dry edible beans has been advocated by governmental nutrition programs for the prevention and treatment of malnutrition in young children and nutritional maintenance of youth and adult populations in LDC's. Dry edible beans are also frequently considered for applications in world food relief programs in LDC's and regions experiencing sustained crop failure.

In many parts of the world, the primary responsibility for bean and cowpea production rests with women and children. While women's role in agriculture vary by country and region, it is not unusual for them to play a major role in seed selection, planting, cultivation, harvesting, storing, and processing and preparation. Women play multiple roles of providing for their family's sustenance, clothing, overseeing the health and nutrition of the children, and frequently supplementing family income. In African and Latin American countries where beans are staple foods, women carry on their shoulders and/or head water vessels and wood for cooking fuel, long distances, frequently in arid or mountainous terrain to their village or rural home sites. Traditional cooking of dry edible beans in these countries involve excessive expenditure of time and fuel. The development of appropriate preparation technologies for use at the household and village-level would facilitate processing and dietary availability of beans and other legumes. Valuable time

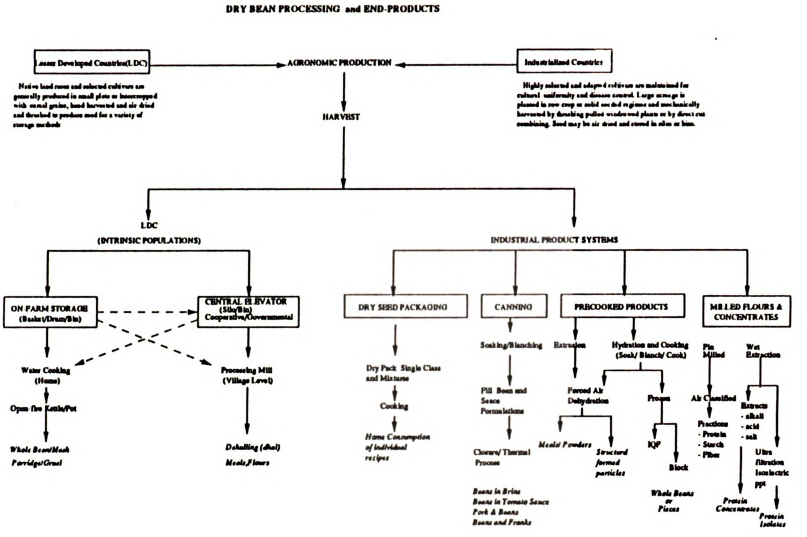


Figure 9. Outline illustrating processing strategies and edible by-products of common dry beans in LDC and industrialized countries

could thus be devoted to more effective child care or additional income-generating activities. Drum-dried bean meals possess potential for pre-cooked, prolonged shelf-life weaning food formulations which can provide both protein and energy to the infants as well as offer preparation convenience for mothers.

The demand for convenient food items has resulted in specialized pre-processed food products and ingredients from dry edible beans, possessing diversified functional properties which may be used directly, or utilized as a component of formulated foods (Uebersax et al., 1991). The technological challenge presented is to achieve these improvements within the scope of minimum process inputs and appropriate technologies. Potential increased consumption of novel bean products is dependent on the individual processing economics associated with the specific product.

Bean Sprouts, Curds and Ferments

Utilization of legumes in LDC's still entail long and tedious preparation procedures. However, traditional methods of legume preparation, such as germination and fermentation, have found wide acceptability in African and Asian countries for their potential in improving digestibility and reducing antinutritional factors. Germination and fermentation have been reported to reduce phytic acid which interferes with protein digestibility and mineral bioavailability (Rao and Belavady, 1978; Silva and Luh, 1979; Sathe and Salunkhe,1982) and oligosaccharides which have been associated with flatulence (Rackis et al., 1970; Calloway, et al., 1971; Murphy et al., 1972; Rao and Belavady, 1978 and Fleming, 1981). Enzymatic treatment of beans have also been reported to remove much of the oligosaccharides (Calloway et al., 1971).

Germination results in at least a ten-fold increase in phytase activity and a simultaneous decrease in phytate, and if done carefully, the bean seed can still be used for cooking in the customary manner (Reddy et al., 1978). Sprouting was found to increase the digestibility coefficient of red kidney beans from 29.5% in the raw to 66.4% in the

cooked kidney bean. Nielsen and Liener (1984) reported a thiol protease with an acid pH optimum to be responsible for the disappearance of the major storage protein, G1, during germination. During the process of sprouting, enzymes released from the embryo hydrolyze portions of the linear and branched starch molecules into short-chained dextrimaltose which does not swell when cooked into a gruel (Ebrahim, 1983). Sprouted grains may also be consumed fresh or when followed by seed coat removal, can be roasted or ground for use in soups or side dishes.

Soybeans are fermented to produce a variety of nutritious Oriental food products including tofu (calcium precipitated curd), natto, miso, tempeh, shoyu and tamari. Indian fermented delicacies like idly and dosa can be prepared from black gram (*Phaseolus mungo*); dhokla and khaman utilize Bengal gram (*Cicer arietinum*).

'Dhal' is prepared by cooking beans until soft, mashing, mixing with water, then boiling to yield a homogeneous gruel. They can also be cooked with vegetables, spices and condiments. Roasting and puffing of dhal and whole seeds to improve flavor and maintain maximum protein quality involve the application of dry heat (100°C to 200°C) for a short period (1-5 min). Dry beans and other legumes could be prepared by methods which include protein curd production or fermentation to improve quality and digestibility. Swanson and Raysid (1984) found that there was a significant increase in protein digestibility and PER in tempeh made from red beans (*P. Vulgaris L.*) and corn. Further research is needed to assess the potential of *Phaseolus vulgaris* in fermented foods.

Bean Meals, Flours and Powders

Beans may be consumed as whole cooked seeds where seed distinctives are readily perceived by the consumer, or as comminuted meals, pastes or curds where seed integrity is lost and the functional properties are a direct function of the seed components and their interaction with water and heat. In products utilizing beans in milled or mashed forms, the consumer may not readily discern the bean type (Uebersax and Occeña, 1991). High

protein food ingredients can be produced as either flour processed by pin milling & air classification, or as concentrates and isolates processed by alkali, salt and acid extraction with subsequent use of isoelectric precipitation or ultrafiltration (Uebersax et al., 1991). Chang and Satterlee (1979) produced bean protein concentrates containing 72% and 81% protein by wet processing using water extraction techniques.

Dry beans have been milled into whole flour and air-classified into high starch and high protein fractions (Kon,1979; Ekpenyong and Borchers, 1980; Reddy and Salunkhe,1980; Aguilera et al., 1982 a and b). High protein flour fractions generally contain twice the original whole bean seed protein content. Bean hulls are readily separated by cracking rolls and air aspiration and then milled into a high fiber fraction. Air classification of roasted beans produced high fiber, starch and protein fractions, each suitable as food ingredients in a variety of food products such as cookies, doughnuts, quick breads and leavened doughs (Defouw, et al., 1982; Dryer et al., 1982; Zabik et al., 1983; Uebersax and Zabik, 1986). Instant precooked bean powders have also been prepared by soaking, cooking, slurrying and drum or spray drying of edible beans (Bakker, 1973).

Functional Characteristics and Applications. Much research has been conducted to investigate the functionality of high protein bean products to broaden their use in various foods. Air-classified high protein bean flour contains a residual starch which offers some particularly desirable functional applications. Lee et al. (1983) reported that wheat flour substituted with 10% navy bean fractions (whole, hull and high protein and starch) resulted in increased water absorption and decreased mixing performance. Whole bean flour and high starch flours produced viscous solutions with controlled swelling at high temperature. Bean slurries remained stable during high temperature holding, and upon cooling, showed moderate increase in viscosity, indicating a tendency toward association of starch molecules. Zabik et al. (1983) demonstrated that dry roasted air-classified navy

bean protein flours substituted for 10% bread flour exhibited increased water absorption but decreased arrival and peak times of the flours processed with increased roasting temperature. Mixing stability of the flours showed a decreasing trend as the bean/bead ratio and roasting time increased. Lorimer et al. (1991) studied the effect of prime and cull navy bean high-protein flours on rheological parameters and microstructure of composite doughs. Composite flours with increased protein exhibited the expected increase in absorption, delay in arrival and peak time and reduction in stability. The microstructure of composites with cotyledon flour from culled beans showed slightly greater gluten disruption than was evident in the doughs prepared from the wheat-prime cotyledon composites.

Kohnhorst et al. (1990) reported that navy and kidney bean flours formed significantly higher volume foams but had lower foam stability compared to soy products at pH 6.5, possibly indicating compositional or structural differences between the proteins. The emulsion capacity, gel strength and foaming properties of navy and kidney bean flours were similar and were significantly higher than that of soy flours.

Navy bean flours substituted for wheat flour in baked products such as pumpkin and banana breads and fried "doughnuts holes" increased protein quality and quantity since they are relatively high in lysine. Dry roasted navy bean flour incorporated into pumpkin bread increased tenderness because of the reduced gluten formation and of the diluted nature of the gluten. Volume decreased with increasing levels of bean flour substitution because of decreased gluten formation. The pumpkin bread with bean flour was darker than the control made from wheat flour because of increased reducing sugars in the navy bean flour, promoting Maillard browning. Pumpkin bread containing 35% navy bean protein substitution appeared to be optimum and contained about 25% more protein than the control (Dryer et al., 1982). Extruded navy bean flour incorporated at either 20 or 35% level of substitution produced acceptable quick breads (Kane et al., 1991). Doughnuts with 13% bean substitution had less fat, were softer and showed less firming

after 6 days storage compared to control doughnuts and other bean substitutions (Uebersax et al., 1981 and 1982). Raised doughnut holes prepared at a 25% navy bean protein flour substitution level exhibited greater tenderness than did the wheat flour control (Zabik et al., 1983).

Volume and crumb color of bread were not adversely affected at a 10% bean protein substitution, and raised the protein level by 20% (Zabik et al., 1983). Silaula et al. (1989) reported that dry-roasted navy and pinto bean high -protein fractions blended with bread or whole wheat flour increased tenderness and reduced lightness of breads. Substitution of legume flour for bread or wheat flour dilutes the gluten and interferes with the formation of a well-defined protein-starch complex. Substitution of 5 and 10% high-protein flour for bread flour increased absorption and lengthened arrival and peak times in farinograph studies but decreased dough stability, causing a faster rate and greater magnitude of breakdown (Lorimer et al. 1991). Navy bean flour incorporated into Chinese steam bread decreased volume but increased tenderness with increased bean substitution (Uebersax and Zabik , 1983).

DeFouw et al. (1982) reported that navy bean hulls incorporated into sugar-snap cookies resulted in less acceptable interior color as the level of hull substitution increased. Texture was not significantly affected by less than 30% substitution with unroasted or moderately roasted hulls. Flavor, top grain and spread were adversely affected as the level of substitution increased. Navy bean protein substitution for wheat flour increased water holding capacity and cookie tenderness, but decreased cookie spread slightly (Zabik et al., 1983).

Breads prepared with wheat flour blend (10% pinto flour) had a slight decrease in volume, while those with bread and pinto flour blend had fewer differences in volume and tenderness (Uebersax and Zabik, 1986a and b). Noodles produced with pinto bean flour at % wheat substitution were more tender and slightly darker, with decreased flavor and acceptability as substitution level increased (Uebersax and Zabik, 1986).

Numerous studies assessing the funtional characteristics of other legume flours have been conducted. For instance, heat processing of winged bean flour have been observed to lower nitrogen solubility, emulsification and foam capacity, but increased water and fat absorption capacity (Narayana and Rao, 1982). Sathe and Salunkhe (1981) reported that Great Northern bean flour had lower oil and water absorption capacities compared to those of soybean and sunflower flours. Emulsion capacity and stability of the flour was the lowest compared to those of the protein concentrates and isolates and albumin and globulin proteins.

Drum - Drying of Legumes. Drum drying of dry beans into precooked flakes and powders could be used to produce ingredients suitable for convenience foods or replacements for cooked bean purees or refried beans. Drum-driers have high drying rates and high energy efficiencies, and are suitable for slurries in which the particles are too large or thick for spray drying. Bakker et al. (1967 and 1969) produced instant pea bean, pea and lentil powders using a modified single-drum-drier. An acceptable drum-dried infant cereal using banana (40% dw), rice (10.5% dw) and soybean (16.5% dw)was developed on a commercial scale to supply the needs of the Costa Rican Program for Food and Nutrition aimed at children under age 2. The process consisted of the following steps: 1) cooking the soybeans at 121°C for 30 minutes; 2) boiling the rice for 5 minutes to gelatinize the rice starch; 3) inactivation of the banana enzymes by boiling with the rice and soybeans for 3 minutes, and 4) drum-drying to 5% moisture (Lastreto et al., 1986).

Description of Equipment and Process System. A basic drum drier, as illustrated in Figure 10, is comprised of one or more hollow steel drums (rollers) which slowly rotate and are heated internally by pressurized steam to 120-170°C. Drum-driers use conduction heat transfer; condensation of the steam inside the drum provides energy

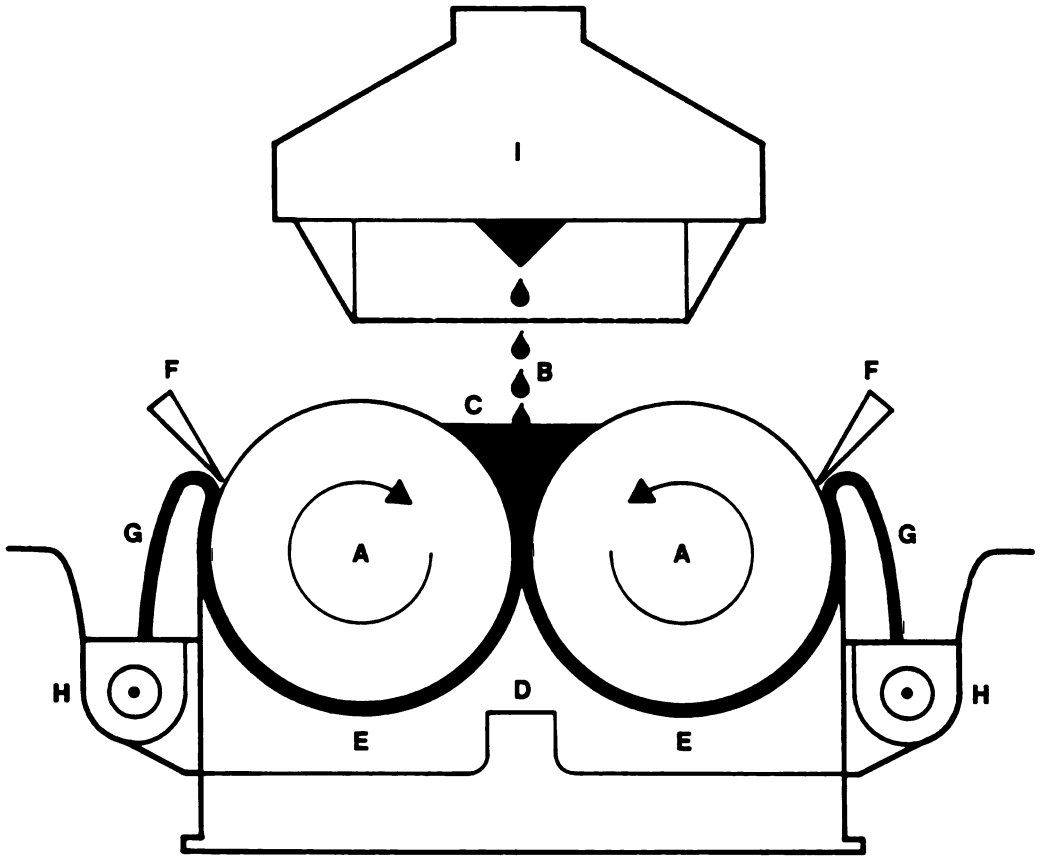


Figure 10. **Schematic Components of a Double Surface Drum-Drier: A** - Steam-Heated Drums; **B, C** - Slurry "Feed and Puddle"; **D** - "Nip"; **E** - Dehydration Surface; **F** - "Doctor" Blade (knife); **G** - Dehydrated Sheet; **H** - Conveyor; **I** - Vapor Hood.

for vaporization of water at the external drying surface (Batty and Folkman, 1983). The drums are mounted to rotate about the symmetrical axis. A feeding device is used to apply a thin, uniform layer of food material to be dried on the hot outer surface. There are a variety of methods for distributing the stock feed over the surface, including a) dipping, b) spraying and c) spreading or use of auxiliary feed rollers. Before the drum completes one revolution (within 20 secs to 30 mins.), the feed material applied on the periphery is dried as the heated drum rotates toward the 'doctor' blade (knife) which contacts the drum surface uniformly along its length, and scrapes the thin layer of dry material from the drum surface.

Drum driers are classified as single-drum, double-drum, or twin-drum systems. A single-drum drier has only one roll while a double-drum drier has two rolls which rotate toward each other at the top. The two parallel drums of a twin-drier may either rotate toward each other or away from each other at the top, and are not spaced close together (Harper, 1979). The single drum is widely used as it has greater flexibility, a larger proportion of the drum area is available for drying, easier access for maintenance and no risk of damage caused by metal objects falling between the drums (Fellows, 1988).

Control of drying process. There are four basic drum drier controls in an atmospheric double drum drier; these include : a) puddle level, b) drum speed (rpm), c) steam pressure and d) drum clearance ("nip"). The speed of the rotation is adjusted so that the desired moisture is obtained when the product is scraped off the drum (Batty and Folkman, 1983). Other control points of the drum-drying process include initial solids content, feed rate and drum-drier temperature. A typical commercial single drum dryer has the following dimensions : 1.65 m in diameter and 4 m long. The drums are run at about 8 to 9 atm. steam pressure (115-135 psig) with surface temperature of about 240^{o}C. The rotation speed of the drums is 3.5 to 4 rpm and the effective drying surface 31 m^2.

Pretreated slurry is pumped through a tangential steam heater where starch is fully gelatinized. The hot slurry enters the flash tank which serves as a surge reservoir for

uniformly supplying slurry to the drum drier. Following application and dehydration, the thin continuous sheets of dried beans falling from the drier knives is broken by a screw conveyor and the resultant flakes collected are passed through a Fitzpatrick comminuting mill to provide uniform particle size.

Developments in drum design to improve the sensory and nutritional qualities of dried food include the use of auxiliary rolls to remove and reapply food during drying, the use of high-velocity air to increase the drying rate or the use of chilled air to cool down the product. Drums may be enclosed in a vacuum system to dry food at lower temperatures which is especially suitable for high-value heat-sensitive food products (Fellows, 1988).

Thermal Extrusion

High starch navy, pinto and black bean flour fractions yield good expansion properties during high pressure and high temperature extrusion (Zabik et al., 1983b; Aguilera et al., 1984). When the cooking and extrusion processes are combined, the increased pressure differential will facilitate expansion and a final dry formed 'bean' particle which readily hydrates and softens when reconstituted is achieved. Die configuration can be designed to produce a variety of intricate product shapes which can be further optimized to produce simulated beans. Appropriate binders and coating agents can be added to improve appearance of pre-cooked or quick cooking bean products resulting in promising prototypes of extrusion cooked - formed 'beans' (Uebersax et al., 1991).

Legume flours can also find application in extruded snack items, breakfast cereals, pasta products and infant foods. Extrusion of millet flour to prepare instant infant foods yielded a product that was much less dispersible (<20% for millet flour at 85°C compared to >70% for corn starch at 35°C). Increased lipid and fiber contents of millet flour probably contributed to these observations. However, a similar decrease in soluble starch was observed in both materials when the extrusion moisture content decreased from 17% to 12%. More amylopectin was dispersed from the millet extrudate when the extrusion

moisture content was 13.6% and 15.0% (Waniska, R.D. and Gomez, M.H., 1992). The formation of amylose-lipid complexes reduces the digestibility and water solubility of cooked starches (Galloway et al., 1989). Amylose in starch will complex with lipids during extrusion cooking. As starch in the presence of lipids is exposed to increasing amounts of heat and pressure, the amount of amylose-lipid complexing increases (Huber, 1991). Extruded navy bean and lentil flours substituted (20 and 35%) for all-purpose flour in pumpkin bread demonstrated the feasibility of extruded starchy legumes as food ingredients (Hart et al., 1991).

Giese (1992) provided a review of new types of pasta developed from unconventional raw materials such as legumes. High temperature extrusion cooking allows added proteins such as egg albumen to form a coagulated network to improve pasta quality. Thin-walled macaroni formulations, incorporating durum wheat semolina with 15% and 25% drum-dried navy bean flour or 15% whole raw navy bean meal, have been successfully developed at Michigan State University (MSU) using a high temperature twin-screw extrusion cooker (Lin et. al., 1993).

Borejszo and Khan (1992) reported lower levels (3.69%) of flatulence causing sugars (raffinose and stachyose) in extruded pinto bean high starch fractions compared to non-extruded samples (5.29%) with higher reduction occurring at higher process temperature (163°C). According to Chauchan et al. (1988), protein efficiency ratio values of extruded rice-legume blends (rice-soybean, 2.25; rice-bengal gram, 2.30 and rice-black gram, 2.28) were improved compared to the respective raw blends (2.10, 1.89 and 1.98 respectively). They also demonstrated that extrusion processing reduced the phytates in the products to the extent of 20.3% to 26.8%. Ummadi et al. (1993) determined the effect of low and high impact extrusion processing on iron dialyzability, forms of iron, tannin content and degradation of phytic acid in navy beans, chickpeas, cowpeas and lentils. Dialyzable iron increased following low impact extrusion in all the legume products, but only in navy beans with high impact extrusion. Low impact extrusion

resulted in increased total soluble and ionic iron. However, high impact extrusion did not result in any change in total soluble iron but increased ionic iron in cowpeas and lentils. Degraded phytate products increased to 51-71% in extruded legumes. Tannin content decreased with low and high impact extrusion.

Microwave Heating of Legumes

Microwave heating has a direct potential for use as a preconditioning treatment for common dry beans to reduce processing time, and to inactivate antinutritional factors (Uebersax et al., 1991).

Mechanism of Microwave Heating. Microwaves are generated by a magnetron (a device that converts electrical energy at low frequencies) into an electromagnetic field with centers of positive and negative charges that change direction at a frequency of 10^9/sec. As microwaves enter the product, they interact with regions of positive and negative charges on water molecules, (electrical dipoles) that rotate the molecules in the electrical field by forces of attraction and repulsion between oppositely charged regions of the field and the dipoles. This results in the disruption of hydrogen bonds between adjacent water molecules and generates heat by "molecular friction" (Decareau, 1988; Mudgett, 1989; Giese, 1992). The molecules in the microwave paths oscillate around their axes in response to reversal of the electric field that occurs 915 or 2450 million times per second, creating intermolecular friction that results in the generation of heat (Cross and Fung, 1989). Food cooked by microwave involves heat generated from within the food through a series of molecular vibrations. In contrast, conventional heating methods transfer thermal energy from product surfaces toward their center 10 - 20 times slower than microwave heating (Mudgett, 1989). In developing products for microwave processing, it is important to recognize that microwaves are a form of energy, not a form of heat, and are only manifested as heat upon interaction with an aqueous material as a result of one or more energy-transfer mechanisms.

Microwave Effects on Food Composition

Moisture. Greater losses in moisture in microwave-cooked compared to conventionally heated products could be due to a greater rise in post-oven temperature, causing more dehydration through evaporation and increased shrinking. Very few studies provided data on water content of foods prior to and following microwave cooking (Schiffmann, 1988).

Protein. Hafez et al. (1985) conducted experiments to determine *in vivo* protein digestibility and metabolizable nitrogen using male Sprague-Dewley rats. They observed that proper microwave treatment increased the digestibility of soybean. Optimum microwave heating time was around 9-12 minutes for 1 kg whole soybeans to improve weight gain, digestibility and intestinal proteolytic activity under experimental conditions maintained during the study. They also did not observe alteration of fatty acid composition of soy oil.

A study by Yoshida and Kajimoto (1988) showed that microwave heating might be effective in the inactivation of the trypsin inhibitor in whole soybeans. Microwave treatment of whole soybeans for 8-10 minutes after soaking to approximately 25% moisture would be optimal to prepare soyflour or soy grits without a burnt odor, and completely inhibiting trypsin activity. The loss of molecular species of soy triglycerides containing more than four double bonds in a triglyceride was less than that obtained from native soybeans prior to soaking (8.6% moisture). In an earlier study, Yoshida and Kajimoto (1986), found that microwave heating for about 5 minutes did not cause a loss of unsaturated fatty acids, nor did it change molecular species of soybean triacylglycerols.

Susceptibility to heat processing has been observed with protein containing significant amounts of carbohydrates. Interactions between functional groups with the protein chain or between the protein chain and other food constituents during heating lead to cross-linkages which are resistant to normal digestive processes.

Overall, nutritional effects of microwaves on animal proteins appear minor, however, many studies have demonstrated that microwave heating may aid retention of total protein in foods (Decareau, 1986). For example, microwave treatment of potatoes favored protein retention over other preparation methods (boiling or oven-baked) investigated (Mudgett, 1982).

Carbohydrates. Overall, much work needs to be done on the effects of microwave heating on the carbohydrate fraction of foods. Aref et al. (1972) observed the inactivation of alpha-amylase in wheat flour after 60-second exposures to microwave energy without pronounced deleterious effects on the principal characteristics of the flour or the subsequently prepared doughs . The exposure also drastically reduced the number of viable organisms in the flour, but seemed to cause a relatively high loss in moisture.

Microwave baking is usually done at 896 to 2450 MHz, the range also used in normal household ovens. The lower frequency is to be recommended in some cases, as this enables both higher penetration depth to be obtained and thus avoids the risk of the core not being sufficiently baked.

The main problem of baking with microwaves is the lack of both crust formation and surface browning. This may be achieved by short-time conventional re-baking for about 4-5 min at a temperature of 200-300°C.

Flour with a high alpha-amylase concentration and low protein content have been used for dough preparation. In microwave baking, the entire loaf is heated rapidly, and the development of CO_2 and steam in the dough is accelerated, thus resulting in breads with a relatively high specific volume despite the low protein content. The temperature range essential for alpha-amylase is reached rapidly, so that the amount of undesired decomposition products remain low.

Microwaves are currently used for commercial leavening and proofing, thus enabling the process of gas development and adhesive formation to be completed within a

period of only 2-4 min. Microwave treatment might require changes in the dough recipe, leading to new final products. A lack of crust formation and differences in the color were observed in whole-grain baked products, and the microwave breads were tough and rubber-like (Schiffmann, 1988). The taste was less significant than that of conventionally prepared bread because the microwave products lacked both caramelization and development of aroma components. Loaf volume and crumb condition were not of the quality obtained in conventional bread production. The following are advantages of microwave baking: 1) provides a final product with a higher nutritive value than conventional baking, provided the same ingredients are used; 2) baking times are reduced to a few minutes - the entire baking process of a 1 -kg loaf only takes 7-8 minutes and 3) microwave equipment requires less space.

Microwavable pasta and pasta made from a primary material other than wheat have been developed. Strategies for improving pasta performance during microwave heating include modifying the wall thickness and adding selected ingredients such as egg albumen to assist shape retention. The increased protein content allows for the formation of polypeptide chains which interact to form a fibrillar network which subsequently entraps starch granules and slows gelatinization (Giese, 1992). Cabello et al. (1992) reported a highly acceptable precooked and dehydrated pasta suitable for microwave heating using 15% substituted navy bean cotyledon flour. The navy bean flour pasta had a richer yellow color which was favored over 100% semolina pasta. Microwave cooked legume pasta made from different levels of extruded drum-dried bean flour demonstrated higher cooked weight and was less firm than 100% semolina control pasta. Microwave preparation (740 watts, 5 mins.) also resulted in lower cooking loss than conventional cooking (100°C, 2 mins.) (unpublished data).

Lipid Oxidation and Total Fats. No general statement can be made from reported literature studies regarding total fat in microwave versus conventionally prepared food products due to the differing procedures, conditions, and products used (Decareau, 1982).

Industrial investigations of the function of fats, oils and emulsifiers in microwave food formulations reported fats/oils and emulsifiers to function optimally, and not to be chemically altered by microwaves (Durkee Foods Corporation, 1989). Emulsifiers interface with water, within the food matrix, due to the polar hydroxyl end of the emulsifier. This interaction may maintain moisture within microwave prepared foods. They also interact with proteins, starches, and gases such as air, further stabilizing and improving texture (Connerton and Shuleva, 1989). Fats/oils can function thermodynamically as a "heat source" to absorb energy, generate heat, and release heat to adjacent non-fat molecules of lower polarity. Lipids remain totally unchanged by the microwave energy, thereby aiding moisture retention within a product.

Minerals. Investigation of some vegetables cooked by oven heat and in a microwave oven revealed negligible loss of water and minerals regardless of cooking method, although microwave heating was suggested to be superior to conventional heating since less nutrients were lost to the aqueous cooking medium. More recent studies showed that nutrient retention in fruits and vegetables was greater with microwave heating than with conventional heating due to less water-to-product ratio and consequent reduced leaching in foods cooked with microwave energy, therefore the microwave heating increased retention of nutrients. Specific studies like those of four potato varieties prepared by various home cooking methods and microwave cooking suggested that the latter enhances macro-nutrient retention.

Mineral determinations for meat drippings showed significant differences of selected minerals (Na, Cl, P, Fe) from oven-roasted pork, beef and lamb drippings. It was suggested that the greater concentration of minerals in the drippings from

conventionally cooked roasts could be related to the lower moisture content of the drippings. Therefore comparisons of absolute mineral losses remain unclear.

Vitamins. Studies have indicated that destruction of thiamine in food systems is primarily related to heat level rather than exposure to microwaves. Studies with pork, beef and lamb suggested that vitamin retention was not related solely to length of exposure to high temperatures. The contribution of vegetables to the riboflavin and niacin content in the diet is minimal, so few studies have investigated microwave effects on these components.

The review of many selected studies indicated that no appreciable losses in ascorbic acid were attributed to microwave heating compared to conventional heating methods. Any increased retention of ascorbic acid in microwave-cooked foods cannot be attributed entirely to the dielectric heating. A combination of factors including ratio of water-to-vegetable, cooking length, and variability of cooking loads may account for the variations in reported data (Harrison, 1980).

Except for significant losses of Vitamin A in microwave-cooked meat, limited data on fat-soluble vitamins are available.

Potential for Dry Edible Beans as Weaning Foods

The Codex Alimentarius Commission (Food and Agriculture Organization/World Health Organization, 1976) prepared the official guidelines "Recommendations for International Standards of Infant Foods." Dried products included in this section are based on cereals (wheat, rice, barley, oatmeal, rye, millet, corn, sorghum) and legumes (ex. soybeans) of low water content processed under conditions that allow their reconstitution with milk or water. If the product is to be mixed with water, it is required that the protein content be at least 15% (db) and protein quality at least 70% of casein value. The addition of protein concentrates, isolates and essential amino acids is allowed only in concentrations

needed to meet these criteria. Supplemental sugar and fruit addition is also allowed under these guidelines.

The Thirty-fourth World Health Assembly of the World Health Organization (1981) adopted the "International Code of Marketing of Breast-milk substitutes" in the form of a recommendation which aims to protect and promote breast-feeding and ensure the proper use of breast-milk substitutes when these are necessary to ensure adequate nutrition. The Code defines "Infant formula" as a breast-milk substitute formulated industrially in accordance with applicable Codex Alimentarius standards, to satisfy the normal nutritional requirements of infants between four and six months of age, and adapted to their physiological characteristics. Infant formula may also be prepared at home, in which case it is described as "home-prepared" formula. "Weaning food" or "complementary food" are defined as any food, whether manufactured or locally prepared, suitable as a complement to breast-milk or to infant formula, when either becomes insufficient to satisfy the nutritional requirements of the infant. Such food is also commonly called as "breast-milk supplement" (WHO, 1981). WHO and United Nations International Children and Educational Fund (UNICEF) promote and support appropriate and timely complementary (weaning) feeding practices, usually when the infant reaches four to six months of age, and further encourage the use of locally available food resources.

In LDC's, research efforts on weaning food formulations have focused on the utilization of indigenous or locally-available protein-rich foods such as legumes and oil seeds. Digestibility and flatulence-producing components are important factors to consider when feeding legumes to children. Processing techniques including dehulling, fine grinding, roasting or any form of prolonged cooking and sometimes germination and fermentation, have been found to reduce flatus from beans.

A challenge limiting starch-based gruels such as rice and corn as primary supplement stocks are their characteristically high ingestion volumes required to achieve

adequate nutrient delivery (low nutrient density) and the high viscosity which might cause choking in young children. The ability of starch to bind more water leads to dilution which increases bulk which subsequently limits the amount of nutrients that could be derived per serving of gruel. Industrial methods which have been reported to modify starch structure to lower its water-binding capacity have included enzyme pre-treatment (amylase), precooking or extrusion and drum-drying, and germination or sprouting (Lastreto et al., 1982 and Marero et al., 1988).

Enzymes released from the embryo of sprouted grains hydrolyze portions of the starch molecules into short-chained dextri-maltose which does not swell when cooked into a gruel (Ebrahim, 1983) and therefore possess great potential in the preparation of flour from sprouted grains for use in weaning foods. Flour from sprouted grains can be used in greater amounts to give the same viscosity as flour from unsprouted grain, thereby improving nutrient and energy density (Mosha and Svanberg, 1983; Desikachar, 1981 and Brandtzaeg et al., 1981). Weaning foods suitable for use in the Philippines were developed by Marero et al.(1988) and prepared from flours obtained from germinated rice-mungbean, germinated rice-cowpea, germinated corn-mungbean and germinated corn-cowpea. The low viscosity and high caloric density formulations were found stable for 6 months, microbially safe and well-tolerated by infants. The blends had amino acid values which approximated the FAO reference pattern, except for the S-containing amino acids. The rice-based formulations were demonstrated to be superior to the corn-based gruels based on chemical score for cystine/methionine, net dietary protein energy and PER. The use of germinated legumes was also reported to increase the micronutrient contents of the weaning food formulations (with the exception of calcium), compared to control formulations without the incorporation of germinated legumes. Kusin et al. (1984) reported that in rural Indonesia, growth faltering from the third month and the high percentage of wasting at age 0-3 years in pre-school children were mainly due to the

inadequacy of weaning foods and the low intakes of vitamins and minerals resulting from low consumption of pulses and leafy vegetables.

In South and Central America and parts of central and eastern Africa, dry beans are commonly served without dehulling, a form unsuitable for children. Thus, young children are merely given the broth or soup in which the beans have been cooked, resulting in poor protein digestibility, diarrhea, and other gastrointestinal distresses. Protein digestibility of the soup was lowered by the tannins present in the seed-coats of colored beans and are partially leached into the cooking medium. The hydroxyl groups of the phenol ring enable the tannins to form crosslinks with proteins which may be implicated with decreased digestibility (Ma and Bliss, 1978 and Haslam 1979; Elias et al., 1979; Aykroyd and Doughty, 1982; Bressani and Elias, 1980; Bressani et al., 1983 and 1988). The Institute of Nutrition of Central America and Panama (INCAP) introduced in the late 1950's "Incaparina", a mixture of predominantly vegetable protein origin having a nutritional value similar to that of milk and suitable for the mixed feeding of infants and young children. The first one tested clinically was "Mixture 8" made with entirely indigenous materials having the following formulation : lime-treated corn flour flour (50%), sesame meal (35%), cottonseed meal (9%), torula yeast (3%) and powdered kikuyu leaf (3%), and was supplemented with vitamin A. "Mixture 9" was the first mixture recommmended for commercial production , and was made of lime-treated ($CaCO_3$; 1%) corn flour (29%), sorghum (29%), cottonseed flour (38%), torula yeast (3%), and supplemented with 4,500 I.U. of vitamin A per 100 grams of the dry product. Alternate formulas were subsequently developed with soy, peanut and sesame as the alternate vegetable protein sources and with rice as the cereal component (Scrimshaw, 1980). These modifications were recommended due to agricultural availability and least cost formulation considerations. Other recommendations to improve the product included the addition of amylase to make a thinner gruel, and marketing it as a precooked product. However, these were not endorsed protocols due to inherent additional costs, departure from the traditional practice of

preparing thick corn gruels or the potential encouragement of feeding by bottle which would have introduced problems of contamination and displacement of breast milk. Other efforts which have have been made to develop low-cost nutritive infant foods included : 1) Del Valle et al.'s (1981) "Soyaven," an infant formula already in commercial production; 2) "Nutrisoy", a blend of corn and soybeans (Easterbrook, Gregg, 1986; malted and roller dried sorghum and cowpea weaning food formulations (Malleshi et al., 1989); 3) sesame protein-based weaning food (Moharram et al., 1989); 4) ultrafiltered chickpea protein concentrate in infant formula (Ulloa et al., 1988; Reddy et al., 1990).

Traditional germinated legume and cereal seeds have also been used in mixtures for feeding young children in India. An Incaparina-type of precooked weaning food, named *Bal-Ahar* ("nutritious child"), played an important role in government nutrition programs in India (Scrimshaw, 1980 and Reddy et al., 1990). It was provided to the consumer without cost and largely displaced imported weaning foods. Traditional processing techniques which have been utilized include roasting, malting (germination), puffing and fermentation (Reddy et al., 1990). Legumes which have been successfully utilized in commercial preparations include soybeans, green and black gram, chickpeas, pigeon peas, cowpeas and groundnuts. Multimixes consisting of local staples (ex. cassava and plantain), legumes and vegetables (ex. dark-leafy greens), or sometimes even meat, raised not only the protein but also the vitamins, minerals and energy content as well. The addition of fat or legume oil is recommended when the legume or pulse component is intrinsically low in fat. Gupta and Sehgal (1991) reported lower levels of phytic acid, polyphenols and saponins and higher *in-vitro* digestibility of low-cost weaning mixtures prepared by mixing malted pearl millet, roasted amaranth, roasted green gram, jaggery (a low-grade molasses residual fraction) and malted barley. Olaofe (1988) compared the mineral contents of imported baby foods with those of selected Nigerian grains (maize, sorgum, cowpea and soya beans) suitable for baby foods and found the iron contents to be in the same range but calcium to be much lower in the Nigerian grains. Corn-flour

infant feeds consumed in Nigeria were reported to have considerably lower amounts of mineral elements than popular brands of infant formula (Fatoki, 1990). Moharram et al. (1989) also reported that weaning foods based on sesame protein in combination with dry skim milk and either corn or rice starch were successfully prepared and found to be feasible for production in developing countries .

Potential for Soybeans as a High-Protein Weaning Beverage

The soybean and suitable processing methodology have been extensively studied for use in weaning foods. This review is presented to provide a basis for use of these technologies in preparation of dry bean (*Phaseolus vulgaris*) based products.

Soybeans as Protein Source. Soybeans (*Glycine max (L.) Merr.*) are the most widely grown of the grain legumes. It is essentially a temperate crop but can be grown up to an elevation of 5000 meters depending upon the location. Production of soybeans is dominant in both developed countries and lesser developed countries (Figure 11). Dry beans, peas and peanuts are of similar total production scale, with noted differentiation among predominant sectors (Uebersax and Occeña, 1991). Soybeans serve as the major source of protein to supplement a variety of grains in the diet in LDC's and are utilized to help alleviate malnutrition, especially in the Asian region. The approximate composition of soybeans is as follows: protein, 40%; lipid, 20%; cellulose and hemicellulose, 17%; sugars, 7%; crude fiber, 5% and ash, 6% (Erickson et al., 1980). Isolated soy proteins are now used commercially in many food applications such as infant formulas, emulsified meats, imitation cheese, bakery products, high-protein diet products and formulated soymilk (Haytowitz and Matthews, 1989).

Production Sequence for Commercial Soymilk Production. The preparation of soymilk requires five basic steps: cleaning the soybeans soaking, grinding, filtering the soy residue and cooking the soymilk. Commercial production of soymilk include

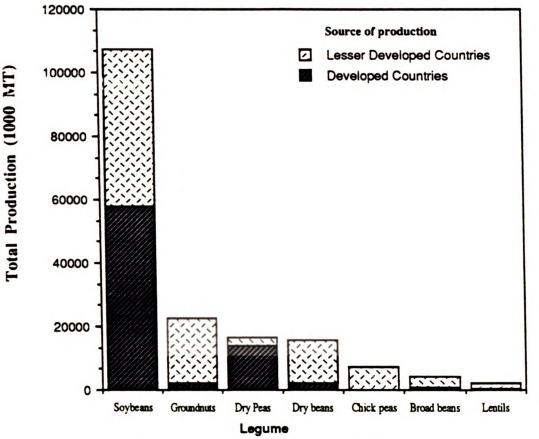


Figure 11. World production of major food legumes (Source: Data adapted from FAO, 1989).

additional steps of dehulling, formulating, homogenization, sterilization and packaging. The flowsheet for commercial soymilk production using the Illinois Method is outlined in Figure 12. The advantages of this method include: very high yield (89% solids, 95% protein); removal of oligosaccharides and beany flavor. However, the use of an expensive, powerful homogenizer to produce a smooth, stable emulsion is a major disadvantage.

Cleaning reduces or eliminates damaged soybeans in which activated lipoxygenase has catalyzed oxidation of the unsaturated fatty acids producing compounds with the characteristic beany flavor. Good quality soymilk can be made without dehulling of the soybeans, but dehulling offers the following advantages: improved protein recovery, improved soymilk flavor and slightly whiter color; improved digestibility; reduced oligosaccharides, soaking time, grinding energy and bacterial count.

Soybeans are generally soaked in about three times their weight of water. Cold soaking requires a longer time (8 to 10 hours at 20^{o}C or 14 to 20 hours at 10^{o}C) for hydration of soybeans compared to hot soaking. Longer soaking periods result in greater loss of soluble components, particularly the water-soluble carbohydrates. Soaking reduces energy input required for grinding, enables better dispersion and suspension of the solids during extraction and increases yield and decreases cooking time. Soaking in alkaline solution containing sodium bicarbonate or sodium citrate are reported to decrease the beany soymilk flavor, tenderize the soybeans leading to reduced cooking time and enhanced homogenization, reduce oligosaccharides and accelerate the inactivation of the soybean trypsin inhibitor.

Blanching or steaming in a solution of sodium bicarbonate initiates inactivation of the lipoxygenase causing the beany flavor, washes out water-soluble oligosaccharides and inactivates trypsin inhibitors. Hot water grinding to achieve a 150 mesh colloidal suspension further inactivates the lipoxygenase. Filtering the insoluble soy residues from the slurry is done using a decanter centrifuge and results in improved flavor, mouthfeel and facilitates removal of oligosaccharides.

SOYMILK PROCESSING PROCEDURE

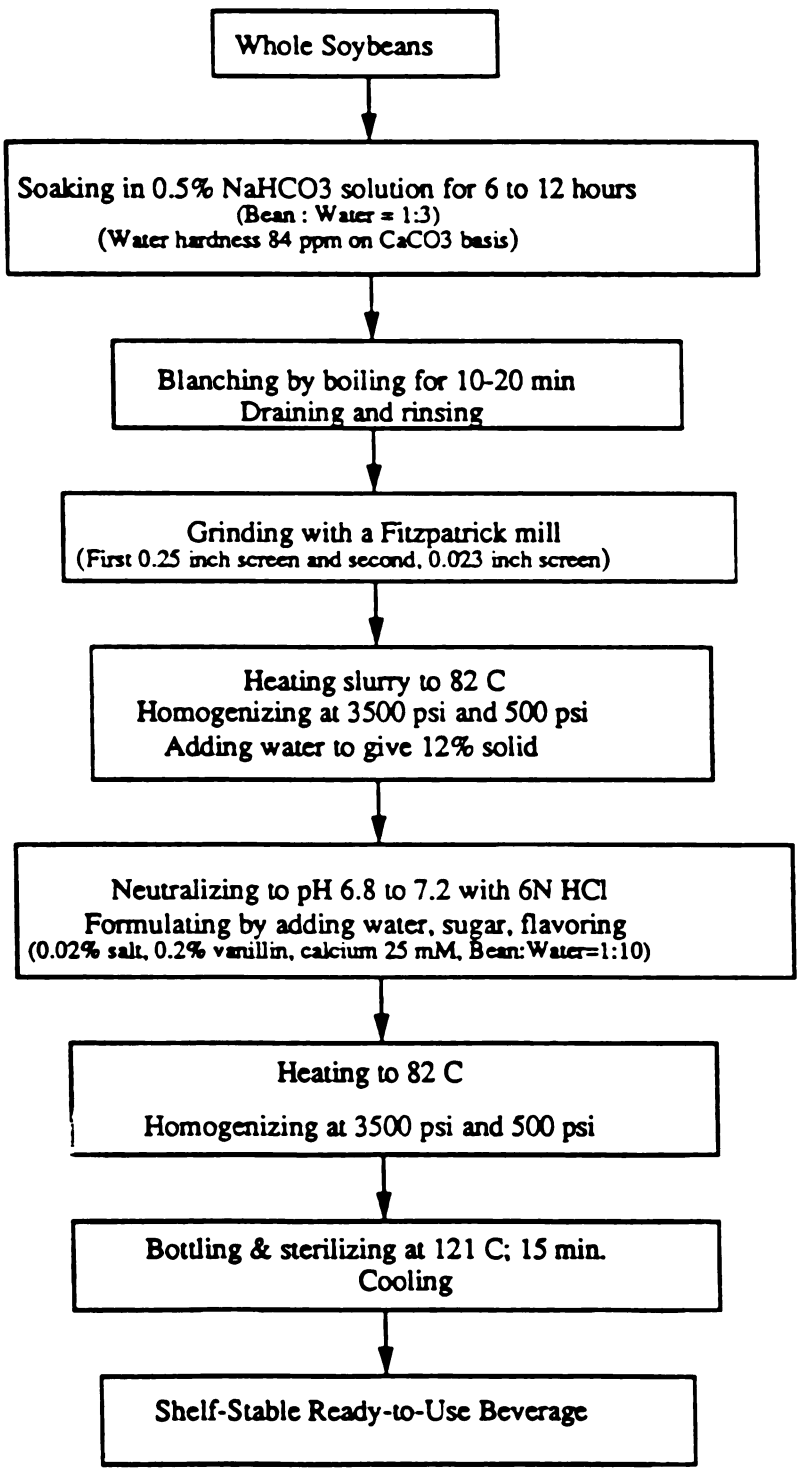


Figure 12. Illinois Method for processing soymilk from whole soybeans (Nelson et al., 1975)

Cooking or heating the soymilk destroys the spoilage microorganisms, inactivates trypsin inhibitors and also reduces viscosity to facilitate extraction and give higher yields of protein and solids. Heating soymilk to 100°C for 14 to 30 minutes or at 110°C for 8 to 22 minutes was reported by Van Buren et al. (1964) to inactivate 80 to 90% of the native trypsin inhibitors.

Appropriate formulation of soymilk using sweetening and flavoring agents increases the acceptability of soymilk. Flavored soymilks already currently commercially produced in Asian nations include vanilla, milk, egg, strawberry, apple, peanut, chocolate, coffee, almond, orange (Taiwan), malt (Hong Kong), pandan (Singapore and Malaysia), and fruit, vegetable, beef, yakult, lactic acid, honey and sesame (Japan). The additon of soy oil, lecithin or an emulsifier improves the mouthfeel of soymilk.

Technological Challenges Associated with Soymilk Production. There are three major problems to be overcome to produce good quality soymilk : 1) elimination of the beany/bitter flavor, 2) inactivation of trypsin inhibitors and 3) removal of flatulence-causing oligosaccharides.

Wilkens et al.(1967) reported that lipoxygenase (or lipoxydase) present in the soybean is responsible for the undesirable flavors and odors upon grinding soybeans and is the cause of the beany flavor in soymilk. Soybean lipoxygenase catalyzes the oxidation of cis,cis 1,4 pentadiene containing fatty acids to form 1,3, cis, trans hydroperoxides. These hydroperoxides decompose to form greenish beany, painty flavors. Several methods have been developed to eliminate off-flavors in soymilk. Lipoxygenase is often inactivated by hot water blanching of soybeans in boiling water prior to cracking or hot water grinding with boiling water to produce a slurry having a temperature of 80°C or above and holding at this temperature for 10 minutes. Removal of the soy oil to prevent it from reacting with the lipoxygenase has also been suggested to yield a bland soymilk. Steinkraus (1973) patented a method for producing defatted soy flour which involves

extracting full-fat soy flour with 95% ethanol, followed by a mixture of equal volume of 95% ethanol and n-hexane. This procedure extracts remaining phospholipids which tend to form off flavors. The beany flavor in soymilk was reported to be reduced by fermentation with *Lactobacillus acidophilus* (Wang et al., 1974) or *Aspergillus oryzae* and *Rhizopus oligosporus* (Ebine, 1976). Nelson et al.(1976) reported that soaking and/or blanching soybeans in 0.5% alkaline solution ($NaHCO_3$) improves soymilk flavor, removes oligosaccharides and decreases cooking time. Badenhop (1969) reported that alkaline soaking reduces a beany flavor compound, 1-octen-3-ol. Acidic grinding (below pH 3) will inactivate lipoxygenase, but is not utilized commercially. Flavor of soymilk has also been improved by slurrying soy flour in hot water or in-line dispersement of soyflour which minimizes water contacting time followed by rapid hydrothermal cooking (154°C for 30-40 sec) (Johnson et al., 1981), or by ultra-high temperature (UHT) sterilization of soymilk by steam injection at 140°C for 4 sec.

Two of the five or more trypsin inhibitors have been purified and studied : the Kunitz (1.4%) and the Bowman Birk (0.4%) inhibitors. Van Buren et al. (1964) indicated that 90% of trypsin inhibitors in soymilk could be inactivated at 100°C for 14 to 30 minutes or at 110°C for 8 to 22 minutes.

Soybeans contain the oligosaccharides raffinose (1.1%) and stachyose (3.8%). Oligosaccharides can be removed by alkaline soaking of soybeans (Nelson et al., 1976), heat treatment and removal of soy fiber residue. Sugimoto and Van Buren (1970) also used an enzyme system of alpha galactosidase and invertase to remove oligosaccharides. These enzymes are a commercial product extracted from *Aspergillus saitoi* free from protease activity. The soymilk-enzyme mixture was incubated for 3 hours at 55°C, then boiled for 10 minutes to stop the enzyme reaction. The enzyme treated soymilk contained no oligosaccharides because they had been hydrolyzed by enzymes to their constituent monosaccharides.

MATERIALS AND METHODS

Sample Preparation

Commercially produced whole and split dry navy beans grown in the 1991 crop year were obtained from the Cooperative Elevator Company , Pigeon, MI. The beans were field-dried, harvested, sorted, packaged and transported to Michigan State University where they were stored in a walk-in cooler maintained at 4°C (39°F) prior to use.

Extractive Pretreatments

Hot Water/Steam Extractions of Whole Navy Beans

Whole dry navy beans were soaked for 16 hours at 25°C (77°F) in stainless steel tanks and kettles. The hydrated beans were subjected to either hot water (60°C/60 mins) (140°F/60 mins) or steam extraction (100°C/15 mins) (212°F/15 mins) procedures as outlined in Figure 13, respectively. "CONTROL" meal contained cooked beans and all original soak and rinse waters, cook water or condensate. Leachate of "EXTRACT" beans was discarded and replaced with fresh formulation water (Figure 14). Cooking involved holding the beans at the designated temperatures and times in covered stainless steel tanks and kettles (Groen Model N60 Sp, Illinois) equipped with either a vortex mixer or rotating paddles to provide gentle agitation which would facilitate extraction and avoid thermal gradients. Extraction procedures were as follows:

a) Aqueous extraction. Hydrated beans were submerged in water at the ratio of a 3:1 water to bean. The mixture was continuously agitated and held at 60°C (140°F) for 30 minutes for split beans, and for 60 minutes for the whole beans. The cooking water was drained and mixed with the soak and rinse waters. The accumulated formulation water was added to the "CONTROL" beans prior to milling in a 1:1 water to bean ratio

HOT WATER EXTRACTION OF DRY NAVY BEANS

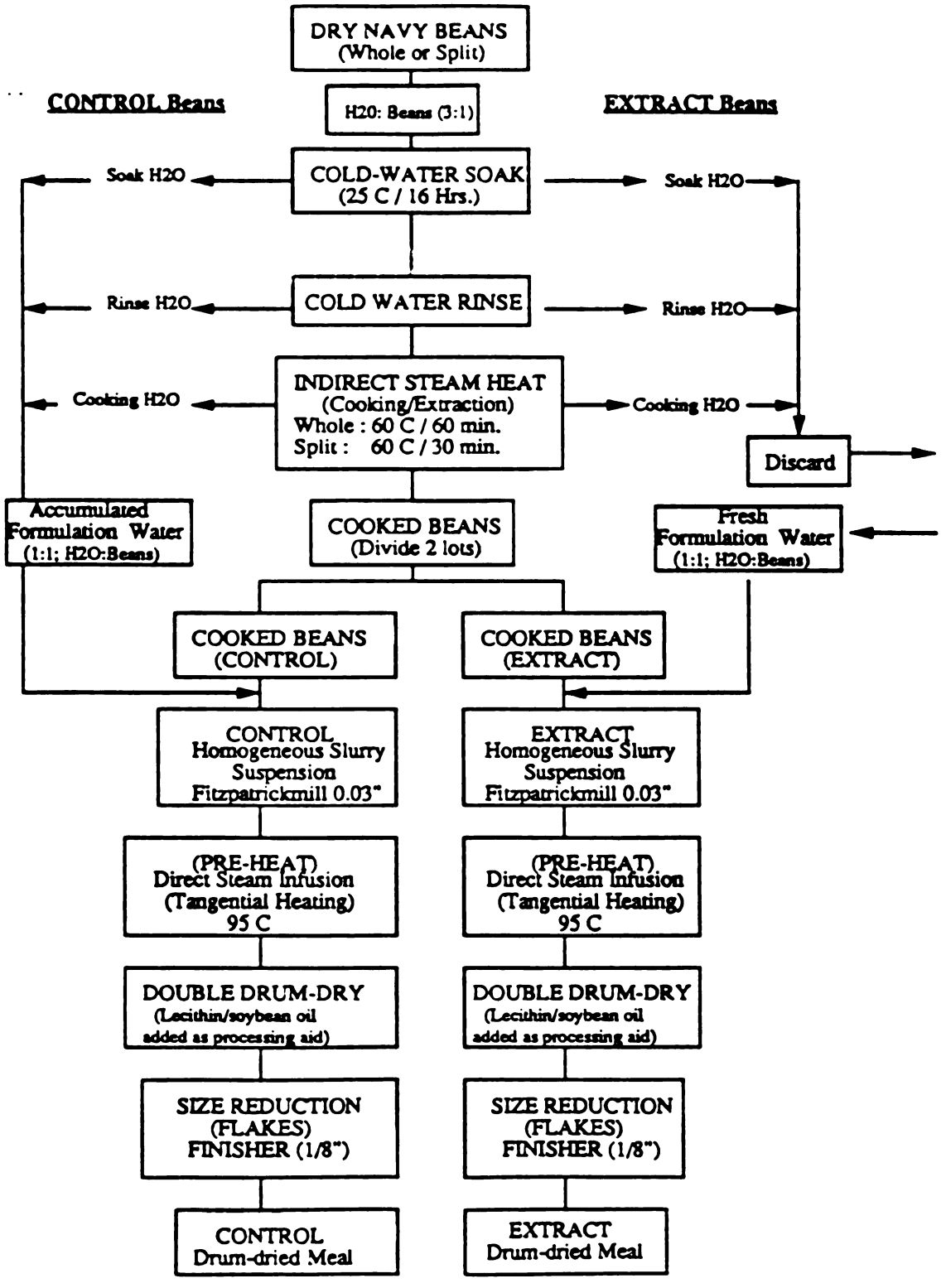


Figure 13. Flowchart for hot water extraction of dry navy beans

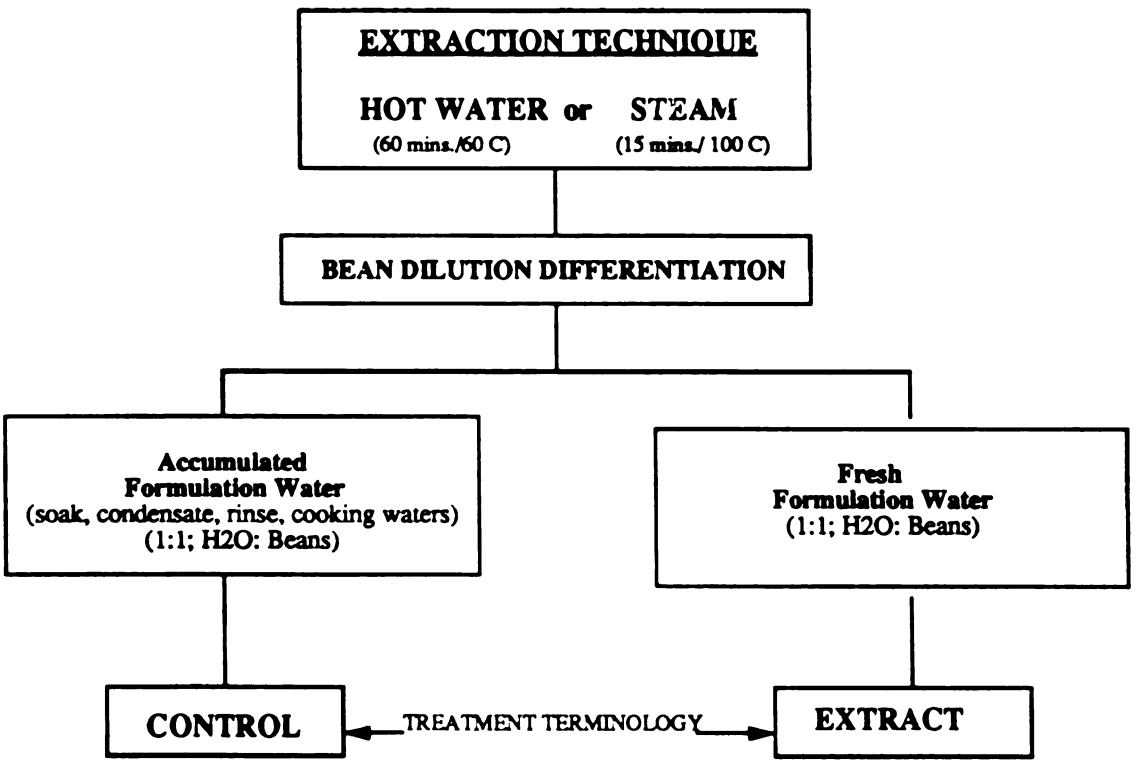


Figure 14. Designation of treatment terminology and extraction treatment differentiation used during cooked bean preparation.

while an equivalent amount of fresh formulation water was added to the "EXTRACT" beans.

b) Steam extraction. Hydrated beans were held in a stainless steel mesh basket and placed into a covered steam kettle. Live steam was injected into the base of the covered kettle and continuously refluxed through the beans during the 60 minute extraction period. Condensate accumulated at the base of the kettle below the bean surface and was drained and added to the accumulated formulation water. After heating, the beans and formulation water (1:1) were milled to a homogeneous slurry with a Fitzpatrickmill (Model D Comminuting Machine, Fitzpatrick Co., Chicago, IL) using a 0.04 inch mesh screen.

Enzymatic Pre-digestion of Whole Navy Beans

Whole dry navy beans soaked for 16 hours at 25°C (77°F) were milled to a slurry and subjected to enzymatic pretreatment. Enzymatic pre-digestion involved the use of both commercial and indigenous enzyme preparations. Commercial enzyme preparations with selective substrate specificity and activity were obtained from Novo Nordisk Bioindustrials Inc. (Danbury, CT).

I. Commercial enzyme preparations were as follows:

a) Viscozyme120L (a multienzyme complex of the carbohydrases cellulase, *B*-glucanase, hemicellulase, arabanase and xylanase; activity of 120 FBG/ml);

b) Celluclast 1.5L (cellulase from fungus *Trichoderma reesei* ; activity of 1500 NCU/g)

c) Neutrase (metallo-bacterial protease produced by a selected strain of neutral *Bacillus subtilis*; activity of 0.5 AU/g) and

d) Alcalase Food Grade (an endoproteinase prepared from *Bacillus licheniformis*; activity of 2.5 AU/g).

II. Indigenous preparation was obtained as follows :

Seed germination was conducted in the seed research facilities of the Michigan Department of Agriculture, East Lansing, MI, using the the standard procedure for dry beans of the Association of Official Seed Analysts (1991). Hundred dry seed were uniformly positioned on 2 cm centers using a manual seed depositor on clean, saturated fiber padding. The whole navy bean seeds were allowed to germinate in chambers for three days under controlled temperature and dual light cycle (20°C at night and 30°C during daylight), and humidity. The sprouted beans were pureed (referred to as "sprout slurry") and held frozen (-4°C/ 24°F) until used to serve as an intrinsic source of catabolic enzymes with substrate diversity.

Small batches (approximately 5 kg) of the "CONTROL" (soak, rinse and heating water added back) and hot water-extracted whole bean bean slurries were individually incubated and predigested for 16 hours (overnight) at 4°C (39°F) with each of the selected commercial enzymes (0.1 % w/w) and "sprout slurry" (5% w/w) prior to drum-drying (Figure 15). A batch with no added enzyme served as a comparative control for the experiment.

Aqueous Extraction and Enzymatic Pre-digestion of Split Navy Beans

Commercial scale-up of the drum-drying process utilizing split navy beans was conducted following the aqueous extraction (Figure 13) and enzyme pre-digestion

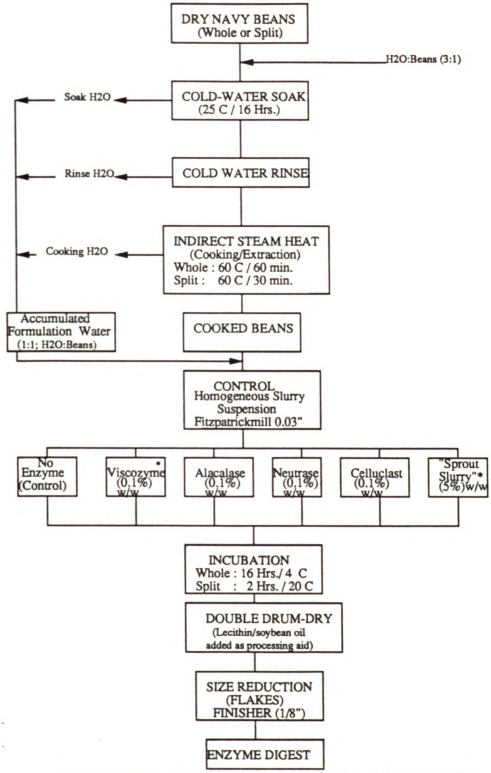


Figure 15. Flowchart for enzyme predigestion oof whole and split navy beans prior to drum-drying

* Commercial-scale runs using split beans also prepared for these designated treatments

(Figure 15) protocols. The cooking time was reduced to 30 minutes holding at 60°C (140°F) to minimize excessive disintegration of the split beans. Two separate batches of the "CONTROL" split bean slurries were incubated (55°C / 2 hours) (131°F/2 hours) and pre-digested with either the Viscozyme (0.1% w/v) or "sprout slurry" (3% w/v) prior to drum-drying.

Drum-Drying of Bean Meals

Various drum-drying systems were utilized during selected phases of this research. Each system was steam heated and operated with batch sizes appropriate to maintain full operating capacity. Nomenclature and description of these drum-drying systems are as follows:

a) Bench-scale. A 6" x 8" (38 cm x 52 cm) fabricated double-drum-drier with a heating surface area of 1134 cm^2 was utilized to produce the enzyme pre-digested whole bean drum-dried meals.

b) Pilot-scale. Aqueous and steam extracted whole bean drum-dried meals were produced using a pilot-scale American drum-drier (Overton Machine Co., Dowagiac, MI) with drum dimensions of 12" x 19 1/8" (77 x 123 cm) and (total surface area of 10.24 ft^2) (9509 cm^2)with drying surface area nip to doctor blade equal to 5.12 ft^2 (4755 cm^2). It was set up to simulate a single drum operation by running one drum as a cold applicator (Bakker-Arkema et al., 1966).

c) Commercial-scale. A 5' 4" x 2' (413 cm x 155 cm) atmospheric double drum-drier (Overton Machine Co., Dowagiac, MI) with a heating surface area of 62.83 ft^2 (58357 cm^2)was used to produce split bean drum-dried meals. Drying conditions of 4.5 rpm and 75 psig per drum were maintained.

After holding the slurries at the desired temperatures and times, total solids content was checked using a Computrac Moisture Analyzer Model MA5A (Quintel Corp.) to control slurry viscosity and ensure a uniform film formed on the dryer surface. The

temperature of the slurry was then brought up to 90-95°C (203°F) by steam injection, prior to pumping the slurry into a stainless steel flash tank (Perma-San Model 75 OVC, Process Equipment Corp., MI) serving as a reservoir for continuously supplying slurry to the drum-drier. The slurry was pumped through a tangential steam heater which gelatinized the starch and reduced microbial load of the slurry. A 0.1% mixture (1:1) of lecithin and soya oil was sprayed on the surface of the drum-drier to prevent the sheets from sticking to the drum-drier surface. The drum-dried bean sheets obtained from the drum heating surface were scraped by the drier knives (doctor blades) and collected using a continuous belt conveyor, weighed and flaked through a finisher (Reeves Pulley Co., Indiana) with a 1/4" (1.61 cm) stainless steel mesh screen. Drum-dried bean flakes were thoroughly mixed to obtain a homogeneous product and stored in sealed polyethylene bags which were held in fiber drums at 4°C (39°F) . The drum-dried bean flakes were further milled into flour using a Udy Cyclone Mill with a 20 mesh screen (Udy Co., Fort Collins, CO), prior to analyses.

Chemical and Biological Analyses

All the analyses were conducted using the described methodologies and were replicated three times, unless otherwise stated.

Proximate Composition

Moisture. Approximately 2.0 grams of the raw bean or drum-dried bean meal sample was weighed into previously weighed aluminum dishes and dried to a constant weight at 80°C (176°F) (ca 7 hrs.) in partial vacuum having pressure equivalent to 25 mm Hg. Percentage moisture was calculated from the weight loss on a fresh weight basis (AACC Method 44-40):

(Eq. 1) % Moisture = loss in moisture (g) / initial weight of sample (g) x 100

Protein. Protein content was determined by the classical Kjeldahl nitrogen analysis using a modification of the AOAC (1984) method. Five millimeters of concentrated sulfuric acid and one catalyst (K_2SO_4 and Selenium) tablet ((Tecator Co., Sweden) were added into each of the digestion tubes containing the pre-weighed samples (ca 150 mg). The tubes were slowly heated until digestion was complete. The protein content was calculated on a dry weight basis using a nitrogen conversion factor of 6.25.

(Eq. 2) % Protein = % Total "Kjeldahl" Nitrogen x 6.25

Fat. Approximately 3.0 grams of the raw bean or drum-dried bean meal sample was refluxed for 4 hours with 60.0 ml petroleum ether using a Goldfish Extractor, or for 25 mins with 40.0 ml petroleum ether using the Soxtec System HT6 (Tecator, England). Solvent was evaporated to yield a fat soluble residue. Percent crude fat was calculated on a dry weight basis (AACC Method 30-25).

(Eq. 3) % Crude Fat = wt. of fat (g)/ dry weight of sample (g) x 100

Ash. Approximately 2.0 grams raw bean or drum-dried bean meal sample was weighed into pre-weighed crucible amd incinerated at 525°C for 24 hours. The ash was cooled in a dessicator and weighed at room temperature. Percent ash was reported on a dry weight basis (AACC Method 08-01).

(Eq. 4) % Ash = weight of incinerated residue (g)/ dry weight of sample (g) x100

Soluble Sugars Quantitated by High Performance Liquid Chromatography (HPLC)

The extraction of the soluble sugars for the drum-dried bean meals was determined using a modified HPLC procedure developed by Agbo (1982) as outlined in Figure 16.

Extraction of Soluble Sugars. Approximately 2.0 g representative raw or drum-dried bean meal sample was placed in a 50-ml polyethylene centrifuge tube. Twenty-five ml of 80% ethanol was added and the mixture was then vortexed. The tube was

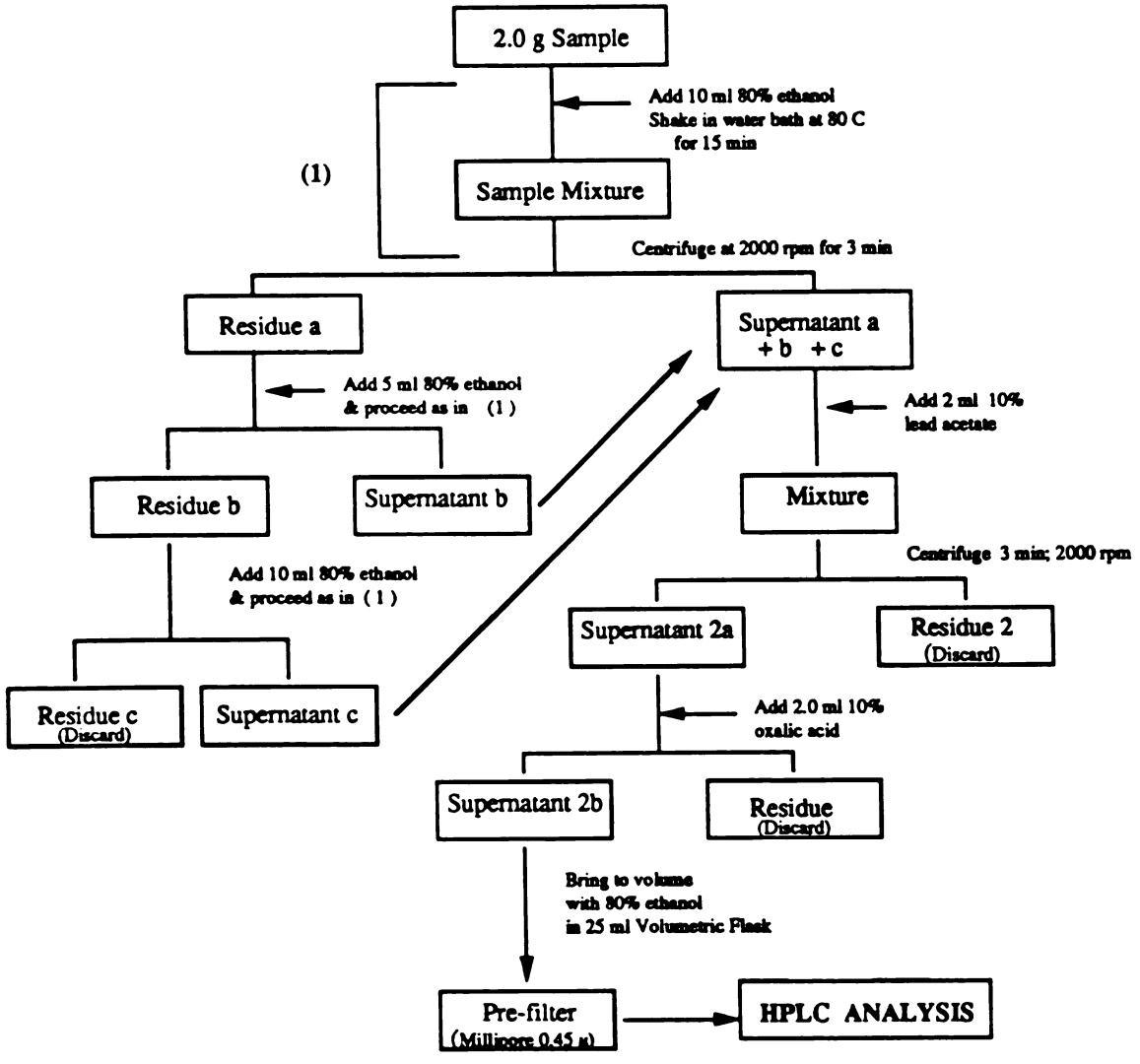


Figure 16. Flowchart for the extraction of soluble sugars from drum-dried bean meals (adapted from Agbo, 1982)

placed in a shaking water bath (Model 1024 Tecator, Hoganas, Sweden) and held at 80°C (176°F) for 15 minutes. The sample was then cooled to room temperature and centrifuged using a Sorvall RC-5B refrigerated centrifuge (Du Pont Inc., IL) at 2000 rpm (755 x *g*) for 3 minutes. One ml of 10% lead acetate (w/v) solution was added to the supernatant to precipitate ethanol soluble proamines, then the tube was again centrifuged for another 3 minutes at 2000 rpm (755 x *g*). One ml of 10% oxalic acid (w/v) solution was added to the filtrate to precipitate excess lead acetate. The sample was again centrifuged at 2000 rpm (755 x *g*) for another 3 minutes. The final supernatant was evaporated down to 3 ml and made-up to a final volume of 5-ml using 80% ethanol. Extracts were stored at -4°C (25°F) prior to analysis.

Separation and Quantitation. The extracted sample was filtered through a 0.45*u*m millipore filter (Waters Associates, Milford, MA). The filtered sample was injected into a 125 A, 10 *u*m, 3.9 x 300 mm I.D. Carbohydrate Analysis column (Waters Associates, Milford, MA). The elution solvent was acetonitrile:water (65:35, v/v) at a flowrate of 2.0 ml/min. A 50 *u*l sample was injected using a 100 *u*l pressure-lock Hamilton microsyringe.

The HPLC consisted of a Solvent Delivery System 6000A, a Rheodyne Injector equipped with a 50-*u*l sample loop and a Differential refractometer R401 (Waters Associates, Inc.). The refractometer was set at 4x to resolve the soluble sugars as discrete peaks.

A mixed standard solution was prepared containing a known amount of standard glucose, sucrose, raffinose and stachyose sugars (Sigma Chemical Co., St. Louis, MO). Quantitation and identification of the sugars were done using the external standard method programmed in the Data Module Model 730 (Waters Associates, Inc.) to calculate the absolute amounts of each component in the injected sample. These data were used to express the percent sugar content based on a dry bean flour weight.

Total Mineral Content

Mineral content of raw beans, drum-dried bean meals and water used during processing was determined using an inductively coupled argon plasma (ICP) emission spectrometer (Jarrell-Ash Model 955 Atomcomp) equipped for simultaneous analysis of 19 elements (Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Mo, Na, P, Pb, Se, Tl and Zn) and maintained by the Department of Pharmacology and Toxicology at Michigan State University. Triplicate samples were combined with 2 ml of concentrated Baker Instra-analyzed nitric acid in 15 ml screw-capped teflon vials (Tuff-Tainer, Pierce Chem. Co., Rockford, Il). The samples were incubated overnight at 70-75°C (167°F), cooled and quantitatively transferred to 10 ml Class A volumetric flasks containing 1.0 ml of 100 ppm Yttrium (internal standard). They were diluted to final volume with water purified by a Millipore water purification system Class 1 (Millipore Corp., Bedford, MA). Along with the bean samples, blanks and standard materials were prepared to serve as controls. Mineral content was determined and values reported in ppm on a dry weight basis.

Insoluble and Soluble Dietary Fiber

The enzymatic method of Prosky et al. (1988) for determining insoluble and soluble dietary fiber is outlined in Figure 17. Four replicate 1.0 g drum-dried bean meal samples were weighed into 400 ml polyethylene beakers to which was added 50 ml of pH 6.0 phosphate buffer. A 0.1 ml heat-stable bacterial *a*-amylase (Sigma Chemical Co., St. Louis, MO) was added, the beaker covered with aluminum foil and the mixture was incubated in a shaking boiling water bath for 15 minutes. The mixture was then cooled to room temperature and pH adjusted to pH 7.5$\pm$0.2 using a 0.275N NaOH solution. Five milligrams of protease was next added and the mixture was incubated for 30 minutes in a 1024 Tecator (Hoganas, Sweden) shaking water bath maintained at 60°C (140°F). The mixture was cooled and pH was adjusted to pH 4.0-4.6 with 0.325M HCl. A 0.3 ml amyloglucosidase solution (Sigma Chemical Co., St. Louis, MO) was added and the

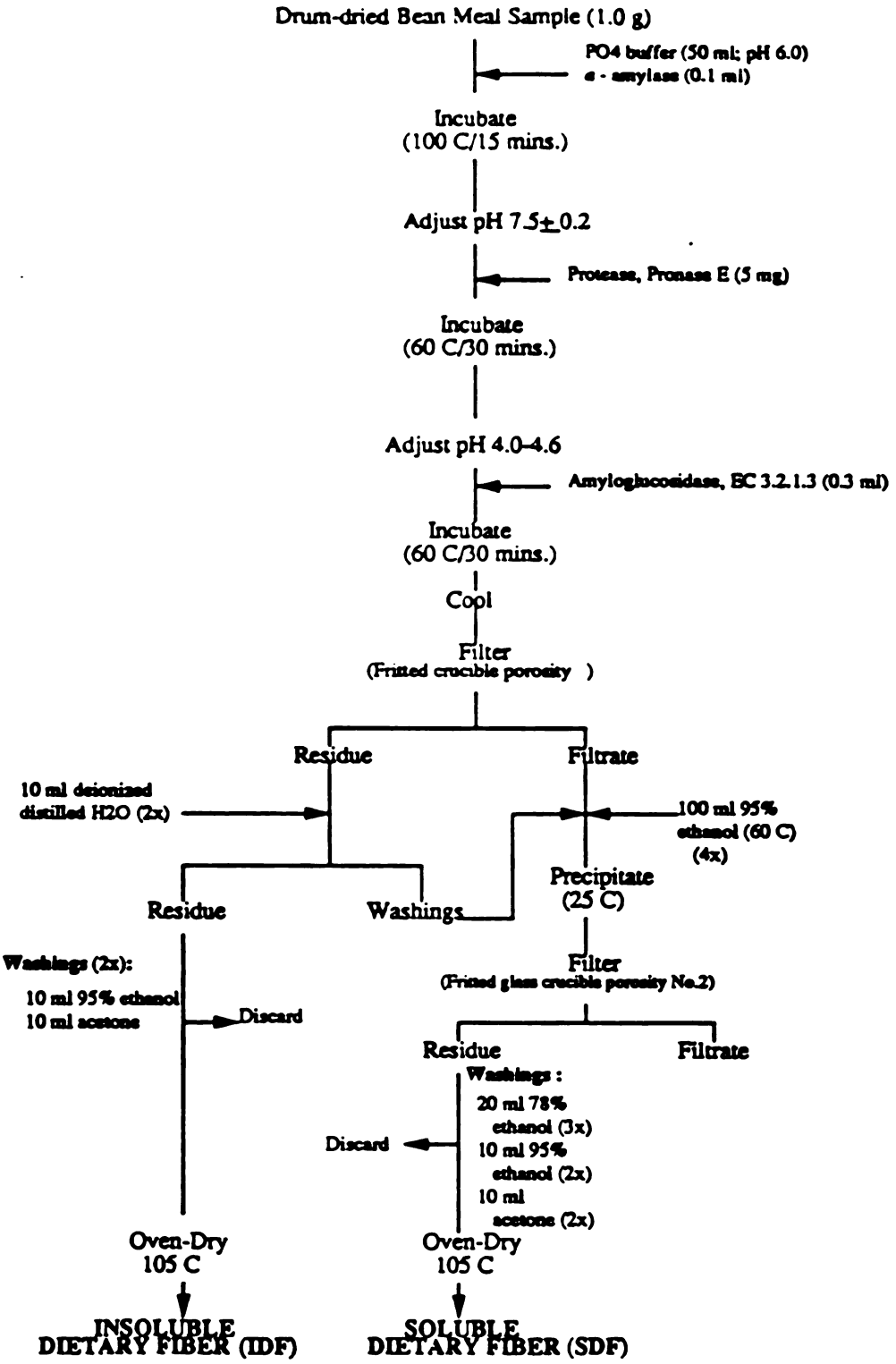


Figure 17. Flowchart of the enzymatic method to determine the insoluble and soluble dietary fiber of drum-dried bean meals (Prosky et al.,1988)

mixture incubated for another 30 minutes at 60°C (140°F). Insoluble and soluble fiber fractions were determined sequentially on this digest.

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

$$\text{(Eq. 5)}\quad \text{IDF (\%)} = \text{mg insoluble residue} - \frac{[\,(\%\text{protein in residue} + \%\text{ ash in residue}) \times \text{mg residue}\,] \times 100}{\text{sample weight. (mg)}}$$

Soluble fiber. The weight of combined filtrate and water washings was adjusted to 100 g with water. Four 100 ml portions of 95% ethanol preheated to 60°C (140°F) was added and allowed to precipitate at room temperature for at least an hour. The enzyme digest was filtered through previously ashed and weighed fritted crucibles containing Celite. The residue was sequentially washed with three 20 ml portions of 78% ethanol, two 10 ml portions of 95% ethanol and finally two 10 ml portions of acetone. The residues were dried and analyzed for protein and ash following the same protocol for the insoluble fiber. Percent Soluble Dietary Fiber (SDF) was calculated as follows:

$$\text{(Eq. 6)}\quad \text{SDF(\%)} = \text{mg soluble residue} - \frac{[\,(\%\text{ protein in residue} + \%\text{ ash in residue}) \times \text{mg residue}\,] \times 100}{\text{sample weight. (mg)}}$$

In-vitro Starch Digestibility

Total and available starch content of the drum-dried bean meal samples were determined using a modification of the enzymatic methods of Holm et al.(1986 and 1985). The released glucose was measured by the colorimetric Glucose oxidase/peroxidase Assay using a glucose diagnostic kit (#510-A, Sigma Chemical Co., St. Louis, MO). The combined enzyme-color reagent contains the two enzymes, glucose oxidase and horseradish peroxidase, and the color reagent, o-dianisidine. The procedure is based upon the following coupled enzymatic reactions:

$$\text{Glucose} + 2H_2O + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic Acid} + 2H_2O_2$$

$$H_2O_2 + \underset{\text{(Colorless)}}{\text{o-Dianisidine}} \xrightarrow{\text{Peroxidase}} \underset{\text{(Brown)}}{\text{Oxidized o-Dianisidine}}$$

The intensity of the brown color measured at 450 nm is proportional to the original glucose concentration. The total amount of glucose in samples was calculated from a standard curve prepared from known concentrations of glucose. The total starch content in each sample was obtained by multiplying the mg of glucose in each sample by a factor of 0.9 to account for the weight of the water gained during the hydrolysis of starch to glucose.

Total Starch. The modified enzymatic/colorimetric methods of Holm et al. (1986) and Tovar et al. (1992) were followed to determine total starch content of the drum-dried bean meals (Figure 18). A 500 mg drum-dried bean sample was weighed into a beaker which was tared containing a magnetic stir bar. The sample was dispersed in 10.0 ml distilled water and 10.0 ml 4N NaOH, and allowed to stand at room temperature for 1.0 hour. Twenty milliliters of 0.08 M phosphate buffer was added and pH was adjusted to 6.0 with 5N HCl. The mixture was incubated with 50 μl of thermostable α-amylase Type

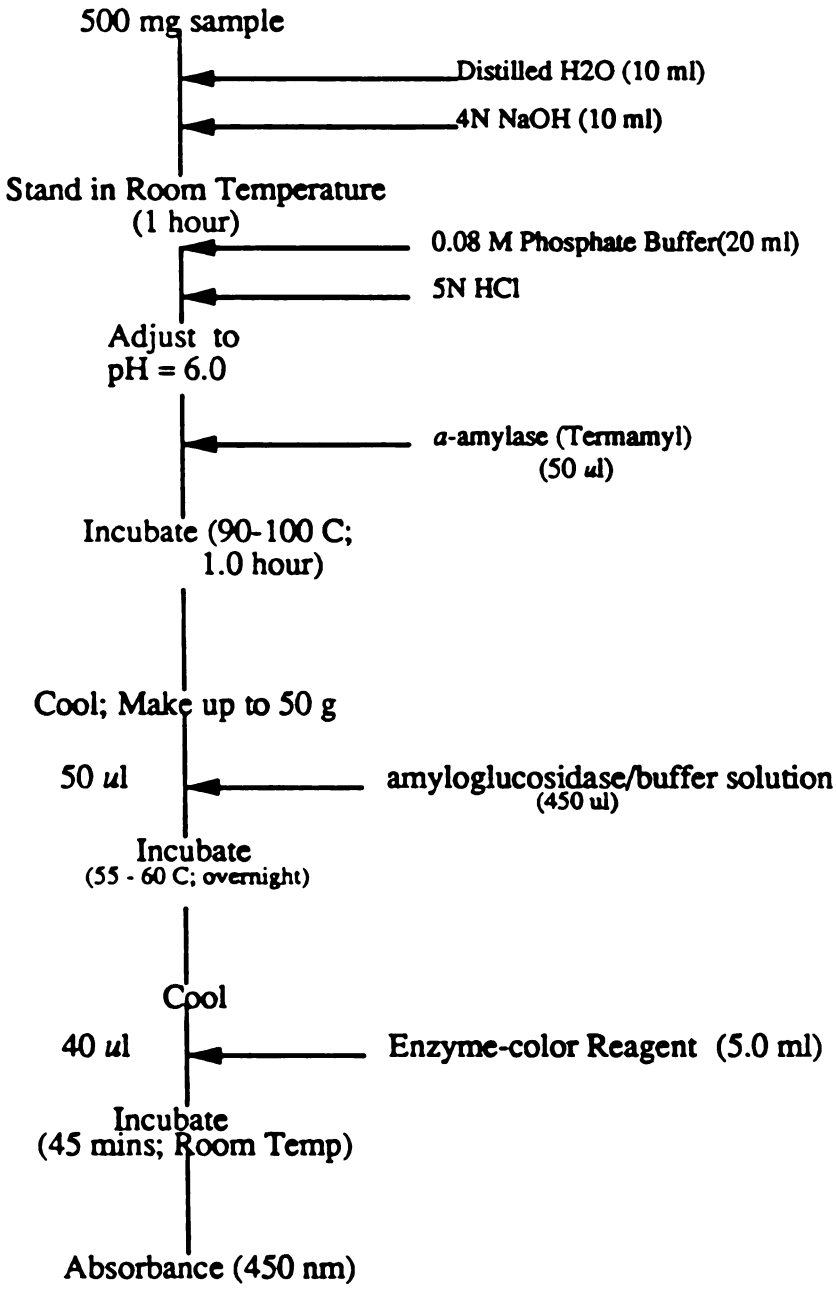


Figure 18. Flow diagram for determination of total starch.

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XII-A (*Bacillus licheniformis*, Sigma Chemical Co., St. Louis, MO) at 90-100°C(203-212°F) for 1.0 hour. The mixture was cooled to room temperature and distilled water added so that the total aqueous weight is 50.0 g. A 50.0 *u*l sample aliquot was pipetted into a screw-capped tube and incubated with 450 *u*l of amyloglucosidase enzyme solution (*Aspergillus niger* (Boehringer Mannheim) 10 mg of lyophilizate/ml of 3.2 M $(NH_4)_2SO_4$;)/buffer solution (dilute enzyme stock solution 1:10 with 0.1M sodium acetate, pH 4.5) and incubated overnight at 55-60°C(140°F). The mixture was cooled to room temperature and 40 *u*l sample aliquot used for the glucose assay.

Available Starch. Figure 19 outlines the protocol for determining *in-vitro* digestibility of starch. A 500 mg sample was weighed into a 100 ml beaker tared with a magnetic stir bar and mixed with 10.0 ml of deionized-distilled water (For raw bean samples and reference cornstarch and all-purpose flour, samples were gelatinized with 10.0 ml distilled water in a 90-100°C(212°F) water bath for 20 mins. and then cooled to room temperature before proceeding to the next steps). HCl (200 *u*l , 5N)was added to bring the pH to approximately 1.5. Pepsin (100 mg) was added and the mixture incubated at 37°C (99°F) for 1.0 hour. Twenty ml of 0.1M phosphate buffer (pH 6.0 - 7.0, and containing 0.1% Thimersal)® and 215 *u*l of 4N NaOH were added and pH was adjusted to 6.0. Deionized distilled water was added so that the total aqueous weight was 50 g. The mixture was incubated with 15.3 *u*l of porcine pancreatic *a*-amylase (Sigma Chemical Co., St. Louis) at 37°C(99°F). The rate of starch hydroysis was monitored by removing a 2.0-ml sample of the digest at appropriate time intervals (15 and 60 min., and 24 hours of incubation). This aliquot was immediately added to 8.0 ml of 95% ethanol. Precipitation was allowed to occur for 1.0 hour at room temperature and then centrifuged at 2000 *g* (3000 rpm) for 10 mins. (For zero time sampling, a 2.0 ml sample was taken prior to adding the porcine *a*-amylase solution). A 50.0*u*l supernatant was pipetted into a screw-capped tube and incubated with 450 *u*l of amyloglucosidase (*Aspergillus niger*, 14 enzyme

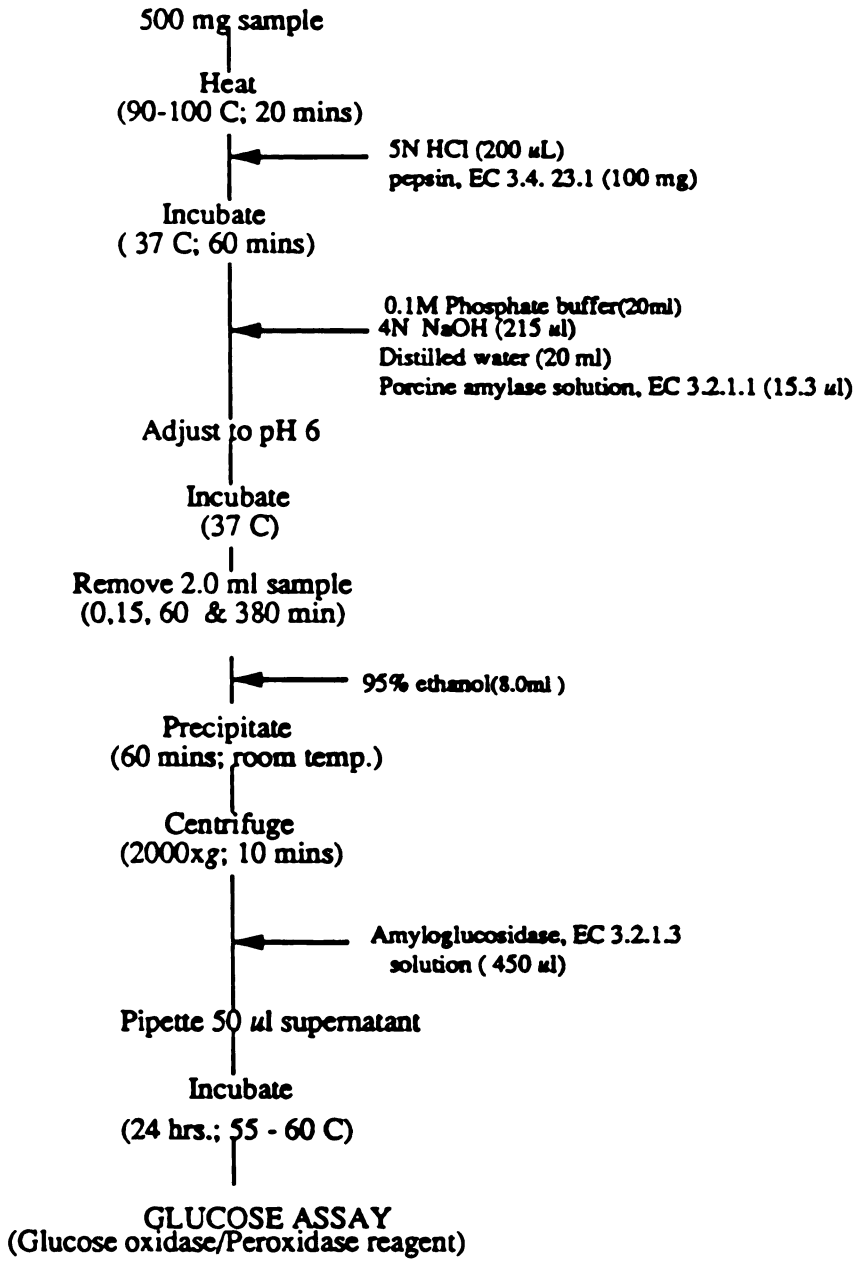


Figure 19. Flowchart for the determination of available starch (Holm, 1984)

units/mg protein, 10 mg protein/ml; Boehringer Mannheim) enzyme solution overnight at 55-60°C (131°F). The mixture was cooled to room temperature and 100 *ul* was used for the glucose assay.

The release of free glucose was measured with a glucose oxidase/peroxidase reagent and the starch content was calculated as glucose x 0.9. Reagent blanks with the starch degrading enzymes were run in each case. Corn, potato and wheat starch suspensions and all-purpose (wheat) flour were assayed as reference samples.

$$\text{(Eq. 7)}\quad \%\ \text{Total or Available Starch} = \frac{\text{ug glucose x dilution factors x 0.9}}{\text{sample weight (mg)}} \times 100$$

In-vitro Protein Digestibility

The sequential multi-enzyme system (AOAC, 1990) for determining *in-vitro* protein digestibility is outlined in Figure 20. Drum-dried bean meal samples and control casein (ANRC Na caseinate) were weighed to contain approximately 10 mg nitrogen. Nitrogen content of the drum-dried bean meal samples was determined by Kjeldahl method (Eq. 2). Ten ml of deionized distilled water was added to the casein control (Sigma Chemical Co., St. Louis, MO) or to the sample in a test tube containing a stirring bar. Protein suspensions were held in a water bath maintained at 37°C (99°F)for at least an hour. One ml of "Enzyme A" solution (porcine pancreatic trypsin, Type IX; bovine pancreatic *a*-chymotrypsin, Type II and porcine intestinal peptidase, Grade I; Sigma Chemical Co., St. Louis, MO) which had previously been equilibrated to pH 8.0±0.03, was added to the protein suspension after it had also been equilibrated to pH 8.0±0.03. After incubation with Enzyme A for 10 minutes at 37°C (99°F), 1.0 ml of "Enzyme B" solution (bacterial protease, Pronase E; Sigma Chemical Co., St. Louis, MO) was added to the protein suspension which was further incubated for 9 minutes in a water bath maintained at 55°C(131°F). At precisely 20 minutes from the addition of "Enzyme A" solution, pH was read at 37°C(99°F). The extent to which pH of the protein suspension

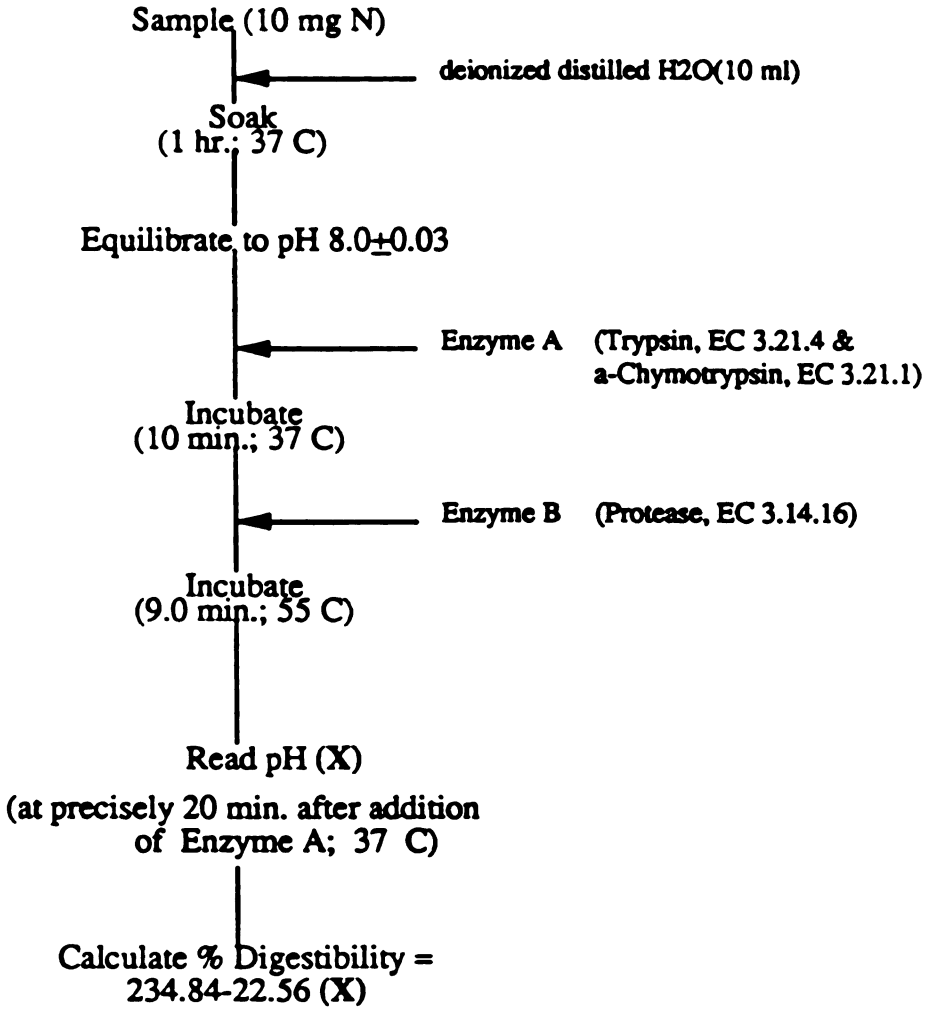


Figure 20. Flow chart for *in-vitro* protein digestibility (AOAC, 1990)

dropped was used as a measure of protein digestibility. Percent digestibility was calculated as follows:

(Eq. 8) % Protein Digestibility = 234.84 - 22.56 (X)

where X = pH at 20 min.

Methionine Content

The improved gas chromatography procedure of MacKenzie (1977) for the analysis of methionine in plant materials was followed. A 200 *ul* of the cyanogen bromide reagent (5% in 50% formic acid) was added to 20 mg of the drum-dried bean meal in a microcentrifuge tube. After 2 hours at room temperature, the tube was centrifuged at 2000 *g* (1300 rpm) for 15 min. and 2 *ul* aliquots of the supernatant were injected directly on the chromatographic column using a Hamilton syringe (No.7001).

All analyses were performed using a SRI 8610 gas chromatograph (SRI Instruments, California) equipped with both flame ionization and flame photometric detectors. The column (4 ft x 1/8" SS) was packed with Porapak QS, 80-100 mesh (Applied Science Labs., State College, Pa, U.S.A.) and conditioned at 160°F (110°C) for 2 hr. with a hydrogen carrier gas. The GC conditions used were as follows: column temperature, thermal gradient 145 to 160°C (293 to 320°F) at 5°C/ min., and 160°(320°F) for 3 min. Quantitation and data reduction were performed by a laboratory data system using an internal standard program.

$$\text{(Eq. 9) nmoles Methionine} = \frac{\text{nmoles methyl thiocynanate}}{\text{(g) protein}} \times 100$$

Amino Acid Analysis

Oxidation, acid hydrolysis and amino acid anlysis of the oxidized samples were performed at the Macromolecular Structure Facility maintained in the Biochemistry Department at Michigan State University. Constant boiling HCl (200*ul* (Pierce Chemical Co., Rockford, IL) was added to the bottom of the vacuum vial. Three alternate vacuum

nitrogen flushing steps were required to ensure the oxygen-free atmosphere. Vapor phase hydrolysis was conducted at 112-116°C for 24 hours. Following hydrolysis, the samples were re-dried with ethanol:water:triethylamine (2:2:1, v/v), and derivatized. The derivatization reagent consisted of a 7:1:1:1 solution of ethanol, triethylamine, water and phenyllisothiocyanate (PITC). After 10 min. derivatization, the samples were vacuum-dried and re-dissolved in 500 ul of 5 mM sodium phosphate, pH 7.8. A Waters 600 HPLC system (Waters Associates, Milford, MA) was used for the analysis of the derivatized amino acids. The two component (A and B) gradient mobile phase system consisted of the following : A, 15 *m*M sodium acetate buffer, pH 5.9, and 0.05% triethylamine and B, acetonitrile:H2O (60:40). An injection volume of 40 *u*l and ultraviolet absorbance at 254 nm were used for detection of the PITC-labelled amino acids.

SDS-PAGE Electrophoresis

Crude extracts of the raw and drum-dried bean meals were subjected to discontinuous SDS-PAGE using an electrode buffer at pH 8.3 following the procedure of Hames (1990). A 7.5% to 24% acrylamide gradient with a 3.75% acrylamide stacking gel was employed (180 x 160 x 1.5 mm slab, Protean 16 cm electrophoresis unit, Bio-Rad Laboratories, Richmond, CA). The sample and standards were loaded onto the gel and the gel was run at 20 mA constant current. The SDS-PAGE gels were stained with silver nitrate to resolve bands, and destained with a 5% MeOH, 7.5% HOAc solution. Standard proteins used for molecular weight estimation were from an electrophoresis calibration kit for molecular weight determination of low molecular weight proteins (Electrophoresis Calibration Kit, Pharmacia-LKB, Piscataway, NJ). The kit consisted of phosphorylase b (94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (20.1 kD), and *a* - lactalbumin (14.4 kD).

Assessment of Antinutritional Factors

Screening for Heat-Stable Trypsin Inhibitor Activity (TIA)

A modification of the spectrophotometric assay of Kakade et al. (1974) by Rayas-Duarte et al. (1992) to determine trypsin inhibitor activity using the substrate BAPNA (N-benzoyl-DL-arginine-*p*-nitroanilide),is summarized in Figure 21. Approximately 5.0 gram drum-dried bean meal samples were homogenized with 100 ml deionized-distilled water using a high-shear Polytron (Brinkman Instruments Co, Westbury, NY), Model PT 10/35, at medium speed (setting=5). The homogenized bean solutions were centrifuged at 15000 *g* (10000 rpm) for 30 minutes at 20°C(68°F) . The supernatants were used to assay for TIA. The total sample volume was adjusted to yield a final volume of 400 *u*l. Control tubes contained 400 *u*l of deionized water. The reagent blank tubes contained 400 *u*l of distilled water + 200 *u*l of 30% acetic acid, while the sample blank tubes contained 400 *u*l of sample + 200 *u*l of 30% acetic acid. Sample aliquots were selected to yield a 40-60% inhibition of trypsin activity compared to a control containing no sample.

A 400 *u*l sample aliquot of trypsin (EC 3.4.21.4 bovine pancreas Type III-S, Sigma Chemical Co, St. Louis, MO) was added to all tubes at 10 second intervals to ensure that all of the tubes were incubated for the same time period. The tubes were then incubated at 37°C (99°F) for a total of 10 minutes before addition of 1.0 ml BAPNA solution (Sigma Chemical Co., St. Louis, MO). The tubes were incubated for another 10 minutes before addition of 200 *u*l of 30% acetic acid to all tubes except the reagent and sample blank tubes. The tubes were vortexed before reading absorbance using a Spectronic-70 spectrophotometer at 410 nm.

One unit of trypsin activity (TA) was defined as an increase of 0.001 absorbance unit at 410 nm of the reaction mixture under assay conditions. One unit

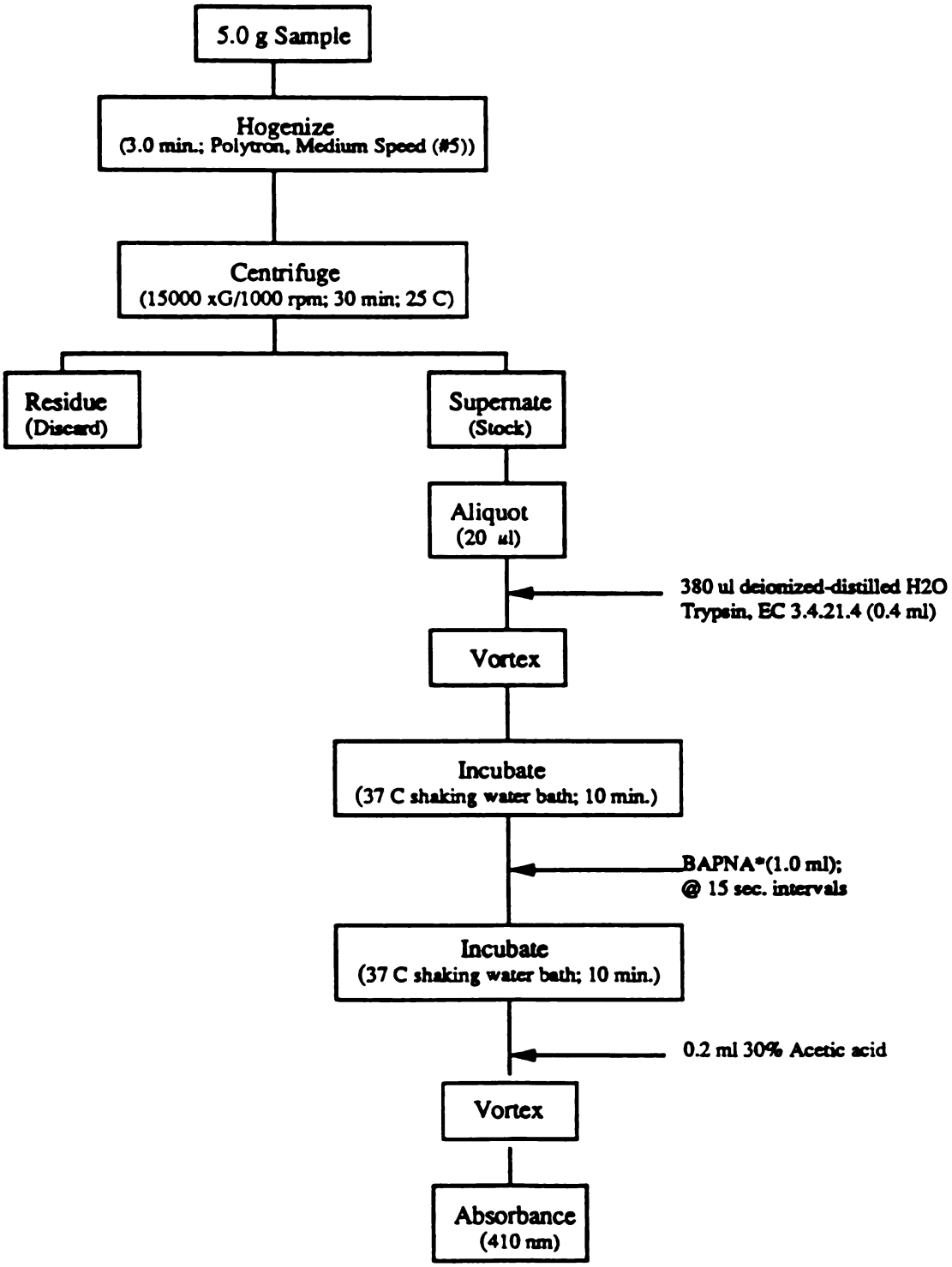


Figure 21. Flowchart for the trypsin inhibitor assay (TIA) (Kakade et al., 1974)

of trypsin inhibitory activity (TIA) was defined at as a 10% decrease in the number of units of TA under assay conditions. Thus, a 50% decrease in units of TA (i.e., 50% inhibition of TA) would equal 5 units of TIA (Rayas-Duarte, et al., 1992).

Screening for Phytohemagglutinin

Phytohemagglutinin (PHA) was determined using Sigma Kit No. P-0526 (Sigma Chemical Co., St. Louis, MO) containing purified *Phasolus vulgaris* erythroagglutinin (kidney bean lectin) as immunogen. A 0.01 g/ml slurry of raw bean or drum-dried bean meal in deionized distilled water was prepared. Different aliquots (10 to 40 *u*l) of the raw and drum-dried bean meal slurries were pipetted into round bottom microtiter wells and reacted with 10 *u*l of antibody. The reaction of the antibody with purified *Phaseolus vulgaris* lectin (Sigma Chemical Co., St. Louis, MO) was used as a reference. For comparative control so as to distinguish sample settling from agglutination, the same quantities of raw and drum-dried bean meal slurries were pipetted into adjacent rows of wells, but no antibody was added to it. The formation of a lectin-antibody precipitate was monitored every 30 minutes.

Phytic Acid

Purified inositol phosphate fractions were prepared by hydrolysis of sodium phytate using a modification of the procedure of Graf and Dintzis (1982) as summarized in Figure 22. A 0.5 g drum-dried bean sample was extracted with 20 ml of 0.5M HCl for 2 hr. at 20°C under vigorous mechanical agitation. The extract was centrifuged and the supernatant decanted, frozen overnight and filtered using No. 1 Whatman filter paper. The inositol phosphates were separated from the filtrate by ion-exchange using plastic columns with glass wool filter and containing 0.65 ml resin (AG 1-X8, 200-400 mesh). The filtrate was passed through the

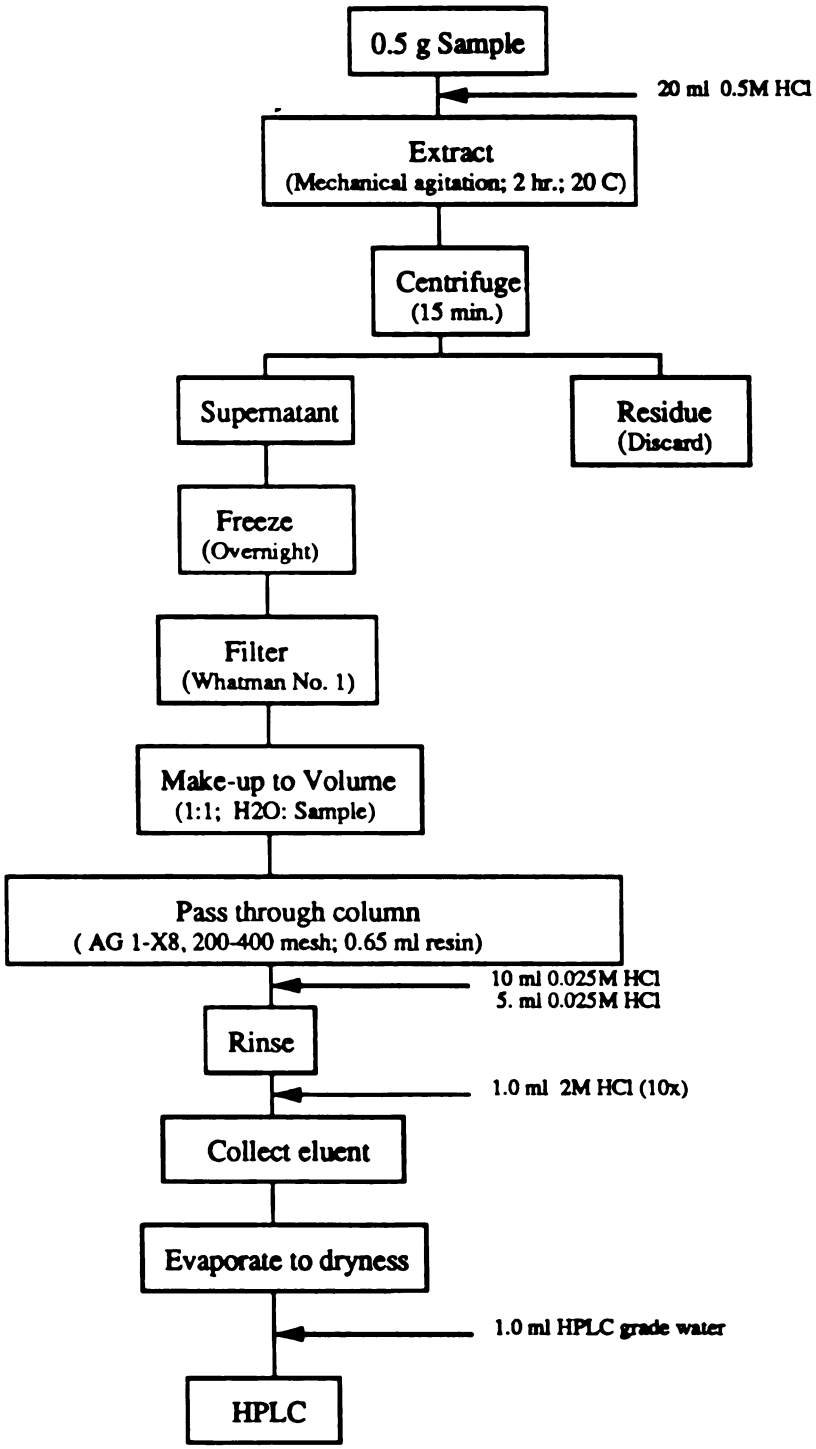


Figure 22. Flowchart for the separation and quantitative determination of phytic acid as inositol phosphates

column (ca 0.4 ml/min) followed by 10 and 5 ml rinsings of 0.025M HCl. The inositol phosphates were eluted from the resin with ten 1-ml portions of 2M HCl, and the eluent evaporated to dryness and diluted with 1.0 ml of HPLC Baker Analyzed reagent water. Inositol phosphates were then identified and quantified by HPLC using the method of Sandberg et al. (1989).

Physical and Functional Properties of Drum-Dried Bean Meals

Color

Drum-dried bean meal surface color was evaluated using a Hunter Lab Model D25-PC2 system (Hunter Associates Laboratory, Inc., Reston, Virginia) which includes an optical sensor and a sensor interface unit (SIU) interfaced to a Zenith computer. The colorimeter measures reflectance on three coordinates labeled L, a_L, and b_L. The L value measures darkness (0) to lightness (100); a_L (green to red) and b_L (blue to yellow) values were expressed as hue angle by calculating the corresponding x, y and z values. A white tile (L= 95.35, a_L = -0.6, b_L=+0.4) was used to standardize the system.

Cellular Structure and Starch Granule Appearance

The microstructure of the drum-dried bean meals and starch granule appearance were observed under Laser Scanning Confocal Microscope (LSM) (Carle Electron Optics Limited, Tokyo, Japan). A polarizing light microscope (Carl Zeiss, West Germany) was also utilized to determine the effect of the extraction pretreatments and the drum-drying process on the gelatinization and birefringence of bean starch. Visual examination and selected representative photomicrographs were prepared as the basis to draw conclusions

Solubility

Drum-dried bean meal solubility characteristics were determined by dissolving approximately 0.5 g sample in 50.0 ml distilled water. The mixture was slowly stirred on a magnetic stir plate at room temperature for one hour, after which it was filtered through a

Whatman No. 1 filter paper. The filtrate was weighed and dried in a vacuum-oven. The following solubility indices were determined:

Water Absorption Index (WAI) was determined by weighing the wet residue.

(Eq. 11) $$WAI = \frac{\text{wet residue (g)}}{\text{sample dry weight (g)}}$$

Water Solubility Index (WSI) was determined by weighing the dry soluble solids.

(Eq. 10) $$WSI = \frac{\text{weight of dried soluble solids(g)}}{\text{sample dry weight/50 ml water}}$$

Soluble Solids (SS, ^{o}B) of the filtrate from the above preparation was determined using an Abbe refractometer (Bausch and Lomb) and recorded as degrees Brix (^{o}B).

Water Holding Capacity (WHC)

WHC, frequently referred to as hydration capacity (HC), was determined using AACC (1984) Method 56-20. Triplicate 2.0 g samples were weighed into centrifuge tubes. Forty ml distilled water was added and the tubes vortexed to suspend the sample. The suspension was allowed to stand for 10 min., inverting the tubes three times at the end of the 5-min and 10-min periods. The tubes were then centrifuged for 15 minutes at 1000 *g* (and the supernatant decanted. The tubes were then inverted and allowed to drain for 5 minutes. The wet samples were weighed and hydration capacity calculated using the formula:

(Eq. 11) $$WHC = \frac{\text{(wt. of tube + sediment) - (tare wt. of tube)}}{\text{sample wt (dry basis)}}$$

Bulk density

Bulk density was determined as the weight of drum-dried bean meal sample tightly packed into a 100 ml graduated cylinder and recorded as g/100 ml.

Nitrogen Solubility Index (NSI)

NSI of the drum-dried bean meals was determined using AACC Method 46-23. A 5.0 g sample was weighed into a 400 ml beaker and mixed with 200 ml distilled water. The mixture was stirred with a mechanical stirrer at low speed for 120 min at 30°C(86°F). The mixture was centrifuged at 15000 rpm (for 10 min and the supernate was filtered through a funnel plugged with glass wool to obtain a clear liquid. Percent water-soluble nitrogen was determined from the liquid using the Kjeldahl method. Nitrogen solubility index, expressing water soluble nitrogen as a percentage of the total nitrogen was calculated as follows:

$$\text{(Eq. 12)}\quad \text{Nitrogen Solubility Index (NSI)} = \frac{\%\ \text{water soluble nitrogen}}{\%\ \text{total nitrogen}} \times 100$$

Incorporation of Drum-Dried Bean Flour in Food Products

Quick Bread

The procedure for baking the pumpkin bread is outlined in Figure 23. The pumpkin bread formula (Rombauer and Becker, 1978) is presented in Table 3. The composite flours consisted of all-purpose and drum-dried navy bean flour blended in 80:20, 60:40 and 40:60 ratios, respectively. Batter samples of 325 grams were poured into 3" x 5" (7.62 cm x 12.7 cm) greased baking pans and baked at 177$\pm$2°C (350°F) for 45 min. in a National Reel Type Test Baking Oven. Bread loaves were removed from the pan 30 min after baking, for 15 min of additional cooling and weighed to determine the percentage weight loss during baking.

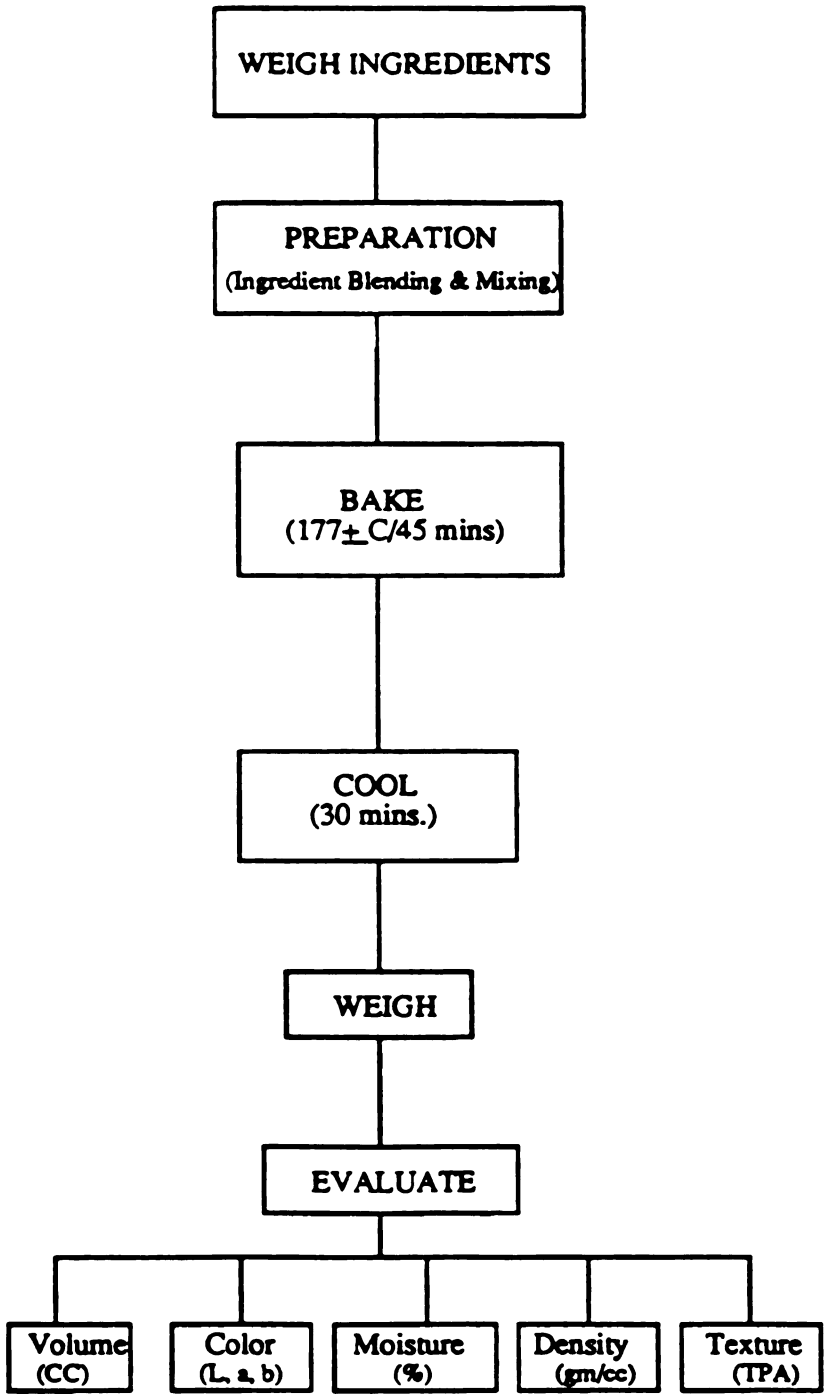


Figure 23. Flowchart of utilization of drum-dried bean flour in pumpkin bread

Table 3. Pumpkin Bread Formula[1]

Ingredient	Weight (g)
All purpose or composite flour[2]	100.65
Baking powder, SAS	0.45
Baking soda	2.00
Salt	3.00
Cinnamon, ground	0.50
Cloves, ground	0.25
Brown sugar, dark	133.35
Vegetable shortening	31.35
Egg, fresh whole	50.00
Pumpkin, Canned	123.00
Milk, whole fluid	60.50
Vanilla	1.23

[1] Rombauer and Becker, 1978 as cited by Alani et al., 1989

[2] Composite flours contained all-purpose/drum-dried bean flour in 80:20. 60:40, 40:60 ratios

Weaning Food

The drum-dried bean meals were utilized to formulate a bean-based weaning food/beverage similar to soymilk. Different concentrations (5%, 8% and 10%) of the "EXTRACT"(aqueous), and "ENZYME-DIGEST" (Viscozyme) drum-dried bean meals were prepared by homogenizing (Tekmar Tissumizer) weighed quantities of the sample with 200 ml distilled water for 2 min. Objective evaluations (Figure 24) were conducted to assess the quality of the bean weaning food/beverage.

Selected emulsifiers (carageenan products and xanthan gums) at differential levels were combined with the 5% EXTRACT and ENZYME-DIGEST drum-dried bean meal samples to enhance stability of the beverage.

Objective Quality Evaluation of the Bean Products

Quick Bread

Viscosity. Batter viscosity was determined using a Brookfield viscometer (model DV-II) equipped with a no. 7 spindle and rotating at 20 to 100 rpm, depending on the sample consistency. Results were recorded as centipoise (cps) at 23±3°C (73°F).

pH. A portable pH meter (VWR Scientific Inc.,San Francisco, CA) was used to measure the pH of the pumpkin batter.

Volume. Bread volume was measured by rapeseed displacement and results were recorded as cubic centimeters (cm^3).

Texture

Tenderness. The standard shear compression cell of the Kramer Shear Press (Model TMS-90, Food Technology Corp., Maryland) equipped with a 300-lb transducer was used and texture reported as Newtons per gram. Bread slices 0.5 cm thick were used for evaluation.

Texture Profile Analysis (TPA). A Texture Press TMS-90 was used, equipped with a parallel plate compression cell TPA-1 (Food Technology Corp., Maryland). The TPA test is a two-bite compression mode programmed to a specified percentage of deformation. A 1.0 cm thick pumpkin bread sample sliced from the middle portion of the loaf was trimmed using a 0.5 cm x 0.5 cm square-shaped dough cutter. The bread slice was placed at the center of the lower compression plate. The upper compression plate of the transducer was initially lowered down to about 1 cm above the sample. By pressing the "RUN" command, the bread slice was compressed twice with a designated 50% compression distance. After each two-stage compression sequence, the TPA force-distance curve and complete TPA analysis data was plotted directly using the TMS-90 Control Panel.

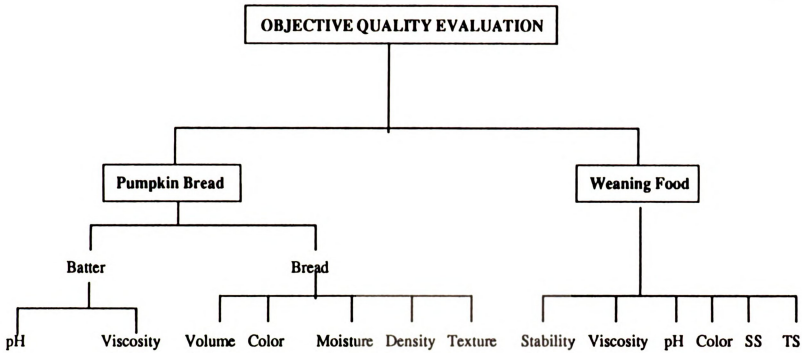


Figure 24. Flowchart of the objective evaluation of drum-dried bean meal products

Density. The trimmed pumpkin bread slice was weighed and its volume calculated by measuring the lenght x width x height to determine density as follows :

(Eq. 13) $$\text{Density} = \frac{\text{Mass of Bread slice}}{\text{Volume of Bread slice}}$$

Moisture. AACC Method 44-40 (1983) was used to determine the moisture of the pumpkin bread. Approximately 20.0 g pumpkin bread sample was dried in a vacuum oven at 90°C (194°F) overnight.

Microstructure. Cellular structure of the pumpkin bread was observed under Laser Scanning Confocal Microscope (LSM) (Carle Zeiss, Inc., West Germany) and observations and conclusions drawn after visual viewing of multiple samples and fields. Representative fields were prepared as photomicrographs.

Weaning Food/ Beverage

Suspension Stability

The different concentrations (5%, 8% and 10%) of drum-dried bean slurries were prepared by adding deionized distilled water to the drum-dried bean meals (Figure 25) and thoroughly mixing using a Tekmar tissue homogenizer prior to conducting the following indices of stability:

Visual Stability

The visual stability of the weaning food/beverage was evaluated by placing the homogeneous bean beverage in a 50-ml conical graduated centrifuge tube and the settled solids measured at 15 minute interval periods. The amount of the separated supernatant was plotted against time and was recorded.

Micro-quantitative method

a. Absorbance/% Transmission of the weaning food/ beverage was measured using a Spectronic 70 spectrophotometer (Bausch and Lomb, Il.). Differential quantities (10, 20, 40 and 60 *u*l) of the homogeneous suspended weaning food/beverage slurry

PREPARATION OF THE WEANING BEAN BEVERAGE

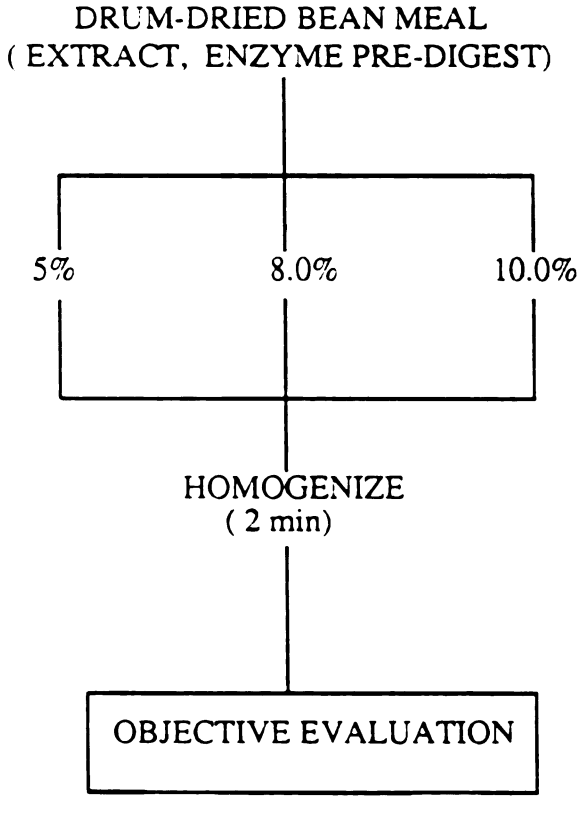


Figure 25. Experimental flowchart for the preparation of the weaning bean beverage

were mixed with a constant quantity (3.0 ml) of distilled water and absorbance read, prior to settling, at 565 nm. This procedure provided a design to quantitate the influence of concentration on stability.

b. For each of the suspended beverage, a 100 *ul* aliquot of the separated supernate was taken at 15 minute intervals during static holding at room temperature. The transmission (%) data was used to calculate an index of stability/settling of solids with time.

Consistency

Apparent Viscosity

Apparent viscosity of the bean beverage was evaluated using a Bookefield viscometer (Model DV-II) with a no. 2 spindle and rotating at 100 rpm for 5 min. Data was recorded as centipose (cps).

Relative viscosity

Relative viscosity of the bean beverage was determined by recording the time required, at room temperature, for the bean beverage sample to flow through the fixed distance between the two marks of an Oswald pipette. Results were reported as time of flow in seconds.

Physico-Chemical Tests

Color of the different concentrations of weaning food/ beverage was evaluated using the Hunter Lab Model D25-PC2 system standardized against the white tile.

pH of the beverage was measured using a Fischer Accumet (Fisher Scienctific, Pittsburgh, PA) pH meter standardized against a standard buffer solution (pH=7.0).

Soluble Solids of the beverage was measured using an Abbe refractometer (Bausch and Lomb, II).

Total Solids (%) of the different concentrations of bean beverage were detemined by weighing 50.0 g samples in tared aluminum pans and allowed to dry to a constant weight in an oven at 87°C(189°F), cooled and reweighed to determine weight loss.

Microwave Preconditioning of Whole Dry Navy Beans

The procedure described by Shirazi et al. (1992) was followed in this study (Figure 26). Whole dry navy beans were ambient soaked at different time periods and allowed to equilibrate at 4°C for 2 days. The beans were then rehydrated to different moisture contents (ranging from 47% to 54%) and subjected to microwave heating at three microwave energy levels (440, 590 and 740 watts) for 2 minutes in a Radarange microwave oven (Model-RS458P, Amana Refrigeration, Inc.). A microwavable pressure cooker, Nordicware's Tender Cooker (Northland Aluminum Products, Inc., Minneapolis, MN), was used to contain the beans and reduce dehydration during heating. The heated beans were stored at -18°C(0°F). The frozen preconditioned beans were subsequently microwave heated for 30 seconds.

Microwave heating time was established after a come-up time ranging from 3 to 7 minutes. Heat distribution patterns were monitored using MIW-02-10740 fiber optic probes of a Luxtron 755 Multichannel Fluoroptic Thermometer. Control beans were boiled in an open kettle for 15, 30 and 45 mins.

Texture of both the microwave and conventionally cooked beans were evaluated using a Kramer Texture Test System (Model TMS-90) equipped with the FT 3000 transducer and the No. C-15 standard multiple blade shear compression cell. Texture was expressed as Newtons/ 50 g bean sample.

Color of 50.0 g beans was obtained using a Hunter Lab Model D25-PC2 (Hunter Associates Laboratory, Inc., Reston, Va.) system standardized against a white tile.

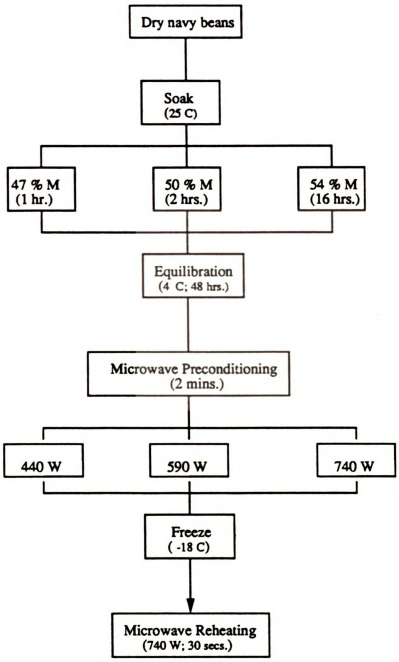


Figure 26. Experimental design for the microwave preconditioning of dry navy beans

Cellular Structure and Starch Granule Appearance of microwave pre-conditioned beans was observed under both Scanning Electron Microscope (SEM) (Model JSM 35CF, Japan Electron Optics Limited, Tokyo, Japan) and Laser Scanning Confocal Microscope (LSM) (Carle Zeiss, Inc., West Germany). Microwave heated bean samples were submerged in liquid nitrogen bath, split into halves and freeze-dried overnight. The dried samples were mounted on circular aluminum stubs with adhesive mounting tab and coated with 20 nm of gold by "Film Vac", sputter-coated and examined under SEM. The same sample preparation was carried out for LSM examination.

Microwave Heating of Bean Starch

Navy beans (Fleetwood cultivar /firm texture) grown during the 1987 crop year in research plots in Saginaw, Michigan, were used in this study.

Bean Starch Isolation

Bean starch was isolated using the method described by Naivikul and D'Appolonia (1979) as modified by Srisuma, N. (1989). Whole bean flour (500 g) was extracted with 1.5 liter of 0.016N NaOH by mixing in a Waring blendor for 2 min. The water-soluble material was removed by centrifugation (2000 *g* for 20 min) (1300 rpm). The precipitate formed was sieved through 60 mesh screen retaining the non-starch components, yielding crude starch. The crude starch was washed with deionized-distilled water (3x), 80% ethanol (2x) and deionized-distilled water (2x). The supernatant and floating sludge were removed and discarded after each washing. The primary starch was air dried at 40°C (104°F) for two days and sieved through a 60-mesh screen to obtain the final bean starch sample. Commercial corn starch (Argo R Pure Corn Starch, CPC International Inc., Englewood Cliffs, NJ) was used as a comparative control.

Bean Starch Chemical Composition

Proximate Analyses. Moisture, ash and protein contents were determined using the standard methods (AACC, 1983 and AOAC, 1984, respectively).

Amylose Content. The amylose content of the isolated bean starch was determined by a modification of the improved colorimetric procedure of Morrison and Laignelet (1983). Approximately 80.0 mg of the bean starch was weighed into a screw-capped test tube to which 10.0 ml of urea-dimethylsulphoxide (UDMSO) was added. After mixing, the tube was placed in a boiling water bath for 90 min. with intermittent mixing until the bean starch-UDMSO solution became transparent and homogeneous. The solution was cooled to room temperature, and a 1.0 ml aliquot of the solution was measured into a 100 ml volumetric flask. Ninety-five ml of deionized-distilled water was added with 2.0 ml of I_2-KI solution (2 mg I_2, 20 mg KI/ml). Final sample volume was adjusted to 100 ml. The absorbance of the sample solution was measured at 635 nm, 15 min. after the I_2-KI addition, using UDMSO-I_2-KI solution as a blank. The blue value is defined as the absorbance/cm at 635 nm of 10 mg anhydrous starch in 100 ml dilute I_2-KI solution at 20°C (Srisuma, 1989). Blue value was converted into amylose content using the following equation :

(Eq. 14) Amylose (%) = (28.414 x Blue Value) - 6.218 .

Heating of Bean Starch

Microwave Heating. Starch slurries were heated in screw-capped test tubes using a Radarange microwave oven (Model - RS458P; Amana Refrigeration, Inc.) with a 700 watt rated power. Desired temperatures were pre-set on the microwave and actual temperatures of the heated bean slurries were monitored using a Luxtron 755 unit with MIW-02-10740 fiber optic probes.

Conventional Heating. Starch slurries were heated in screw-cap test tubes (14 mm I.D.) and placed in a thermostat-controlled water bath. Temperatures were monitored using heating probes inserted inside the tubes and connected to a temperature measuring device (Model T-199, Omega Engineering Inc., Stramford CT).

Bean Starch Physical Properties

Starch Swelling Power and Solubility. Swelling power and solubility of bean starch were determined for the temperature range from 70°C(158°F) to 90°C (194°F), using a modified version of the method of Leach et al. (1959).

Degree of Syneresis. Ten grams of 8% (w/w) starch slurry was prepared in a culture test tube (14 mm I.D.). The slurry was mixed well, heated in the microwave oven or in the water bath, and intermittently mixed (15 secs interval, 3x) using a vortex mixer. After holding the temperature at 70°C for two minutes, the sample was cooled in a 20°C(68°F) water bath for 2 minutes. The starch gel was then incubated at room temperature for 7 days prior to further analyses. Degree of syneresis (DS) of bean starch was determined by measuring the liquid exuded after the storage period. DS was expressed as the percentage weight loss of the gel after discarding the exudate.

Gel Rheological Properties. After weighing the samples prepared above, the gel rheological properties were evaluated using back-extrusion (Hickson et al., 1982). A cylindrical plunger (8.51 mm diameter) attached to the load cell of the Instron Universal Testing Machine (Model 1122) was set to travel through the gel at a constant speed of 50 mm/min. The force required to drive the plunger was registered by a load cell attached to a Hewlett Packard data acquisition microcomputer.

Scanning Electron Microscopy. Cellular structure and starch granule appearance of microwave and conventionally heated starch samples were compared using Scanning

Electron Microscopy (SEM). Starch slurry (10%, w/w) in a culture test tube was heated in the microwave oven and held at 70°C (158°F) for 2 minutes. Same procedure was followed for conventional heating of samples in a water bath maintained at 70°C (158°F). After heating, the starch paste was prepared for SEM examination using a method modified from Pearse (1960 a & b), Bancroft (1975 a, b &c) and Varriano-Marston et al. (1985). This involved dropping the starch paste in a - 70°C(-94°F) n-propanol bath (temperature maintained by solidified acetone and liquid N_2), transferring the frozen starch droplet into liquid N_2 bath and freeze-drying the starch droplet. The dried samples were mounted on circular aluminum stubs with adhesive mounting tab and then coated with 20 nm of gold by "Film Vac," Sputter-coated and examined under a Scanning Electron Microscope (JEOL Model JSM 35CF, Center for Electron Optics, Michigan State University).

Statistical Analysis

The StatView II (1987) computer program for the Macintosh II was used for data calculations and statistical analyses. All mean values are reported plus or minus one standard deviation.

One-way and two-way analyses of variance were determined using the ANOVA program. Mean squares were reported after rounding to two decimal places. Comparisons between treatment means were provided by Fisher's PLSD (Protected Least Significant Difference) test, such that different letters among treatments denote significant differences ($p < 0.05$). Significant F ratios were indicated by asterisks: $p<0.05$ (*). Regression analysis was also determined for the measurement of suspension stability of the bean weaning food/beverage.

CHAPTER I EXTRACTIVE PRETREATMENTS TO IMPROVE DRY BEAN DIGESTIBILITY

Introduction

Processing strategies designed to eliminate antinutritional and flatulence factors in dry edible beans include soaking, extractive pretreatments, germination, dehulling and various cooking and drying methods (Uebersax et al., 1991) Soaking of legume seeds reduces the toxicant content, soluble sugars and minerals due to leaching losses. Enzymatic treatment of beans have been reported to remove much of the flatulence-causing oligosaccharides (Calloway et al., 1971). Germination and fermentation have also been reported to reduce phytic acid which interferes with protein digestibility and mineral bioavailability, and oligosaccharides which have been associated with flatulence. Cooking of dry edible beans is essential to render palatability and inactivate toxic substances. Steam blanching inactivates enzyme systems (Liener, 1962; 1978), enhances digestibility by inactivating biologically active inhibitors, and stabilizes flavor by controlling the oxidation of chemical constituents (Drumm et al., 1990).

Dry bean utilization could be substantially enhanced if they are conveniently available as shelf-stable flours. Drum-drying of bean meals presents the following advantages: 1) allows for utilization of split and culled beans which reduces costs and eliminates the need for maintaining the integrity of beans during digestion and extraction procedures, 2) enables use of selective extracts, and 3) products are pre-cooked and readily hydratable as a nutritious product with controlled product consistency.

The Null Hypothesis tested in this study is stated as follows :

> H_0: the pretreatment of dry beans by physical and biological methods will not enhance their digestibility through removal or inactivation of interfering components prior to drum-drying.

The objectives of this research were : 1) to develop hot water/steam extraction and enzymatic pretreatments procedures 2) produce drum-dried bean meals (DDBMs) and 3) establish chemical and biological compositional differentiation among the pretreated drum-dried bean meals. Two separate studies are discussed in this Chapter : Study I-1 involved the "commercial-scale" development of DDBMs prepared from split dry navy beans and the evaluation of the effects of hot water and enzymatic pretreatments on DDBM composition. Study I-2 was the "bench-scale" development of DDBMs from whole dry navy beans and the characterization of the effects of differential enzymatic pretreatments on resultant DDBMs. Whenever appropriate, comparisons between split and whole DDBMs were discussed.

Materials and Methods

Whole or split dry navy beans were soaked for 16 hours at 25°C in stainless steel tanks and kettles. The different extractive pretreatments were carried out after static hydration of the beans. Weights of materials were recorded during the semicontinuous process to establish a mass balance. The experimental flowchart for this study is presented in Figure 27.

The chemical and biological analyses conducted to evaluate the effectiveness of the different pretreatments for enhancing digestibility of the drum-dried bean meals have been described in detail. Proximate composition was determined using standard methodologies for Moisture (AACC Method 44-40), Protein (AOAC, 1984), Fat (AACC Method 30-25) and Ash (AACC Method 08-01). Soluble sugars of raw beans and drum-dried bean meals were determined by HPLC following a modification of the extraction procedure developed in the MSU Legume Laboratory (Agbo, 1982). Mineral content of raw beans, drum-dried bean meals and water used during processing was determined using an inductively coupled argon plasma (ICP) emission spectrometer (Jarrell-Ash Model 955 Atomcomp) equipped for the simultaneous analysis of 19 elements and maintained by the

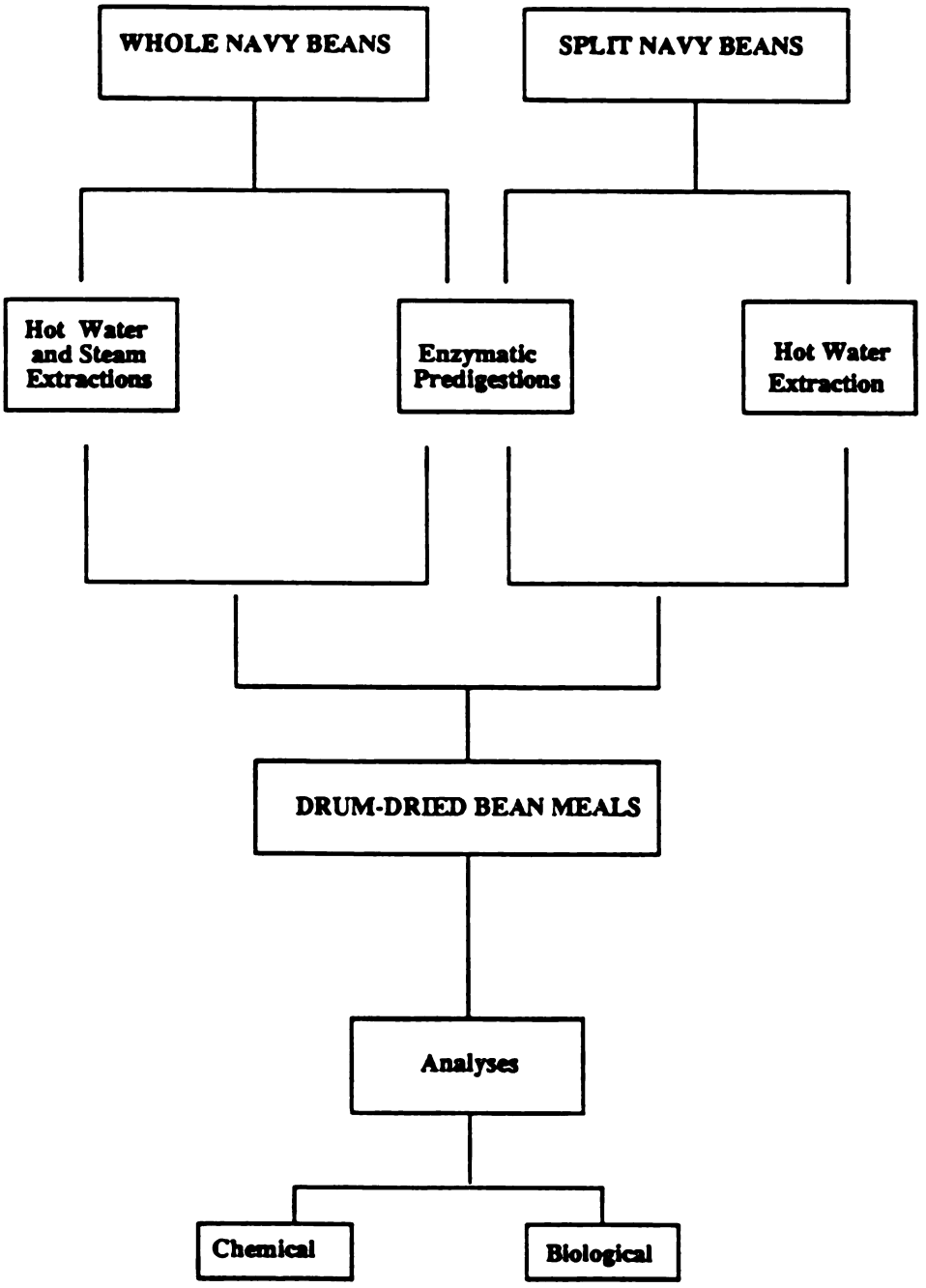


Figure 27. The experimental flowchart for Study I-1: Extractive pretreatments to improve dry bean digestibility.

Department of Pharmacology and Toxicology at Michigan State University. The enzymatic method of Prosky et al. (1988), with modifications from Sigma Chemical Co. (St. Louis, MO), was used to determine the insoluble and soluble dietary fiber content of the drum-dried bean meal samples. A modification of the enzymic/colorimetric method of Holm et al. (1986) was followed to determine starch content of the drum-dried bean meals. The rate of starch hydroysis was evaluated as described by Holm et al. (1985). *In-vitro* protein digestibility was determined using a sequential multi-enzyme system (AOAC, 1990). The procedure of MacKenzie (1977) for the analysis of methionine in plant materials using improved gas chromatography was followed. A modification of the ion-exchange method of Graf and Dintzis (1982) was used to extract the phytates and the HPLC method of Sandberg and Ahderinne (1986) was followed to separate and quantitatively determined the inositol tri-, tetra-, penta- and hexaphosphates of the DDBMs. The method of Kakade et al.(1974) was used to assay for trypsin inhibitory activity. Phytohemagglutinin was determined using Sigma Kit No. P-0526 (Sigma Chemical Co., St. Louis, MO) containing purified *Phaseolus vulgaris* erythroagglutinin as immunogen.

Results and Discussion

Steam extraction (100^{o}C/15 mins) of whole dry navy beans was conducted in preliminary runs and compared with the aqueous pretreatment. Hot water extraction resulted in a substantial reduction in crude ash, soluble sugars and total mineral content due to leaching of soluble components. Based on the results obtained, the hot water pretreatment was recommended as the method of extraction to use prior to drum-drying because of greater potential to leach out undesirable soluble components and the relative adaptability for appropriate application in village-level situations with LDC's. Data obtained for steam extracted DDBMs are summarized in Appendix A and will be referred to whenever discussion is deemed appropriate.

Yield

Figure 28 illustrates the moisture-solids balance for the drum-drying of bean meals collected during commercial-scale runs. Soaked and hydrated navy bean splits were subjected to hot water extraction prior to drum-drying of pre-heated bean slurries. The mass balance was prepared to provide an overall yield estimate. Material balances are useful in comparing actual operations with theoretical values for determining nutrient loss as well as waste generation. An initial 400 lbs of dry navy beans with @13% moisture was hydrated and hot water extracted. The beans were divided into two lots: 1) Half of the cooked beans (200 lbs.) served as CONTROL with all the accumulated formulation water added back to yield a final 118 lbs. of drum-dried bean flakes (63% F.W.; 68% solids). 2) The other half of the bean lot (200 lbs.) was the EXTRACT with leachate discarded and fresh formulation water added back to obtain a final yield of 143 lbs. drum-dried bean flakes (72% F.W.; 77% solids).

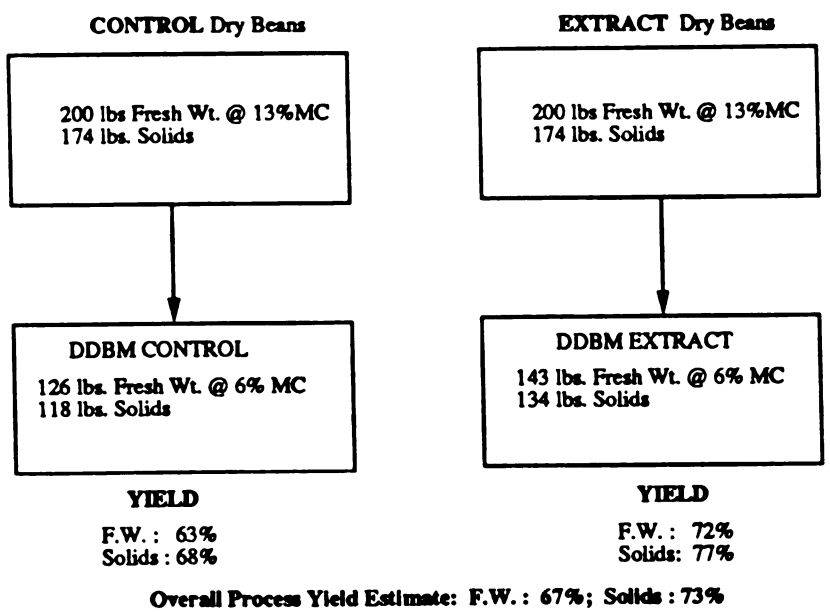


Figure 28: Moisture-solids balance for drum-dried bean meals

Moisture loss brought about by the extreme high temperature during the drum-drying process (≈ 240°C) contributed to the losses in DDBM yield. Additional losses were attributed to the physical loss of slurry during milling and pumping and due to losses of flaking sheets at the drum-dryer. These data were obtained during the experimental process sequences and represent two independent batches. Therefore this mass balance is useful to describe the general process yield. However, no inferences may be drawn regarding the extraction treatments.

Proximate Composition

Tables 4 and 5 summarize the proximate composition of split and whole DDBMs, respectively. Moisture contents of split DDBMs were significantly different ($p < 0.05$) (ranging from 5.1% to 12.6%). Enzyme pre-digestion with Viscozyme resulted in significantly higher ($p < 0.05$) protein (26%) and carbohydrate contents (74%) than raw split beans and the other split DDBMs. Crude fat content was also significantly different ($p < 0.05$) between the split DDBMs, although it was generally low for both the raw beans and the DDBMs. Processing had a significant influence ($p < 0.05$) on ash content of the DDBMs.

Crude protein content of whole DDBMs was not significantly affected by the type of enzyme utilized for pre-digestion. Pre-digestion with the proteases (Alcalase and Neutrase) both resulted in slightly lower fat values of the whole DDBMs. Ash content of whole beans increased with processing and enzyme pretreatment, except for the Celluclast pre-digested DDBM which was lower than the raw whole bean.

The lower carbohydrate content of the EXTRACT DDBM could have resulted from loss of soluble components upon removal of the leachate. Sprouting has been reported to result in an increase in ash content apparently caused by loss of starch (Chavan and

Table 4. Proximate chemical composition of split bean drum-dried meals[1]

Treatment[2,3]	Moisture (%)	(%db) Protein (%)	Fat (%)	Ash (%)	Carbohydrate[4] (%)
Raw	12.55+0.12e	23.75+0.88a	1.59+0.06c	3.88+0.06a	70.79+0.86ab
Control	5.07+0.09a	22.48+0.27a	1.22+0.03a	4.10+0.10b	72.21+0.28b
EXTRACT	11.82+0.06d	24.05+1.34a	1.48+0.05b	4.28+0.11c	70.18+1.47a
Viscozyme	5.59+0.42b	26.24+1.36b	2.15+0.07d	4.28+0.13c	74.38+ 1.08c
Sprout	6.24+0.04c	23.20+0.91a	1.49+0.04b	4.12+0.00b	71.19+0.97ab

[1] n=3
Means within a column followed by different letters are significantly different ($p < 0.05$)

[2] Hot Water Extraction

[3] Raw, Split Bean Meal; Control, Non-extracted; EXTRACT, Fresh formulation; Viscozyme, Commercial preparation; Sprout, 1% germinated slurry

[4] Calculated by difference: 100 - (% Moisture + % Protein + % Fat + % Ash)

Table 5. Proximate chemical composition of non-extracted (CONTROL) with subsequent enzyme-predigested treated whole drum-dried bean meals[1]

Treatment[2]	Moisture (%)	(% db) Protein (%)	Fat (%)	Ash (%)	Carbohydrate[3] (%)
Raw	13.91±0.21e	25.07±0.85a	1.76±0.02c	3.39±0.07a	64.89±0.9d
CONTROL	7.85±0.53b	24.37±0.03a	1.28±0.02b	4.06±0.02b	62.44±1.0b
Sprout	9.94±0.56c	25.72±0.00a	1.19±0.03a	4.16±0.01b	58.99±1.1a
Viscozyme	5.42±0.85a	24.09±1.44a	1.99±0.04c	4.11±0.03b	64.39±0.8c
Alcalase	11.09±0.53d	23.46±2.67a	0.95±0.03a	4.15±0.05b	59.56±0.5a
Neutrase	7.60±0.34b	23.64±0.38a	0.98±0.05a	4.26±0.04c	63.53±0.4c
Celluclast	5.20±0.43a	23.22±0.46a	1.13±0.01a	3.85±0.05a	66.61±0.6e

[1] n=3
Means within a column followed by different letters are significantly different ($p < 0.05$)

[2] Raw,Whole bean meal; CONTROL, non-extracted; Sprout, 1% germinated bean slurry; Commercial Enzyme preparations (Viscozyme, Alcalase, Neutrase and Celluclast)

[3] Calculated by difference: 100 - (% Moisture + % Protein + % Fat + % Ash)

Kadam, 1989), but this effect was not observed for the Sprout DDBMs. Ash content has been reported to decrease after cooking due to leaching, but this was not the case for DDBMs, which were significantly higher in ash content than the raw bean. However, the values were still within the reported range of values for dry beans (3.5% to 4.1%).

Soluble Sugars

Soluble sugars (particularly the oligosaccharides raffinose and stachyose) were present in lower amounts following hot water extraction compared to steam extraction (Appendix A). Figure 29 illustrates that sugar content of split bean CONTROL and EXTRACT DDBMs are not significantly different ($p < 0.05$). However, the differential enzymatic pretreatments employed had an influence on the soluble sugar content of whole DDBMs, especially the simple sugars (glucose and sucrose) which increased with processing (Figure 30). The aqueous extraction in this study resulted in a more significant reduction of the soluble sugars compared to the steam pretreatment, indicating leaching of soluble components (Appendix A). However, there was no significant difference ($p < 0.05$) in theoligosaccharide content between CONTROL DDBM and EXTRACT DDBM, although the latter did possess slightly less stachyose. This suggests that the hot water extraction conditions (60°C/30 mins.) may not have sufficiently leached out the soluble components. A temperature of 60°C was deemed sufficient to initiate bean starch gelatinization, mobilize soluble constituents and yet not result in a mushy product which would decrease yield and ease of bean separation. The enzymatic pretreatments (Viscozyme and Sprout) lowered the stachyose content of split DDBMs, particularly in the case of the Sprout split DDBM. According to Chavan and Kadam (1989), germination or sprouting of grains causes a significant reduction in raffinose saccharides. Viscozyme exhibited a darker surface color (brown hue), indicating occurrence of Maillard reaction as a result of increased glucose content. Borejszo and Khan (1992) reported reduction of raffinose and stachyose by high temperature extrusion of pinto bean high starch fractions, possibly due

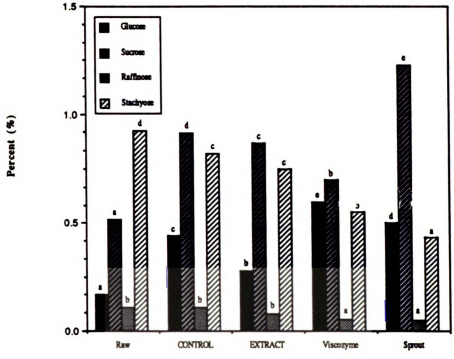


Figure 29. Soluble sugars of split drum-dried bean meals ($p < 0.05$)

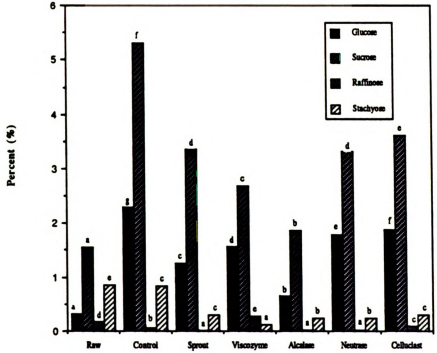


Figure 30. Soluble sugar content of enzyme predigested whole drum-dried bean meals ($p < 0.05$)

to Maillard reaction between charged protein groups and reducing sugars. The enzyme predigested whole DDBMs also exhibited lower stachyose compared to the CONTROL DDBM, clearly indicating the potential of selective enzyme pretreatments to reduce oligosaccharides. Processing increased glucose content of the DDBMs as a result of starch gelatinization. The CONTROL DDBM exhibited the highest glucose content, brought about by the retention of the formulation waters; glucose content of the EXTRACT DDBM was lower as a result of the removal of the leachate. The Sprout split DDBM demonstrated almost two times the sucrose content of the CONTROL DDBM. According to Chavan and Kadam, (1989), during germination of wheat, starch content is decreased whereas reducing sugars are increased. The sucrose peak may actually be a composite of sucrose and some other disaccharide (such as maltose) having almost the same retention time as sucrose.

Total Mineral Content

Tables 6 and 7 summarize the total mineral content of split and whole DDBMs, respectively. Calcium and iron concentrations of split beans increased with processing. Sprout split DDBM demonstrated a significantly higher ($p < 0.05$) calcium value than the other split DDBMs while CONTROL DDBM exhibited a higher iron content compared to the other DDBMs. Processing had no significant influence on the zinc, phosphorus, magnesium, manganese and copper contents of split DDBMs.

Calcium content of Alcalase pretreated DDBM is significantly lower ($p < 0.05$) compared to that of the other enzyme pretreated whole DDBMs. Sprout pretreated DDBM demonstrated a significantly higher ($p < 0.05$) iron value than the other pretreated DDBMs. The differential enzymatic pretreatments likewise had no significant effect on the zinc, manganese, copper and potassium values of the whole DDBMs. However, iron, phosphorus, aluminum and sodium concentration increased with processing.

Table 6. Total mineral content (ppm) of split bean drum-dried bean meals[1]

Treatment[2,3]	B	Ba	Ca	Cu	Fe	Mg	Mn
Raw	9.47±0.38a	0.88±0.02a	1110.00±3.39a	7.69±0.03a	128.00±0.78a	1540.00±5.15a	14.70±0.03a
CONTROL	9.72±0.05a	1.41±0.08b	1557.30±11.1b	9.86±0.04b	184.52±3.92e	1714.50±3.22c	17.97±0.03c
EXTRACT	8.05±0.07a	1.48±0.01b	1687.30±1.17c	9.09±0.01b	131.52±0.25b	1644.50±4.21b	16.57±0.02b
Viscozyme	8.45±0.13a	1.31±0.04b	1407.30±2.31b	9.83±0.09b	150.52±0.46d	1744.50±5.85c	18.67±0.03d
Sprout	9.01±0.30a	1.41±0.04b	2057.30±6.82d	8.42±0.03a	142.52±0.45c	1784.50±7.90d	17.47±0.05c

[1] n=3
Means within a column followed by different letters are significantly different ($p < 0.05$)

[2] Hot Water Extraction

[3] Raw,Whole Bean Meal; CONTROL, Non-extracted; EXTRACT, Fresh formulation; Viscozyme, Commercial preparation; Sprout, 1% germinated slurry

Table 6 (cont'd).

Treatment[2,3]	Mo	P	Zn	Al	Cr	Na	K
Raw	2.19±0.09a	4870.00±8.65a	30.80±0.19a	78.90±2.43c	2.34±0.38a	2.41±0.37a	13700.00±100.00b
CONTROL	2.51±0.13b	5240.00±35.4b	34.75±0.32b	125.00±6.51d	3.84±0.25b	78.89±1.92b	13898.57±49.20b
EXTRACT	2.57±0.07b	4770.00±11.8a	30.45±0.09a	23.30±0.09a	5.73±0.07d	369.29±2.05d	10198.57±53.90a
Viscozyme	3.06±0.14c	5420.00±26.7b	32.85±0.17b	54.40±2.40b	4.92±0.09c	760.29±1.58e	11798.57±52.50a
Sprout	2.45±0.14b	5060.00±22.4b	32.45±0.22b	59.40±2.50b	3.07±0.12b	92.09±1.71c	11298.57±55.40a

[1] n=3
Means within a column followed by different letters are significantly different ($p < 0.05$)

[2] Hot Water Extraction

[3] Raw,Whole Bean Meal; CONTROL, Non-extracted; EXTRACT, Fresh formulation; Viscozyme, Commercial preparation; Sprout, 1% germinated slurry

Table 7. Total mineral content (ppm) of of non-extracted (CONTROL) with subsequent enzyme-predigested treated whole drum-dried bean meals[1]

Treatment[2,3]	K	P	Mg	Ca	Na	Fe
Raw	11800.00±152.00c	3760.00+13.60b	1400.00+15.2a	1250.00+5.37a	2.57+0.14a	61.90+0.41a
Control	11400.00±21.20b	4820.00±17.70c	1520.00±7.79b	1440.00±4.90c	277.00±1.45e	142.00±0.63b
Viscozyme	12000.00±11.30d	5190.00±40.70c	1600.00±7.65d	1550.00±1.50d	940.00±0.82f	144.00±0.39b
Sprout	10700.00±26.20a	520.00±25.00a	1470.00±6.51a	1420.00±5.81c	196.00±1.73d	252.00±0.39c
Alcalase	11598.57±11.40b	4980.00±14.80c	1585.00±9.63c	1237.00± 0.21b	132.00±0.31c	286.29±2.13c
Celluclast	12498.57±46.80e	5190.00±24.60c	1685.00±8.54e	1687.00±5.98f	131.00±1.01c	721.29±0.53d
Neutrase	11698.57±0.00b	4890.00±6.13c	1585.00±4.40c	1470.00±2.92c	120.00±0.46b	991.29±50.00e

[1] n=3, Means within a column followed by different letters are significantly different (p <0.05)

[2] Hot water Extraction

[3] Raw,Whole bean meal; CONTROL, non-extracted; Sprout, 1% germinated bean slurry; Commercial Enzyme preparations (Viscozyme, Alcalase, Neutrase and Celluclast)

Table 7. (cont'd.)

Treatment	Zn	Al	Mn	B	Cu	Mo	Ba
Raw	24.80±0.22a	9.65±0.30a	17.50±0.04c	6.55+0.27a	9.55+0.05b	0.84+0.08a	1.10+0.02a
Control	32.30±0.07c	44.90±1.67b	17.40±0.06c	9.22±0.50c	8.08±0.05a	1.62±0.15b	1.17±0.02a
Viscozyme	33.90±0.15c	72.00±2.63d	16.70±0.11b	9.56±0.23c	8.40±0.05a	1.79±0.30b	1.27±0.01a
Sprout	30.20±0.14c	60.00±1.35c	16.00±0.09b	8.44±0.07b	8.39±0.05a	1.83±0.19b	1.07±0.02a
Alcalase	33.45±0.26c	49.90±0.26b	16.57±0.06b	8.08± 0.34b	8.73±0.02a	2.12±0.04c	0.95± 0.01a
Celluclast	32.75±0.19c	48.90±2.50b	16.87±0.06b	9.06±0.12c	8.81±0.08a	2.43±0.12c	1.14±0.04b
Neutrase	31.25±0.08b	46.10±1.15b	15.67±0.06a	8.70±0.28b	8.33±0.08a	2.29±0.16c	0.99±0.02a

[1] How Water Extraction

[2] n=3, Means within a column followed by different letters are significantly different ($p < 0.05$)

[3] Raw,Whole bean meal; CONTROL, non-extracted; Sprout, 1% germinated bean slurry; Commercial Enzyme preparations (Viscozyme, Alcalase, Neutrase and Celluclast)

High mineral values of dry beans cannot be equated with high bioavailability of some minerals because of the presence of significant amounts of phytic acid which impedes the absorption of multivalent cations like calcium, magnesium, zinc and iron. Lola and Markakis (1975) reported that majority of the total phosphorus content of dry beans is present as phytic acid or its calcium, magnesium and potassium salts. According to Tecklenburg et al.(1984), total phosphorus content correlated well with phytic acid content and protein content. Results of this study demonstrated a slight increase in phosphorus and a significant increase in calcium for the Sprout split DDBMs, contrary to the observation of (Chavan and Kadam, 1989) that calcium, total phophorus and phytate-P decreased during sprouting of millets.

Dietary Fiber

The insoluble dietary fiber comprised approximately 73% to 80% of Total Dietary Fiber (TDF), with the soluble fiber making up approximately 25% of TDF. CONTROL and EXTRACT split DDBMs were not significantly different ($p < 0.05$) in their soluble and insoluble fiber components (Figure 31). Dietary fiber content of whole DDBMs was influenced by the enzyme used for predigestion (Figure 32). According to Hughes (1991), dietary fiber extracts or isolates derived from whole foods possess a ratio of soluble to insoluble dietary fiber similar to that of food from which they are derived, but are higher in total dietary fiber. Dry beans contain approximately 7% soluble dietary fiber and 13% insoluble dietary fiber. Dry bean dietary fiber is primarily structural (includes lignin, cellulose and hemicellulose) in nature, and not easily isolated or extracted because it is intimately involved in maintaining cellular structure and cannot be easily separated from the other cellular components. Nonstructural dietary fibers include the soluble pectin, gums and mucilages. Srisuma et al. (1991) reported that the cell wall strcuture of bean cotyledon possesses a high content of matrix polysaccharides (Hot water soluble polymer, 25.7% -

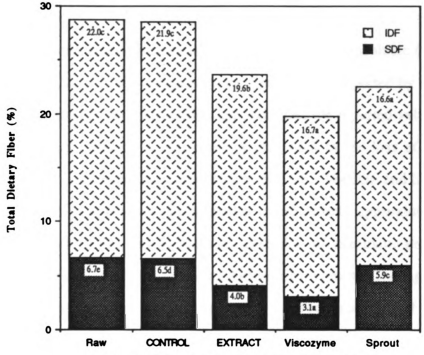


Figure 31. Insoluble and soluble dietary fibers of split bean drum-dried meals

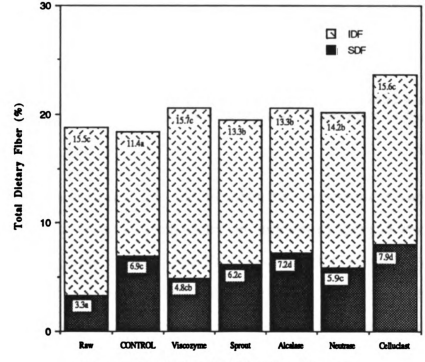


Figure 32. Insoluble and soluble dietary fibers of whole drum-dried bean meals

322.5% and Hemicellulose B, 14.6% to 19.2%) with less cellulose, Hemicellulose A and lignin.

Results of this study demonstrated a higher content of insoluble dietary fiber for the DDBMs (16 - 22.1% IDF) compared to the figure (13%) reported by Hughes (1991) for dry beans, although the soluble fiber approximated the reported value of 7%. The higher TDF content obtained in this study is attributed to the loss of moisture with processing, thereby increasing the concentration of TDF. The IDF and SDF contents of DDBMs subjected to enzymatic pretreatments more closely approximate the figures reported by Hughes (1991), and indicate the potential of enzymatic predigestion as a processing pretreatment to influence product dietary fiber content. The higher TDF value obtained for the raw bean meal also suggest the inherent presence of indigestible or resistant starch which was made more accessible to enzymatic digestion during drum-drying and enzymatic pretreatments. Dietary fiber has been implicated in the flatulence problem associated with consumption of dry beans, and has been attributed to the soluble component pectin.

Effect of Extractive Pretreatments on the *In-vitro* Digestibility and Microstructure of Starch in Drum-dried Bean (*Phaseolus vulgaris*) Meals

Starch Content

Table 8 and figures 33 and 34 summarize the effects of various extractive pretreatments on the apparent starch content of drum-dried split and whole bean meals, respectively. All the raw and drum-dried bean meal (DDBM) samples were significantly lower ($p < 0.05$) in total starch content than the reference samples, cornstarch (95%) and all-purpose flour (wheat) (75%). Starch content of raw whole and split navy bean meals were estimated to be 48 and 60%, respectively. Split CONTROL (54%), Viscozyme (61%) and Sprout (57%) DDBMs had slightly higher starch contents than their

Table 8. Effect of enzymatic pretreatments on the total starch content of bean drum-dried meals[1]

Treatment[2,3]	Starch content (% db)
A. Split Drum-Dried Bean Meals	
Raw	68.02 ± 2.71b
CONTROL	57.29 ± 2.21a
EXTRACT	60.16 ± 0.04a
Viscozyme	64.58 ± 3.09b
Sprout	60.35 ± 1.00ab
Reference Samples	
Cornstarch	99.76 ± 2.81d
All-Purpose Flour	75.04 ± 1.22c
$LSD_{(0.05)}$	5.07
B. Whole Drum-Dried Bean Meals	
Raw	55.77 ± 1.24ac
CONTROL	56.08 ± 7.31a
Sprout	56.07 ± 0.25ab
Viscozyme	61.92 ± 3.16b
Alcalase	49.77 ± 7.86a
Neutrase	59.74 ± 2.3bc
Celluclast	60.16 ± 3.39bc
Reference Samples	
Cornstarch	99.76 ± 2.81e
All-Purpose Flour	75.04 ± 1.22d
$LSD_{(0.05)}$	9.33

[1] n=2; Values are the means of two assays
Means within a treatment group followed by different letters are significantly different ($p < 0.05$)

[2] Hot Water Extraction (60°C/60 minutes)

[3] Raw,Whole bean meal; CONTROL, non-extracted; Sprout, 1% germinated bean slurry; Commercial Enzyme preparations (Viscozyme, Alcalase, Neutrase and Celluclast)

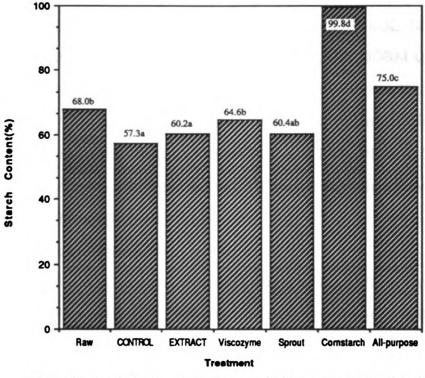


Figure 33. Total starch content of raw and split bean drum-dried meals

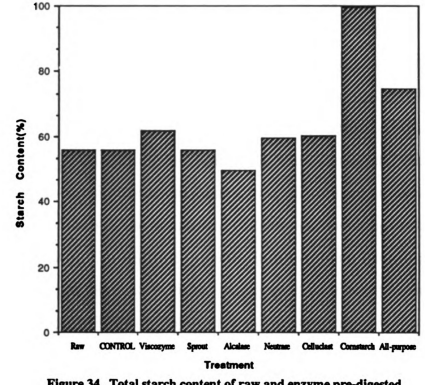


Figure 34. Total starch content of raw and enzyme pre-digested whole bean drum-dried meals

corresponding whole drum-dried bean meals (52%, 59% and 51%, respectively) due to hull loss in split beans. The starch content of the split CONTROL DDBM (54%) was not significantly different ($p < 0.05$) from the EXTRACT DDBM samples (53%), but significantly lower ($p < 0.05$) than the enzyme predigested samples, Viscozyme (61%) and Sprout (57%). The enzyme pre-digested whole DDBMs yielded significantly higher ($p < 0.05$) apparent starch contents (55% to 59%) than the CONTROL DDBM (52%), except for the Alcalase (44%) and Sprout (51%) treated DDBMs which were not significantly different ($p < 0.05$).

In-vitro Starch Availability

Tables 9 and 10 summarize the digestibility of starch (%) in variously treated split and whole DDBMs held under different incubation periods (0, 15, 60 and approx. 380 mins.). Figures 35 and 36 depict the *a*-amylolysis curves of split and whole DDBMs, respectively. All the split and whole DDBMs were hydrolyzed at a significantly lower ($p < 0.05$) rate than the boiled (20 min. at 100^{o}C) cornstarch used as a reference sample. The other reference, boiled all-purpose flour, exhibited a lower degree of hydrolysis than cornstarch, but was not significantly different ($p < 0.05$) from the EXTRACT and enzyme pre-digested split (Viscozyme and Sprout) DDBMs. The boiled raw bean flours (split and whole) were not significantly different ($p < 0.05$) from the CONTROL DDBMs and exhibited the lowest degree of hydrolysis. The various extraction pretreatments employed prior to drum-drying all resulted in an increased rate of amylolysis, with the enzyme pre-digested DDBMs demonstrating the largest increment in starch availability. The degree of hydrolysis increased in the following order : a) for split bean, raw = CONTROL < EXTRACT = Sprout < Viscozyme; whereas b) for whole beans, raw = CONTROL = Sprout <Viscozyme < Neutrase = Alcalase = Celluclast (Figures 36 and 37).

Table 9. Effect of extraction treatments on *in-vitro* digestibility of starch in raw whole navy beans and whole bean drum-dried meals[1]

Treatment[2]	Incubation time (min)			
	0	15	60	Overnight
		Degree of Hydrolysis (%)		
Raw	2.100 ± 0.12a	9.460 ± 0.71ab	13.530 ± 0.25a	17.490 ± 0.34a
HWC	2.630 ± 1.05a	10.880 ± 0.99ab	19.360 ± 2.45ab	26.120 ± 0.78a
HWE	5.660 ± 0.03b	15.670 ± 1.36b	35.910 ± 2.74c	54.090 ± 1.36bc
SC	3.230 ± 0.61a	6.700 ± 3.34a	22.380 ± 12.17ab	36.470 ± 13.03ab
SE	5.850 ± 0.03b	15.730 ± 0.21b	31.000 ± 0.03bc	39.160 ± 0.003ab
Cornstarch	4.320 ± 2.85a	40.080 ± 12.41b	57.600 ± 8.75d	69.070 ± 1.80d
All-Purpose Flour	4.390 ± 3.36a	43.250 ± 7.88b	48.920 ± 5.14b	53.350 ± 6.40b

[1] n=2; Values are the means of two assays
Mean values and standard deviations (different letters within each column indicate significant differences at $p < 0.05$)

[2] Raw, Split Bean Meal; CONTROL, Non-extracted; EXTRACT, Fresh formulation; Viscozyme, Commercial enzyme preparation; Sprout, 1% germinated slurry

Table 10. Effect of enzymatic pre-digestion on *in-vitro* digestibility of starch in raw whole navy beans and whole bean drum-dried meals[1]

Treatment[2]	Incubation time (min)			
	0	15	60	Overnight
		Degree of Hydrolysis (%)		
Raw	2.100 ± 0.12a	9.460 ± 0.71a	13.365 ± 0.43a	17.253 ± 0.34a
CONTROL	3.310 + 0.03a	14.020 ± 1.65ab	21.970 ± 0.64ab	21.970 ± 0.64ab
Sprout	2.160 ± 0.06a	8.920 ± 0.59a	19.467 ± 0.37ab	24.885 ± 0.40a
Viscozyme	2.284 ± 0.002a	17.900 ± 3.63ab	21.302 ± 1.15b	23.015 ± 1.15a
Alcalase	4.880 + 0.23a	18.620 ± 1.06ab	38.040 ± 2.02c	50.680 ± 1.14d
Neutrase	4.700 + 2.33a	17.900 ± 8.40ab	37.590 ± 3.17c	47.790 ± 10.38bc
Celluclast	5.390 + 0.43a	27.100 ± 0.10b	40.740 ± 2.22c	53.420 ± 3.51d
Cornstarch	4.320 ± 2.85a	40.080 ± 12.41c	57.600 ± 8.75bd	69.070 ± 1.80e
All-Purpose Flour	4.390 ± 3.36a	43.250 ± 7.88c	48.920 ± 5.14d	53.350 ± 6.40d
$LSD_{(0.05)}$	3.78	13.16	8.34	9.76

[1] n=2; Values are the means of two assays
Mean values and standard deviations (different letters within each column indicate significant differences at $p < 0.05$)

[2] Raw,Whole bean meal; CONTROL, non-extracted; Sprout, 1% germinated bean slurry; Commercial Enzyme preparations (Viscozyme, Alcalase, Neutrase and Celluclast)

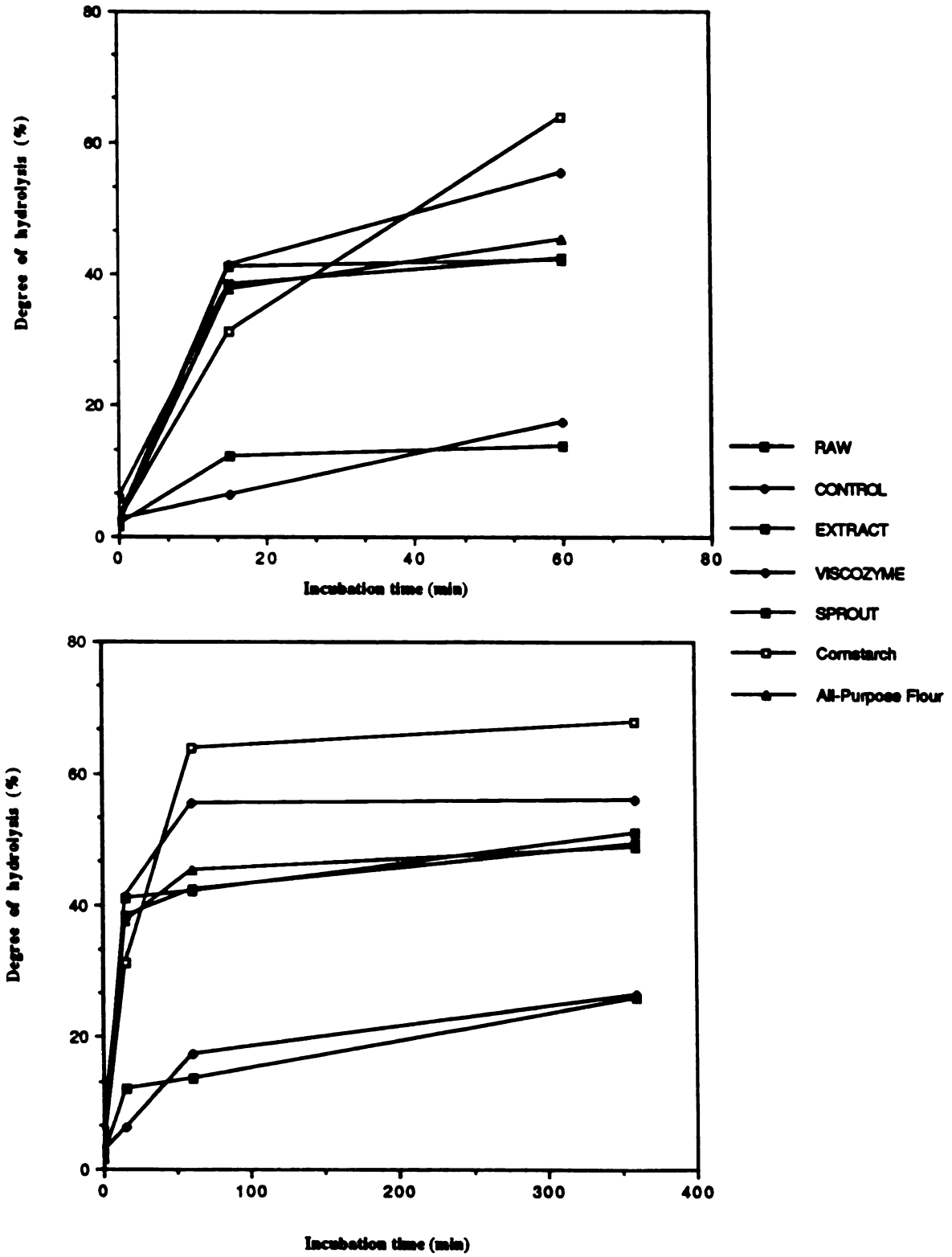


Figure 35. Hydrolysis of starch in raw and split bean drum-dried meals by pancreatic a-amylase

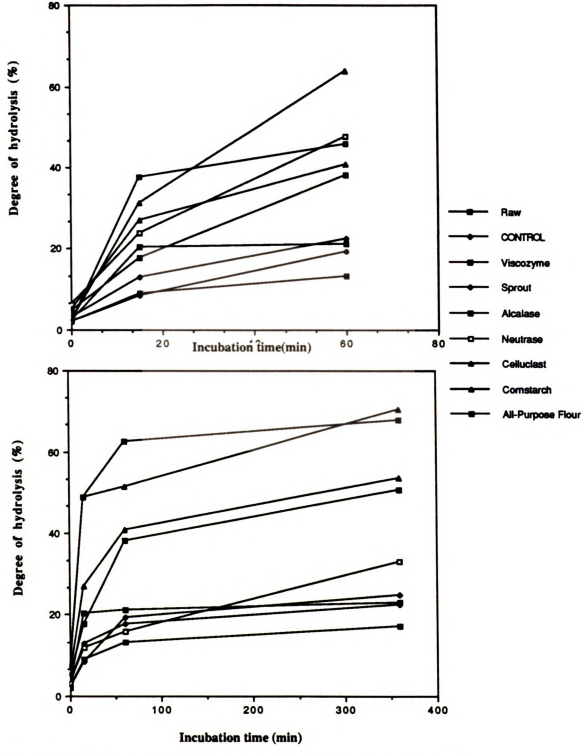


Figure 36. Hydrolysis of starch in raw and whole bean drum-dried meals by in-vitro pancreatic a-amylase

Degree of hydrolysis for whole beans which were pretreated with hot water versus steam cooking increased as follows: raw < HWC < SC < SE < HWE (Appendix C). An additional gelatinization step (boiling for 20 min.) prior to the starch assay, of previously pre-gelatinized split bean DDBMs further increased the rate of amylolysis (Appendix C).

Total and available starch contents of the drum-dried bean meals were determined by following the method of Holm et al. (1986 and 1985, respectively). The following steps were incorporated prior to enzyme digestion : a) solubilization with 4N NaOH at room temperature for 1 hour to measure total starch content and b) pepsin incubation at 37°C for 1 hr. at pH 1.5 for the determination of "potentially available" starch. According to Tovar et al. (1990b), these pretreatments enhanced accessibility of starch to amylases and destroy the integrity of remaining intact cell walls. The alkaline pretreatment used provides for the dispersion of crystalline raw starch and retrograded amylose fractions, resulting in higher starch yields (Englyst and Cummings, 1990). Underestimation of starch content as a consequence of encapsulated starch have been previously reported for precooked legume flours (PCFs). The NaOH or KOH treatment thus provides a way to measure "total starch content" by solubilizing so-called "resistant starch". A preincubation with pepsin has a thinning effect on the cell surface with accompanying alteration of fiber/protein associations occurring in the cell walls (Tovar et al., 1990a).

Total starch yields of the CONTROL and EXTRACT split DDBMs were not significantly different ($p < 0.05$), indicating that the aqueous extractive pretreatment employed did not have a significant effect on starch content ($p < 0.05$), as it did for relatively more soluble simple sugars. Total starch yields of the CONTROL and EXTRACT DDBMs were significantly lower ($p < 0.05$) compared to that of the raw bean meal. Tovar et al. (1990b) reported that differences in susceptibility to enzymatic attack in PCF's and raw flours are mainly due to the release of starch granules during milling of uncooked seeds, whereas starch-containing cotyledon cells remain in the PCF's.

The enzyme pre-digested split bean DDBMs, Viscozyme (commercial), possessed significantly higher ($p < 0.05$) starch contents than the CONTROL and EXTRACT DDBMs. The Sprout DDBM was not significantly different from the CONTROL and EXTRACT DDBMs. Both Viscozyme and Sprout contain cell wall degrading enzymes which could hydrolyze cell wall or starch with a corresponding increase in released glucose. The reference samples, cornstarch and all-purpose flour, possessed significantly higher ($p < 0.05$) starch contents than the boiled raw seed and DDBM flours.

The enzyme pre-digested whole bean DDBM flours did not differ significantly ($p < 0.05$) in their apparent starch contents. However, the cell-wall degrading enzymes, Viscozyme and Celluclast, resulted in the highest starch yields, followed by the protease, Neutrase. These results again indicate the need for the disruption of cotyledon cell wall in order for the starch to be exposed to the amylases. The Sprout DDBM possessed similar starch content as the unextracted sample, CONTROL, indicating that the indigenous amylases present during germination did not sufficiently hydrolyze the starch.

The "potentially available" starch represents the amount of starch which can be hydrolyzed by amylolytic enzymes, and is starch measured after pepsin or homogenization pretreatments of cooked beans (Tovar et al., 1990a; Tovar, 1992). According to Holm et al. (1985), preincubation with pepsin increased the starch availability of raw and boiled wheat to *a*-amylase, indicating a large fraction of the starch was encapsulated in a protein matrix. The disintegration of protein aggregates released highly available gelatinized starch that was readily hydrolyzed by a subsequent incubation with *a* -amylase. The release of starch from the protein matrix indicates that gelatinization alone is not the only factor involved in increasing the starch susceptibility to enzyme action.

Laser Scanning and Polarizing Light Microscopy

Abundant free starch granules with adhering protein bodies were observed in the navy bean flour from milled raw seeds (Plate 1a). The raw bean starch granules exhibited birefringence, as evidenced by the presence of the characteristic "Maltese cross" pattern when viewed under polarizing light microscopy (Plate 1d). The differential extractive pretreatments employed prior to drum-drying resulted in microstructural changes as demonstrated by extensive disruption of the cotyledon cells and exposure of the starch granules (Plates 2a and b, and 3a to e). No evidence of defined cellular structures nor intact starch granules were identified in bean meals subjected to enzymatic pre-digestion of the slurries prior to drum-drying, particularly those meals pre-digested with the commercial enzymes Neutrase and Celluclast (Plates 3i to j) which appeared amorphous. For comparative purposes, LSM micrographs of flour obtained from canned and subsequently freeze-dried whole navy beans are shown in Plate 4. Abundant agglomerates of intact cellular structures entrapping starch granules were evident in these hydrated and thermally processed canned samples. Similar intact cellular structures were observed under LSM of canned whole navy beans (Plate 5). Further, when viewed under polarizing light microscopy, the "Maltese crosses" were still present with relatively greater frequency "crosses" observed in the "firm" (92 RNK 8P) breeding line compared to the "soft" (92 RNK 17E) breeding line, indicating incomplete and differential gelatinization of starch in these thermally processed samples (data not shown).

Plate 1a illustrates the free starch granules released by the breakdown of cell walls of raw navy bean seeds during milling. A high proportion of the starch granules iembedded in a protein matrix (Plate 1d), as evidenced by the preponderance of fluorescent protein bodies. The "Maltese cross" observed under polarizing light microscopy (Plate 1d) is a manifestation of birefringence exhibited by ungelatinized crystalline starch granules. The short boiling step (20 mins. at 100^{o}C) employed prior to the enzyme digestion of the

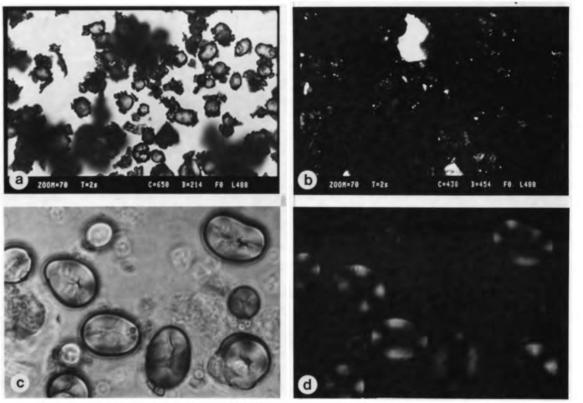


Plate 1. Raw split meal (a-b) under LSM microscopy and (c-d) under polarizing light microscopy

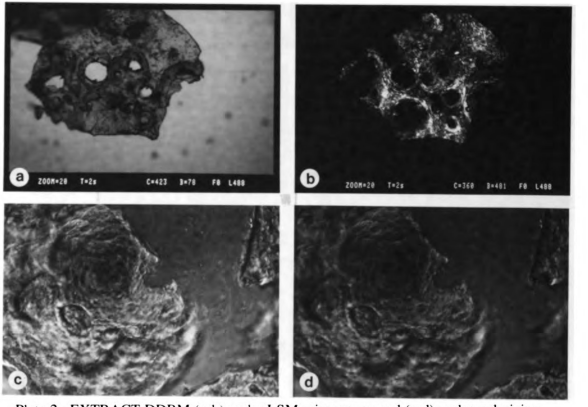


Plate 2. EXTRACT DDBM (a-b) under LSM microscopy and (c-d) under polarizing light microscopy

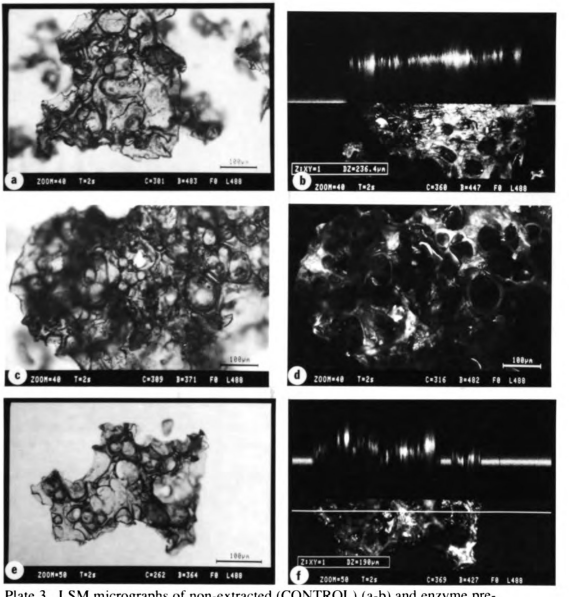
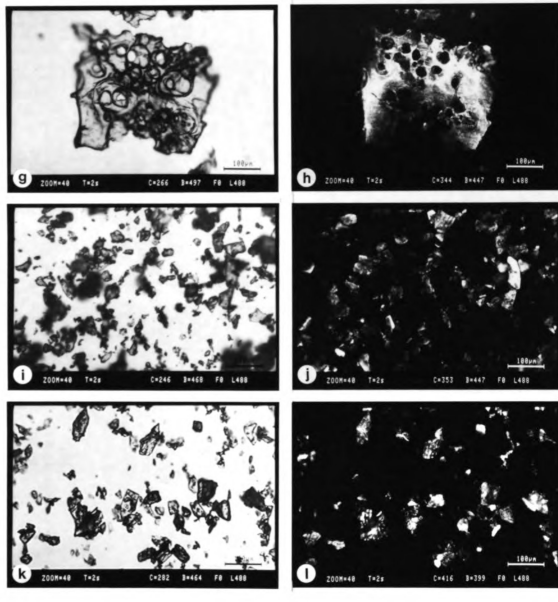


Plate 3. LSM micrographs of non-extracted (CONTROL) (a-b) and enzyme pre-digested whole bean drum-dried meals: Viscozyme (c-d), Sprout (e-f), Alcalase (g-h), Neutrase (i-j) and Celluclast (k-l)


g
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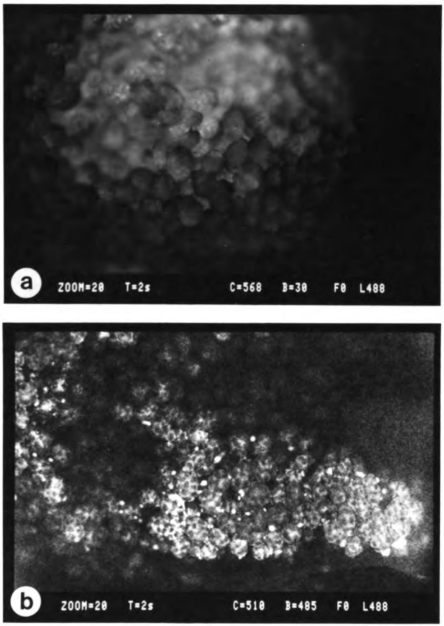


Plate 4. LSM micrograph of canned and subsequently freeze-dried whole navy bean

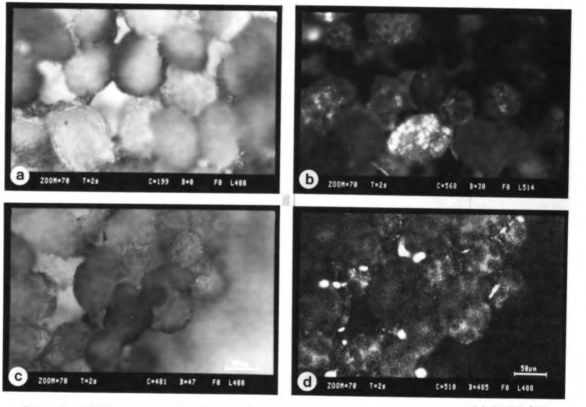


Plate 5. LSM micrographs of (a) soft canned and (b) firm canned bean (92 RNK 8P)

raw bean may have resulted in partial swelling of the bean starch granules. The *a*-amylases cannot easily penetrate through the protein matrix encapsulating starch (Holm et al., 1986) or within incompletely gelatinized granules due to steric hindrance (Wursch, 1986). These factors could explain the lowest degree of hydrolysis (Figure 5) demonstrated by boiled raw bean seeds.

Birefringence was not exhibited by pregelatinized EXTRACT DDBM under polarizing light microscopy (Plate 2), indicating complete gelatinization during the drum-drying process which involved exposure to temperatures as high as 149°C(300°F). Wursch et al. (1986) observed that *a*-amylase digested starch granules progressively from outside of the cell walls. Thus, the absence of the intact cotyledon cells in DDBMs which may be attributed to wet homogenization (Fitzmill) of heated beans prior to drum-drying has resulted to a greater surface area being available to the amylases, rendering the DDBMs susceptible to amylolytic attack with resultant increased digestion rates.

According to Tovar et al. (1992), acidic preincubation of PCF's with pepsin, simulating the gastric phase of digestion, resulted in a greater rate of hydrolysis. The resulting thinned or even missing cell surface and higher amylolytic rate observed in PCF's may be regarded as an enzymatic rather than merely a pH dependent effect. Wursch et al. (1986) also reported a minor change in digestion of leguminous starch during incubation under acidic conditions in the absence of pepsin. Results of LSM in this study demonstrated that enzymatic pre-digestion of bean slurries prior to drum-drying affected the integrity of the cell walls and facilitated the amylolytic process by exposure of starch (Plate 3a-f). DDBMs from whole beans incubated (prior to drum-drying) with the proteases Alcalase (food grade) and Neutrase, exhibited a higher degree of hydrolysis than those pre-digested by Viscozyme and Sprout, indicating potential denaturation of the protein matrix and increased accessibility of *a*-amylase to the substrate. The LSM micrographs (Plate 3d and e) illustrate the disruption of the starch granules increased the total granule

surface area exposed to enzyme action. These results further confirm the observation of Wong et al., (1985) and Tovar et al., (1989) of a possible occurrence of protein/starch interactions which decrease carbohydrate digestibility.

The presence of intact cellular structures encapsulated by a protein matrix (fluorescing) in whole beans canned and subsequently freeze-dried (Plate 4) confirm the observations of Tovar et al. (1992) that freeze-dried PCF's contained more intact cells than vacuum-dried flour. Plate 5 also demonstrates the same observation with canned whole beans ("firm" versus "soft" cultivars) hydrated and thermally processed, clearly demonstrating that the high retort temperature 115°C (240°F) was not sufficient to disrupt cell walls, or to completely gelatinize legume starch, and that gelatinization alone is not the only factor involved in increasing starch susceptibility to amylolytic activity. These results are contrary to the observation of Hughes and Swanson (1989) that flours prepared after autoclaving and warm-air drying of common beans do not contain intact cells. Commercially canned beans have been previously reported to contain resistant starch (Siljestrom and Bjorck, 1990). During cooking, some of the amylose leaches out from the starch granule, remaining within the intact cell and filling spaces between protein bodies and starch granules. If amylose retrogradation occurs, another indigestible matrix is formed in and around the protein and starch granules, thereby contributing to slow protein and starch digestion (Bennink and Srisuma, 1989). During methodology development and screening, it was observed that canned and freeze-dried samples (as in Plate 4) had to undergo a gelatinization step (boiling for 20 mins.) prior to the starch availability assay to enable normal digestion rates. Lower rates of digestibility were observed for samples tested without this additional heat treatment(data not shown). Tovar et al.(1990b) reported that a second boiling treatment promotes a further rise in the enzymatic susceptibility of starch. This could also account for the increased degree of hydrolysis observed with

pregelatinized split DDBMs subjected to a boiling step (20 mins.) prior to the starch assay (data not shown).

The reference cornstarch exhibited the highest degree of hydrolysis, while the all-purpose (wheat) flour was not significantly different ($p < 0.05$) from the enzyme pre-digested DDBMs. Holm et al. (1985) reported that pure starch was more available to salivary *a*-amylase *in-vitro* than starch present in flour. The cohesive properties of wheat proteins result in the formation of glutinous lumps when these samples are being dispersed, making it difficult for the enzymes to degrade the starch completely to glucose.

In-vitro Protein Digestibility

In-vitro protein digestibility of raw split navy beans improved significantly ($p < 0.05$) with processing (Figure 37). The effect of the differential aqueous extractive pretreatments employed prior to drum-drying on *in-vitro* protein digestibility of the split DDBMs were not significantly different ($p < 0.05$).

The differential enzymatic pretreatments (Figure 38) did not significantly enhance ($p < 0.05$) *in-vitro* protein digestibility of whole raw bean meals, with the exception of the Celluclast predigested DDBM which did increase. The CONTROL DDBM (no enzyme pre-digestion) demonstrated a significantly higher ($p < 0.05$) protein digestibility value than the enzyme pre-digested DDBMs. Heat treatment greatly improves the digestibility of most dry bean protein fractions. The improved performance upon heating is partially attributed to inactivation of antinutritional factors such as protease inhibitors and lectins (Nielsen, 1991) and opening of the protein structure through denaturation (Hsu et al., 1977). However, processing can also cause a decrease in protein digestibility via the nonenzymatic browning reaction and thermally induced cross-linking reactions. Hence, a sensitive *in-vitro* protein digestibility method should detect digestibility changes due to protease inhibitors, substrate-bound inhibitors and processing effects. The multienzyme technique utilized in this study for estimating *in-vitro* apparent protein digestibility can

IN-VITRO PROTEIN DIGESTIBILITY OF DRUM-DRIED BEAN MEALS

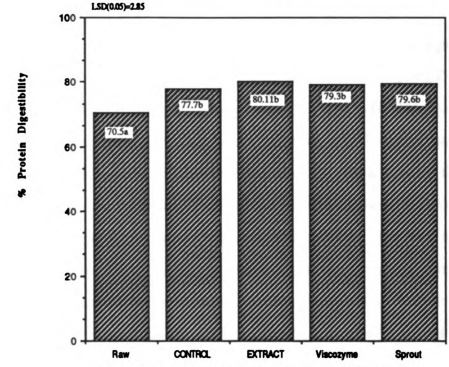


Figure 37. In-vitro protein digestibility of split bean drum-dried meals

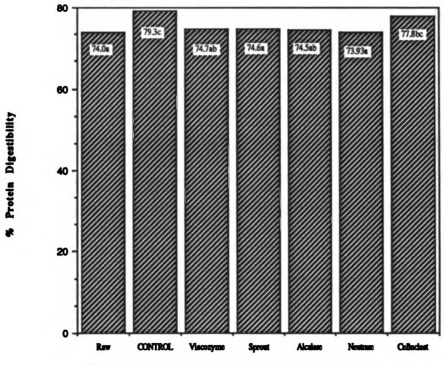


Figure 38. In-vitro protein digestibility of whole bean drum-dried bean meals

detect the effects of trypsin inhibitor and heat treatment on protein digestibility (Hsu et al., 1977). Results of this study demonstrated the improvement in protein digestibility of the DDBMs with heat processing. Although the effects of the aqueous extractive pretreatments were not significantly different, the EXTRACT split DDBMs exhibited a slightly higher (%) protein digestibility over the CONTROL DDBM which indicated the importance of discarding leacheate, thereby removing soluble antinutritional factors. The high temperature involved in drum-drying gelatinized starch, opening the carbohydrate-protein structure and facilitating hydrolysis of proteins by the proteolytic enzymes. This same phenomenon was reported for thermoplastic extrusion involving high temperature, short time heat processing, thereby increasing digestibility of puffed corn grits (Hsu et al., 1977). The slightly higher (%) protein digestibility exhibited by the Split DDBMs over the whole DDBMs demonstrated the significance of the removal of bean hulls (containing less digestible proteins) to improving bean digestibility. Results also indicated that the enzymatic pretreatments with Viscozyme and Sprout (both containing amylases which increased reducing sugars), could have resulted in Maillard reaction as evidenced by the resultant browning reaction in the DDBMs, thereby lowering digestibility of whole DDBMs compared to CONTROL DDBM. According to Hsu et al. (1977), digestibility of bread was decreased due to the Maillard reaction between the reducing sugars which was created from the hydrolysis of starch by the enzymes in the barley malt, and the proteins. The effect of enzymatic predigestions on improved protein digestibility has potential, although it is also clearly demonstrated that the nature of the specific enzyme involved is critical.

Sodium Dodecyl Sulfate (SDS) Gel Electrophoresis

Figure 39 is a graphical representation of the electrophoresis gel which was run for the whole DDBMs subjected to enzymatic predigestion. Gel Lane (A) represents the

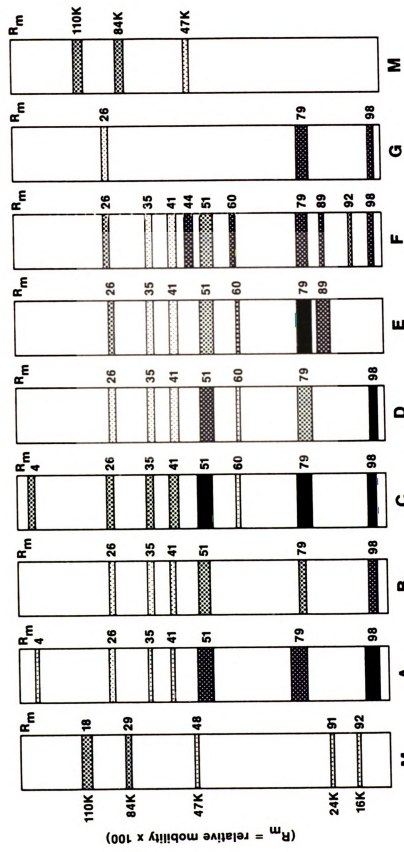


Figure 39. Graphical representation of the SDS gel electrophoretic pattern of enzyme predigested whole drum-dried bean meals ; **M** - Marker; **A** - Raw; **B** - CONTROL; **C** - Sprout; **D**- Viscozyme; **E** - Celluclast; **F** - alcalase; **G** - Neutrase ; M - Marker

protein components present in uncooked or untreated whole (raw) dry bean. CONTROL DDBM (B) represents the processed sample with the retained formulation water. General banding patterns between raw (A) and cooked (B) bean flour are quite similar with little indication of major band position shifts. However, the cooked sample bands were generally less intense than those obtained for raw beans. Gel lanes (C - F) present the CONTROL DDBM (B) upon exposure to selective enzyme pretreatments of the slurry.

It was noted that each enzyme pretreatment resulted in a new band (R_m 60) not evident in the raw or untreated DDBM. Sprout DDBM (C) demonstrated an increased number of protein bands and intensity compared to CONTROL DDBM. It is speculated that these effects (C) have resulted from protein synthesis during the germination or sprouting process when enzymes are generated and overall enzymatic activity increased. However, no dramatic increase in total crude protein was noted for the Sprout-treated sample (Table 5). Whole DDBM subjected to Viscozyme (D), a multienzyme complex made up of cell-wall degrading enzymes, also demonstrated increased numbers of protein bands and a general increase in intensity of banding patterns, which could be a result of the release of the matrix proteins associated with the cell wall. The banding pattern obtained for Celluclast (E), a cellulase which is also a cell-wall degrading enzyme, could also represent release of cell wall associated proteins. Alcalase (F) and Neutrase (G) are both proteases and clearly demonstrate increased in hydrolytic degradation of large molecular weight proteins and partitioning of peptides. Neutrase (G) exhibited increased evidence of hydrolysis and extensive degradation through the formation of lower molecular weight peptides following enzymatic predigestion. These data demonstrate no major changes in protein patterns as a result of cooking; however, selective hydrolysis of the native proteins was evident upon enzymatic digestion and may be associated with differential *in-vitro* protein digestibility.

Amino Acid Composition

Table 11 summarizes the amino acid composition of split raw beans and DDBMs. Methionine content of split beans increased with processing; CONTROL DDBM was almost two times the amount of the EXTRACT and Viscozyme DDBMs. Cystine was not present in any of the DDBMs. Lysine in EXTRACT DDBM was two times that found in raw split beans; lysine in CONTROL DDBM was similar to that in raw beans, while a decrease was observed in the enzyme pre-digested Viscozyme. Isoleucine, valine and phenylalanine concentration increased with processing of DDBMs, with the EXTRACT DDBM containing the highest concentration followed by the CONTROL and either a decrease or a similar level observed in Viscozyme compared to the raw bean meal. The sulfur amino acids cystine/cysteine (Cys) and methionine are of primary concern in protein utilization because they are first-limiting in dry beans. Cys has been found to be more heat-sensitive than methionine. Measuring Cys of beans receiving heat treatment is important in estimating the deterioration of nutritive value due to heating. In navy beans, Cys destruction was 30% at 100°C for 10 mins. but increased with increased cooking time (Dhurandhar and Chang, 1990). In this study, cysteine content for both the raw beans and DDBMs was 0.00 *p*mole, which could be a result of an analytical problem. Lysine content for the Viscozyme-treated DDBM was lower than that of the other DDBMs. This could be attributed to the non-enzymatic browning (Maillard) reaction, demonstrated by the brown hue of the flakes. Lysine losses resulting from Maillard reaction involve the reaction of the *E*-amino group on the lysine residue which becomes blocked by a sugar, yielding a biologically unavailable lysine residue. Heating of dry bean protein improves the rate of release of methionine by *in-vitro* treatment with enzymes (Rayas-Duarte et al., 1988). Methionine increased with processing, and was highest in the CONTROL DDBM indicating retention of the amino acid with the original formulation water. This

Table 11. **Amino acid content (*p*moles) of split drum-dried bean meals.**

Amino acid	RAW	CONTROL	EXTRACT	VISCOZYME
Aspartic	1837.68	3030.18	4264.42	2047.05
Glutamic	2952.91	5052.50	7155.03	3374.45
Hydroxyproline	43.58	0.00	97.94	43.23
Serine	1390.13	2360.80	3586.96	1706.30
Glycine	1609.90	2848.92	4553.57	2007.64
Histidine	480.48	822.74	1337.58	566.54
Arginine	557.13	920.70	1398.47	631.51
Threonine	1111.19	2027.04	3349.60	1431.46
Alanine	1349.96	2406.43	3468.09	1609.24
Proline	1352.50	2488.36	3814.26	1652.58
Tyrosine	120.37	198.79	658.42	145.10
Valine	979.08	1867.45	2931.52	1188.85
Methionine	122.84	290.43	142.60	145.94
Cysteine	0.00	0.00	0.00	0.00
Isoleucine	677.03	1224.71	2007.57	805.98
Leucine	1256.67	2191.53	3659.46	1479.15
Phenylalanine	601.14	1009.86	1796.19	675.71
Lysine	310.94	396.31	670.65	250.51

Table 12. Methionine content of split bean drum-dried meals[1]

Treatment	Methionine (nmoles Met / unit of protein)
A. Split Bean Drum-Dried Meals	
Raw	191.26± 5.25a
CONTROL	203.21±29.07a
EXTRACT	196.99± 1.53a
Viscozyme	201.95±13.70a
Sprout	207.96± 2.49a
B. Whole Bean Drum-Dried Meals	
Raw	192.70±8.89a
CONTROL	220.67±0.67c
Viscozyme	226.17±6.56d
Sprout	205.09±2.41b
Alcalase	215.50±6.36c
Neutrase	210.66±2.36c
Celluclast	210.66±2.83c

[1] n=2; Values are the means of two assays
Means within a column followed by different letters are significantly different ($p < 0.05$)
[2] Hot Water Extraction (60°C/30 minutes)
[3] Raw, Split or Whole Bean Meal: CONTROL, Non-extracted; EXTRACT, Fresh formulation; Commercial Enzyme preparations (Viscozyme, Alcalase, Neutrase and Celluclast); Sprout, 1% germinated bean slurry

observation confirms previous reports that methionine was not decreased by conventional cooking or alkaline treatment (Table 12).

Trypsin Inhibitory Activity (TIA)

Table 13 summarizes the units of TIA present in split raw and DDBMs. Processing significantly reduced ($p < 0.05$) TIA from 18,703 units of TIA per gram of raw split beans to a range of 1000 to 3300 units of TIA per gram of split DDBM. The EXTRACT DDBM was significantly lower in residual TIA compared to the other split DDBMs.

The antinutritional factor trypsin inhibitor (TI) has been reported to be heat resistant, and 50-80% of the original trypsin inhibitor activity is retained after various heat treatments. TIs are high in cystine. Trypsin activity of navy beans (1.9 to 2.7%) was retained even after cooking for 40 min at 100°C. Heat inactivation of TI activity in soaked whole navy beans was more effective compared to dry roasting (Dhurandar and Chang, 1990). The significant reduction ($p < 0.05$) of trypsin activity in the split DDBMs indicated that they have received adequate heat processing. The drum-drying process called for an extremely high temperature of approximately 240°C; in addition, pretreatments including a 16-hr soaking period and hot water extraction (60°C/30 min) facilitated effective reduction of TI.

Phytic Acid

Table 13 summarizes the results of the HPLC analyses of split bean DDBMs for separation and quantitative determination of inositol tri-, tetra, penta and hexaphosphates (IP_3 - IP_6). Fractional data are expressed as a percentage of the sum of inositol phosphates. Soaking has been reported to decrease the content of phytate in cereals and legumes. This is readily apparent in the degradation of phytate (IP_6) in the EXTRACT DDBM (leachate discarded), with a corresponding increase in the formation of lower

Table 13. Heat stable Trypsin Inhibitor Activity (TIA)[1,2], inositol phosphates (%) and phytate content (%) in split bean drum-dried meals

Treatment[3,4]	units of TIA /g DDBM	Inositol Phosphates(%)				Total[5] Phytate (%)
		IP_3	IP_4	IP_5	IP_6	
Raw	18,703±318d	6.72	6.33	16.21	70.74	100
CONTROL	3,292±30c	ND	ND	25.39	74.61	100
EXTRACT	1,088±11a	16.27	9.34	20.71	53.68	100
Viscozyme	2,506±41b	4.78	1.21	15.75	78.26	100
Sprout	3,221±23c	ND	4.95	18.22	76.83	100

[1] n=3, Means within a column followed by different letters are significantly different ($p < 0.01$)

2 Rayas-Duarte et al., 1991

[3] Hot Water Extraction

[4] Raw,Whole Bean Meal; CONTROL, Non-extracted; EXTRACT, Fresh formulation; Viscozyme, Commercial preparation; Sprout, 1% germinated slurry

[5] Total Phytate = Sum of IP_3, IP_4, IP_5 and IP_6 (Inositol tri-, tetra-, penta- and hexaphosphates)

inositol phytates. No difference in phytate content was exhibited by the raw, CONTROL and enzyme pre-digested DDBMs. These data demonstrate the potential of discarding the soak water in reducing phytate content in processed bean products. The heat treatment employed in drum-drying did not effectively reduce the phytate content of DDBMs.

Lectin (Phytohemagglutinin)

Table 14 summarizes results of a diagnostic assay for screening phytohemagglutinin activity. Only the raw bean split demonstrated the presence of agglutination within half an hour when reacted with the antibody (erythroagglutinin) for lectin. The EXTRACT DDBM showed no visible precipitation after reacting with the antibody, after a holding period of one hour at room temperature.

Phytohemagglutinin is also heat stable, but the absence of agglutination when reacted with an antibody again indicated sufficient heat treatment of the DDBMs to inactivate the bean lectin. Lectin activity in raw navy beans was half as much in raw red kidney beans, and cooking for 10 min at 100^{o}C inactivated all lectin activity in navy beans. Wet heat was more effective in inactivating lectins than dry heat (Dhurandar and Chang, 1990). Coffey et al. (1992) reported that purified phytohemagglutinin is sensitive to enzymatic treatments, particularly proteolytic digestion, hence the hemagglutinating activity and enzyme pre-digested DDBMs warrant investigation. Further, erythroagglutination by lectin is affected by the molecular properties of the lectin, cell surface properties, metabolic state of cells and conditions of assay such as temperature, cell concentration and mixing, hence the lectin assay method to use should take these factors into consideration.

Conclusion

The composition of the drum-dried bean meals varied with the process pretreatments utilized. CONTROL and EXTRACT DDBMs did not differ significantly in their oligosaccharide content, but were significantly different in their *in-vitro* starch

Table 14. Visual reaction for screening of dry bean lectin (phytohemagglutinin)[1]

Sample	Sample Volume (*ul*)	Visual Reaction
Antibody (Ab)	10	+
Raw Navy Bean Splits		
	10	-
	20	-
	30	-
	40	-
Raw Beans with Ab (*ul*)		
	10	+
	20	++
	30	+++
	40	++++
DDBM EXTRACT		
	10	-
	20	-
	30	-
	40	-
DDBM EXTRACT with Ab (10 *ul*)		
	10	-
	20	-
	30	-
	40	-
Deionized Water	10	-

[1] n=3

[2] + indicates presence of a precipitate; number of + indicates the degree of precipitation
- indicates absence of a precipitate

digestibilities. Protein digestibility of the DDBM was significantly enhanced by the drum-drying process with increases approximating 9 % over the unprocessed bean.

The enzymatic pretreatments of DDBMs prior to drum-drying significantly increased starch availability by disrupting intact cellular structures and exposing starch. It was proposed that the denaturation of the protein matrix increased the accessibility of the *a*-amylases to starch molecules. Sprouting of beans demonstrated potential as an extractive pretreatment to improve dry bean digestibility and warrants further investigation for applications in appropriate village-level situations in LDC's.

Results of this study suggest that dry bean digestibility has been substantially improved by the extractive pretreatments employed. Oligosaccharide content was reduced by the aqueous and enzymatic pretreatments. Trypsin activity has been significantly reduced and phytohemagglutinin was inactivated by the heat treatments utilized. Results indicated that indigestible starch may be the main factor affecting dry bean digestibility, which could also be implicated with the flatulence problem associated with dry bean consumption.

H_0: the pretreatment of dry beans by physical and biological methods will not enhance their digestibility through removal or inactivation of interfering components prior to drum-drying.

Reject the H_0 as stated and conclude that pretreatment and processing influences bean digestibility.

CHAPTER II FUNCTIONAL CHARACTERISTICS OF DRUM-DRIED BEAN MEALS AND THEIR INCORPORATION IN SELECTED FOOD SYSTEMS

Introduction

Decline in overall bean consumption in the United States and potential dietary benefits of beans have prompted investigations into the use of legume flours in food systems (Zabik et al., 1983a and b). Legumes are successful in increasing the quality of protein through amino acid complementation and through increasing the total quantity of protein in formulated food products. The utilization of bean flour from dry roasted split navy beans has been investigated for bread making (D'Appolonia, 1978). The effect of the addition of navy bean flour and concentrate on the rheological properties of dough and baking quality of bread were studied by Sathe et al. (1981) and Lorimer et al. (1991). Lee et al. (1983) reported on the physicochemical characteristics of dry-roasted navy bean flour fractions and demonstrated differential functional performance of fractions based on processing conditions. The substitution of navy bean flour for wheat flour in pumpkin and banana breads and doughnut holes produced acceptable and nutritious products (Uebersax et al., 1982; Uebersax and Zabik, 1986).

Drum drying of dry beans into precooked flakes, meals or powders could be used to produce versatile ingredients in baked products, convenience food formulations or replacements for cooked bean purees or refried beans. There is also potential in the development of improved weaning food formulations especially suitable for the LDC's.

The Null Hypothesis tested in the studies is stated as follows:

H_0: Drum-dried navy bean meals do not possess potential for use in food systems.

The purpose of this research was to characterize the functional properties of pretreated drum-dried navy bean meals produced using selective predigestion or extraction

treatments and to evaluate the suitability of these bean meals in model formulated food systems (quick bread and weaning beverage).

This chapter is comprised of three studies as outlined in Figure 40. Study II-1 involved the characterization of the physical and functional properties of the drum-dried bean meals (DDBM); Study II-2 focused on the utilization of these various drum-dried bean meals in a quick bread (pumpkin) product, and Study II-3 involved the incorporation of DDBM in a weaning beverage formulation. Drum-dried meal prepared by aqueous extraction of split navy beans termed "EXTRACT" (soak, rinse and cook waters discarded and replaced with fresh formulation water) and enzyme pre-digested (Viscozyme) split bean drum-dried meal were utilized in Study II-3.

Materials and Methods

Split dry navy (*Phaseolus vulgaris*) beans grown in the 1991 crop year were obtained from the Cooperative Elevator Company, Pigeon, MI. The beans soaked and hydrated for 16 hours. (25°C) were subjected to hot water extraction (60°C/30 mins.) prior to milling to a homogeneous slurry. Heated bean slurries were dried using a double drum-dryer.

The physical and functional properties of the drum-dried bean meals, including water holding capacity (WHC) and nitrogen solubility (NSI) were evaluated using standard AACC (1984) methods (Figure 40). The microstructure of the DDBMs was observed under Laser Scanning Confocal Microscopy (LSM).

The recipe of Rombauer and Becker (1978) was followed in making the pumpkin quick bread. The composite flours consisted of wheat flour and drum-dried bean meals blended in 80:20, 60:40 and 40:60 ratios, respectively (Figure 41). The drum-dried bean meals were ground to a flour using the Fitzmill with a 0.04 mesh screen. All formulation ingredients were weighed to the nearest 0.01 g, sealed in clear polyethylene bags, randomly coded and held at -23.9°C until blended, mixed to a batter and baked. Three

FUNCTIONAL CHARACTERISTICS OF DRUM-DRIED BEAN MEALS AND THEIR INCORPORATION IN SELECTED FOOD SYSTEMS

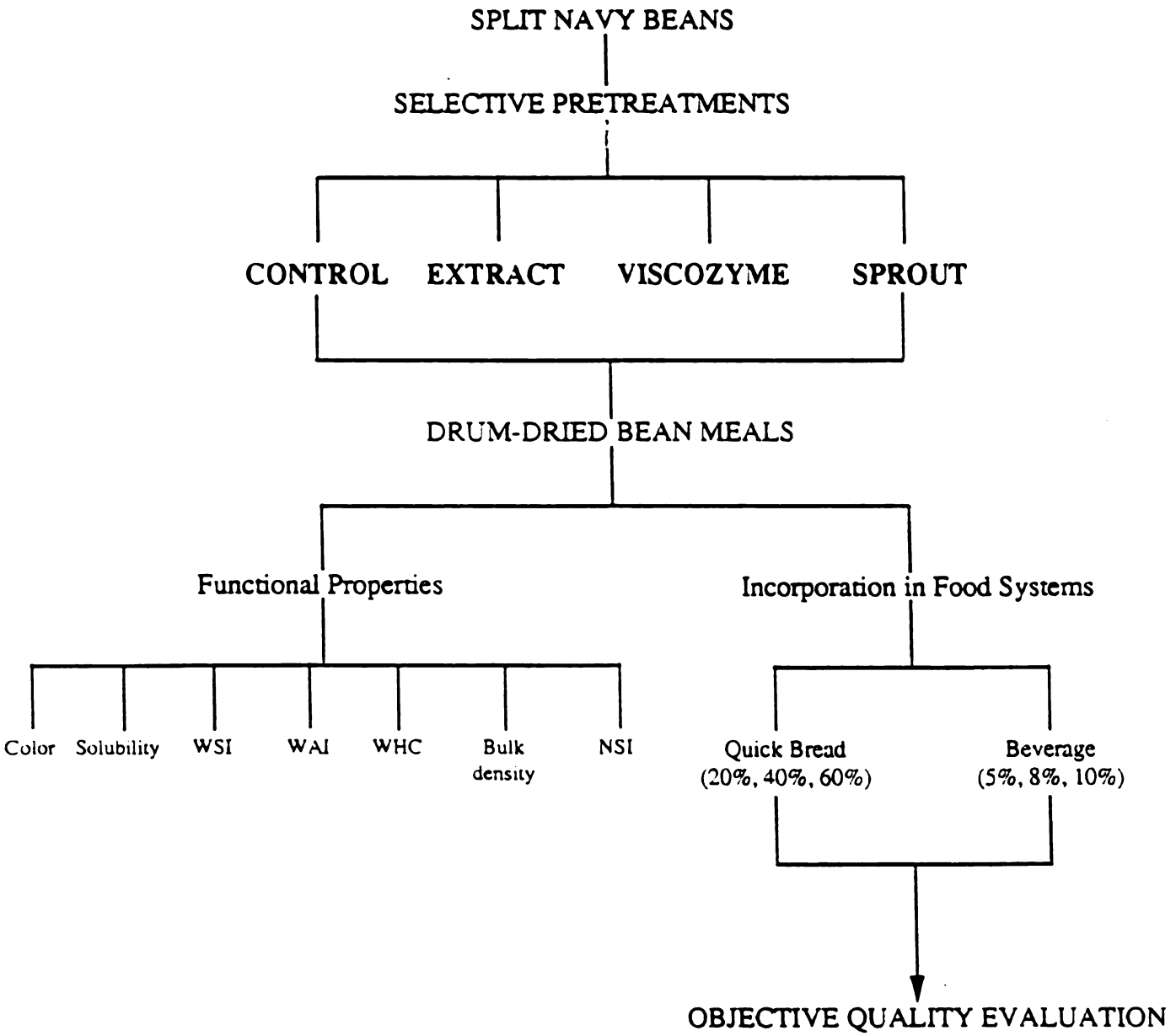


Figure 40. The experimental flowchart for Study II : Functional Characteristics of Drum-dried Bean Meals and their Incorporation in Selected Food Systems

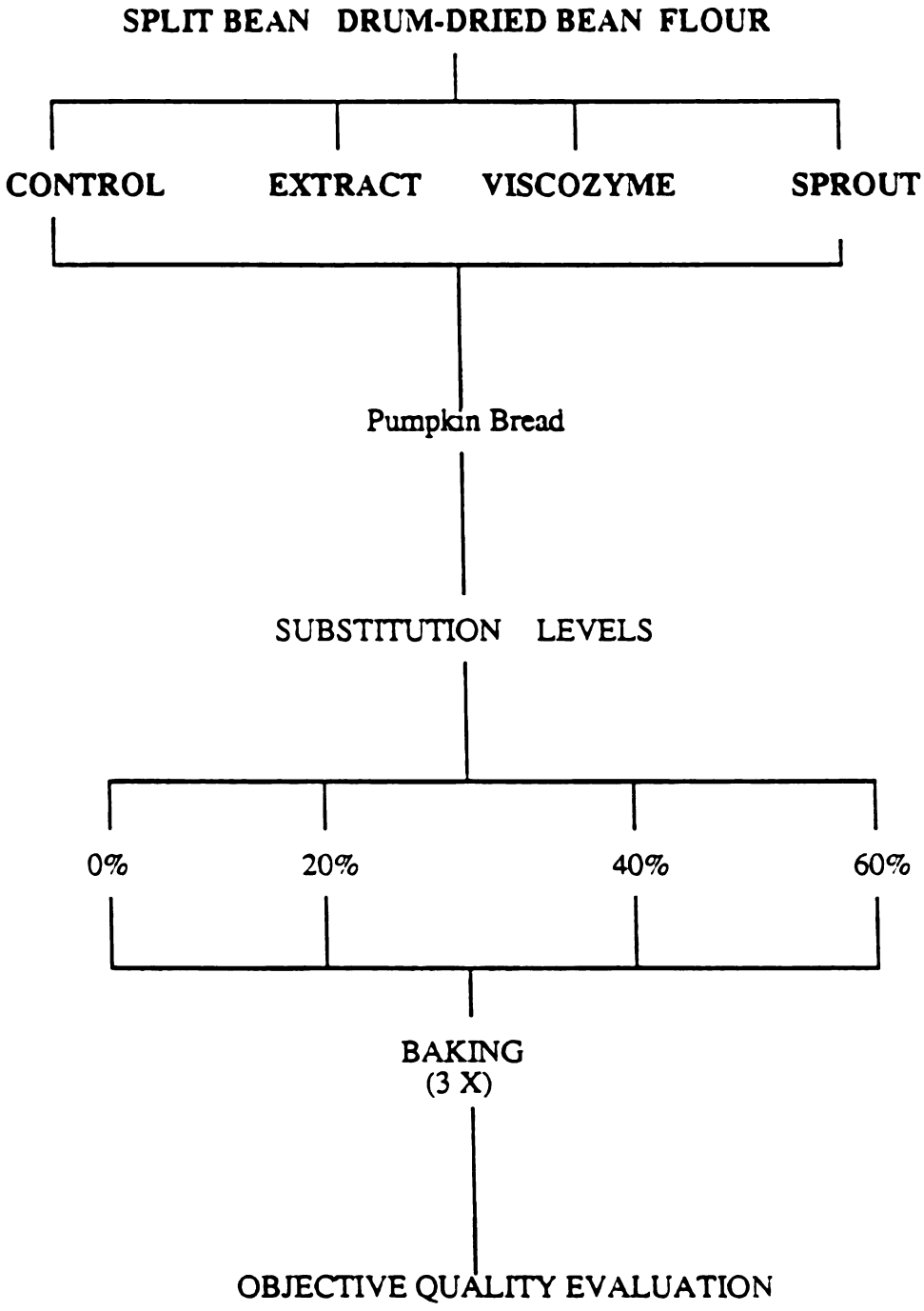


Figure 41. Flowchart of the experimental design for Study II-2: Incorporation of drum-dried bean flour in pumpkin bread

replications of each variable were baked in a rotary oven at 177°C (350°F) for 45 mins. Objective quality evaluations were conducted on both the batter and baked products (Figure 41).

Different concentrations (5%, 8% and 10%) of the weaning food beverage were prepared by homogenizing DDBM slurries with 200 - ml deionized distilled water in a 400-ml polyethylene beaker for 2 minutes. A Tissumizer (Tekmar Co., Cincinnati, OH) with the large probe size (# 18G) was used. Figure 24 summarized the evaluation of the physical quality characteristics of the fully prepared bean beverage.

Results and Discussion

Physical and Functional Properties of Drum-Dried Bean Meals

Plate 6 illustrates the general appearance of the raw split beans and split bean DDBM, as well as representative crystalline structures of the DDBM observed under a stereomicroscope (Wild Type 256530, Heerbrugg, Switzerland). Plate 7 shows the fine crystals of the EXTRACT DDBM compared to the coarse crystals of Viscozyme DDBM. The microstructure of the DDBMs and starch granule appearance were also observed under LSM (Plate 2) and discussed in detail in the study on starch digestibility (Chapter I).

The physical and functional properties of the split DDBMs are summarized in Table 15. The DDBMs exhibited a neutral color, and although CONTROL samples appeared slightly darker than the EXTRACT samples due to the retention of the original formulation water, they were not significantly different ($p < 0.05$). The DDBMs possessed a bland flavor, and did not exhibit the distinct and frequently objectionable "beany" flavor.

The different extractive pretreatments did not have a significant effect ($p < 0.05$) on the water holding capacity (WHC) and soluble solids content (°Bx) of the DDBMs, but had a significant influence ($p < 0.05$) on solubility indices (WSI and WAI) and bulk density (g/100 ml) . WAI and WSI give indications of likely cold-paste viscosity and gelatinization, respectively (Holm et al., 1985). The enzymatic predigestion treatments

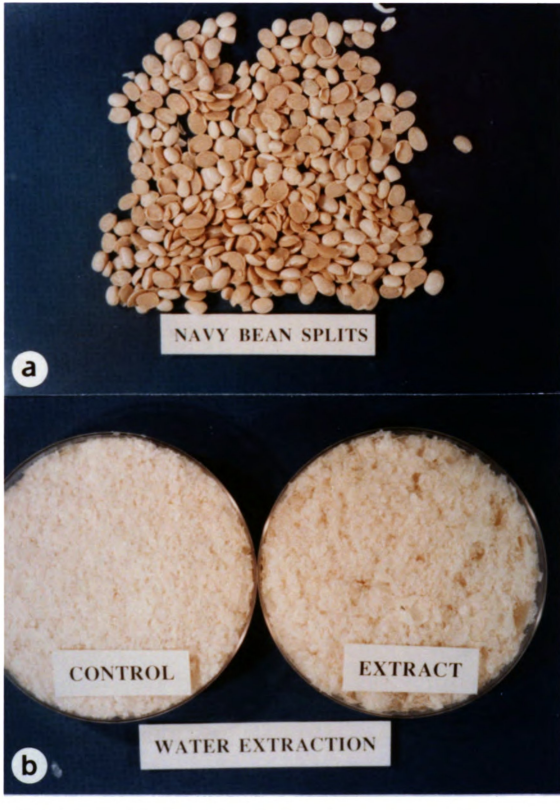


Plate 6. General appearance of (a) raw navy bean splits and (b) EXTRACT DDBM

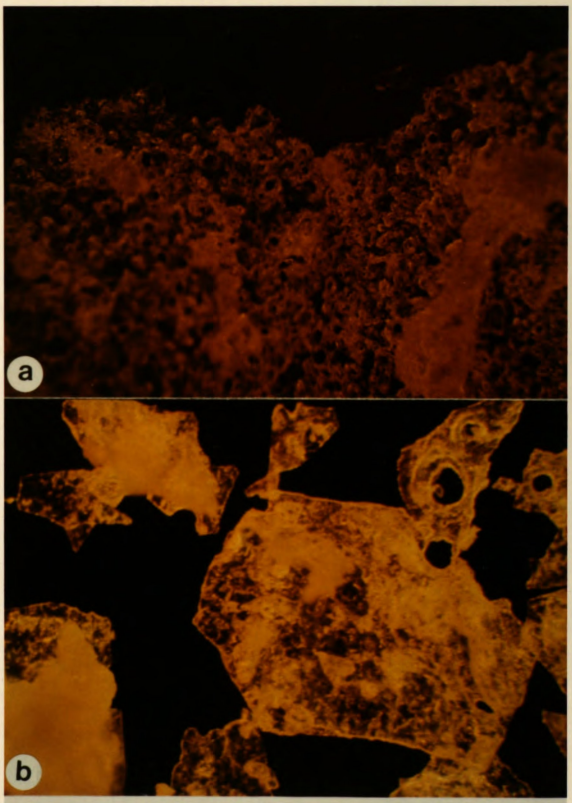


Plate 7. Crystalline structure of drum-dried bean meal under a stereomicroscope (50x): (a) EXTRACT (b) VISCOZYME

Table 15. Physical and functional properties of split bean drum-dried bean meals

Sample/Treatment	Color[1]			WHC[1]	WSI[1]	WAI[1]	NSI[2]	Bulk Density[2]	°Brix[1]
	L	a_L	b_L	(g/g)	(g/g)	((g/g)	(%N/%N)	(g/100)	(SS)
CONTROL	72.90±0.01a	-0.90±0.01a	14.90±0.01a	5.36±0.01a	0.13±0.02a	8.83±0.50a	0.25±0.07b	35.88±0.10a	0.30±0.00a
EXTRACT	72.90±0.01a	-0.90±0.01a	16.70±0.02b	6.37±0.04b	0.17±0.01b	8.89±0.30a	0.22±0.02b	36.73±0.10a	0.40±0.00a
Viscozyme	72.10±0.02a	0.10±0.01a	16.90±0.02b	7.10±0.16c	0.19±0.02b	11.59±0.20b	0.24±0.02b	70.87±0.20b	0.40±0.00a
Sprout	71.90±0.02a	-1.10±0.01a	16.60±0.01b	6.41±0.02b	0.19±0.01b	11.83±0.40b	0.01±0.002a	70.94±0.30b	0.40±0.00a

[1] n=2; Mean values and standard deviations (different letters within each column indicate significant differences at $p < 0.05$)
[2] n=3; Mean values and standard deviations (different letters within each column indicate significant differences at $p < 0.05$)

[3] CONTROL, Non-extracted; EXTRACT, Fresh formulation; Viscozyme, Commercial preparation; Sprout, 1% germinated slurry

(Viscozyme and Sprout) significantly increased ($p < 0.05$) the water absorption index (WAI) of the DDBMs. WAI serves as an index of product functionality and is therefore important to obtain a maximum value. Avin et al., (1992) reported an increase in WAI of red kidney beans with increased extrusion temperature, but no significant influence on density and water solubility index (WSI).

Incorporation of Drum-Dried Bean Meals in Food Products

Quick Bread

A quick bread (pumpkin) formulation was utilized in this study because it minimizes the problems previously encountered with incorporation of legume rich breads and bread products (decreased flavor, volume and color) by using spices, ensuring consistent specific volume through the method of preparation and decreased color change. Quick breads are baked as breads, yet they lack a strong gluten development and contain a high ratio of sugar to flour, as in a cake system; hence, incorporation of protein substitutes is feasible (Dryer et al., 1982).

There were no significant differences ($p < 0.05$) in the color of the pumpkin breads with increasing levels of drum-dried bean meal substitution (Plate 8 and Table 16). Since these products are characteristically dark, no objective difference was observed in the color of the products due to the promotion of Maillard browning reaction generally associated with increased levels of reducing sugars inherent in the bean flour.

Bread loaf volume decreased with increasing levels of drum-dried bean meal substitution, with differences most significantly apparent ($p < 0.05$) between the Reference (all-purpose flour, 100% level) and breads prepared with 60% drum-dried bean meal (particularly, CONTROL and EXTRACT) (Plate 9). Bread volume (cm^3) decreased with a corresponding increase in crumb density (Table 16) when levels of DDBM substitution were increased, because of increased gluten formation and its inherently more compact product structure. A preliminary and essential phase prior to the development of the gluten

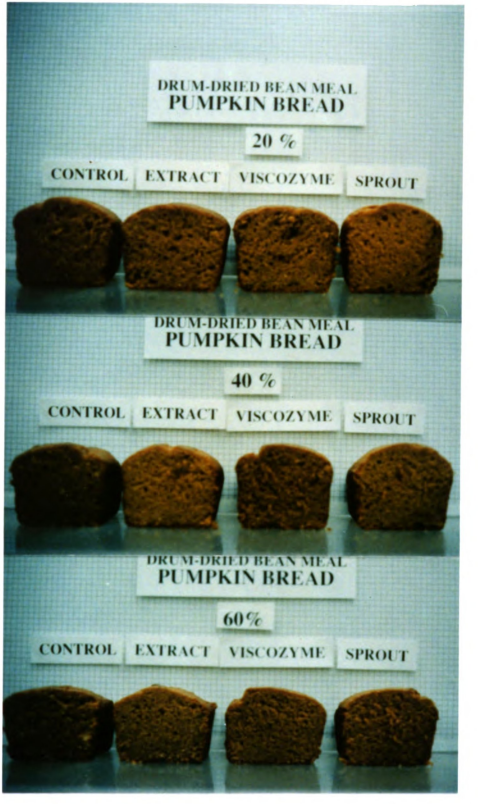


Plate 8. Appearance of pumpkin quick breads prepared with different levels (20%, 40%, 60%) of pretreated DDBMs (CONTROL, EXTRACT, Viscozyme, Sprout)

Table 16. Physical characteristics of pumpkinbread substituted with 0 to 60% drum-dried bean meals.

Treatment	Batter		Bread					
	pH	Viscosity (cps)	Color L	a_L	b_L	Volume (cm^3)	Density (g/in^3)	Moisture (%)
REFERENCE (100%)		9466.7+225.5	28.0	2.4	9.4	585+14.43	0.66+0.01	62.2+0.1a
CONTROL								
20%	7.6	15133.3+152.8	28.3	2.4	9.4	525+ 0.00c	0.68+0.02a	57.0+0.1a
40%	7.6	18766.7+2926.32	28.3	2.4	9.4	491+14.43b	0.81+0.01b	58.4+0.2a
60%	7.6	22700.0+109995.5	28.3	2.4	9.4	458+14.43a	0.85+0.01b	63.6+0.3a
EXTRACT								
20%	7.8	20366.7+1167.6	28.3	2.4	9.4	542+14.43b	0.74+0.2a	64.9+0.4a
40%	7.7	30866.7+416.3	28.3	2.4	9.4	492+14.43a	0.78+0.2a	63.9+0.3a
60%	7.6	10583.3+707.7	28.3	2.4	9.4	442+14.43a	0.79+0.2a	66.6+0.3a
VISCOZYME								
20%	7.5	15133.3+321.5	28.3	2.4	9.4	542+10.31c	0.68+0.3a	64.6+0.1a
40%	7.5	13266.7+305.5	28.3	2.4	9.4	500+ 9.78b	0.82+0.4b	61.1+0.2a
60%	7.5	67120.0+9596.5	28.3	2.4	9.4	482+10.50a	0.88+0.1b	61.9+0.4a
SPROUT								
20%	7.7	12900.0+624.5	28.3	2.4	9.4	555+11.54b	0.67+0.01a	59.9+0.3a
40%	7.6	44100.0+600	28.3	2.4	9.4	510+10.90b	0.71+0.02a	62.9+0.2a
60%	7.6	97000.0+83108.4	28.3	2.4	9.4	492+11.20a	0.83+0.01b	61.0+0.5a

1 n=3; means within a treatment followed by different letters are significantly different ($p < 0.05$)

2 CONTROL, Non-Extracted DDBM; EXTRACT, DDBM leachate removed, fresh formulation water; REFERENCE, 100% wheat flour (0% DDBM)

3 Standardized to a white tile: L=92.2; a_L = -0.96, b_L = 0.1

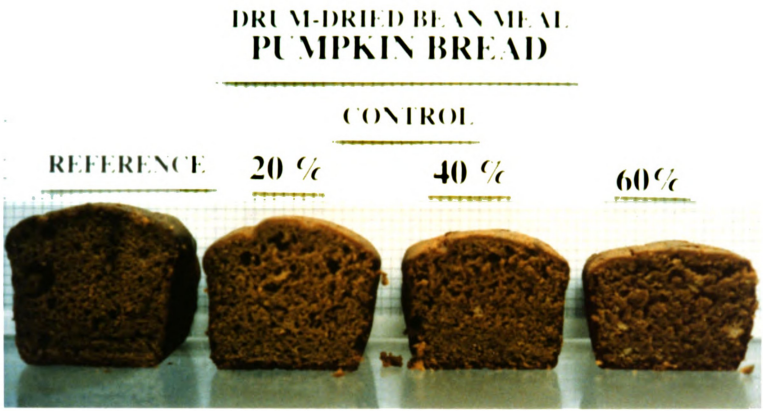


Plate 9. General appearance of pumpkin quick breads prepared with CONTROL DDBM substituted at 20% to 60% reference sample in 100% wheat flour formulation.

is the hydration of the protein and starch of the flour. With higher levels of substitution (60%), it was possible that the DDBMs were not fully hydrated by the available fluid.

Shear force values increased (i.e., decreased tenderness) with increased level of bean meal substitution; however, no statistical differences were detected among the various substituted-pumpkin breads (Table 17). It is likely that increased shear force values are a result of the decreased gluten formation and the resulting decrease in size and number of the air cells present within the crumb structure. With higher levels of DDBM substitution (60%), bread density is greater as a result of decreased air cells needed to develop and expand gluten, which in turn leads to increased shear resistance force.

Table 18 summarizes the textural data obtained from the Texture Profile Analysis (TPA) of the pumpkin breads. The TPA is a two bite method of texture analysis in which the food is compressed twice and a complete texture profile of the sample is calculated from the data recorded. A generalized TPA curve, a typical pumpkin bread (CONTROL DDBM) curve and the definition and calculations of textural parameters are illustrated in Figure 42. Hardness is the area under the primary force deformation curve. Adhesiveness refers to the area under the third curve measured during the second bite in the downstroke mode. Cohesiveness is obtained from the ratio of area 2 (adhesive force) to area 1(hardness) in the upstroke mode during the first bite. Stringiness is measured as the distance of area 3 (adhesiveness) from area 1 (hardness) measured in the upstroke mode, while springiness is measured as the distance of area 2.

A typical pumpkin bread curve consists of only two peaks with areas corresponding to the parameters hardness and adhesiveness. Hardness values increased with increasing levels of drum-dried bean meal substitution, for all the different pretreated DDBMs. Increased adhesiveness, gumminess (hardness x cohesiveness) and chewiness (gumminess x springiness) were also observed with increased DDBM substitution levels. Conversely, springiness and cohesiveness of the pumpkin breads decreased as substitution with drum-dried bean meals was increased. These TPA results confirm observations that as DDBM

Table 17. Multiple Blade Shear Compression Mode of pumpkin breads with 0 to 60% drum-dried bean meal substitution [1]

	Texture Shear Force (Newtons)	Slope (Newtons/cm)	Work (Newtons-cm)
REFERENCE (100%)	120.29±10.96	19.14±2.32	394.97±38.4
CONTROL			
20%	114.93±12.26a	18.50± 1.93a	362.20±41.57a
40%	136.33±16.05a	21.40± 1.90a	402.20±45.99a
60%	133.67±23.15a	20.93± 2.32a	424.20±62.87a
EXTRACT			
20%	130.97± 4.62a	21.77± 1.55a	367.70±17.24a
40%	136.30± 8.00a	23.90± 4.48a	394.70± 6.99a
60%	138.97± 4.62a	39.50±9.96a	429.93±22.77a
VISCOZYME			
20%	120.30±13.86a	19.30± 2.51a	316.73±20.60a
40%	130.97± 9.24a	21.13± 1.44a	400.23±13.48a
60%	138.97± 4.62a	20.47± 9.66a	352.10±107.11a
SPROUT			
20%	122.97±12.22a	20.07± 1.92a	348.03±23.94a
40%	139.00±24.48a	71.73±6.49b	383.90±51.29a
60%	160.37±16.05a	91.53± 9.94a	451.33±25.14a

[1] n=3; means followed by different letters are significantly different ($p < 0.05$)

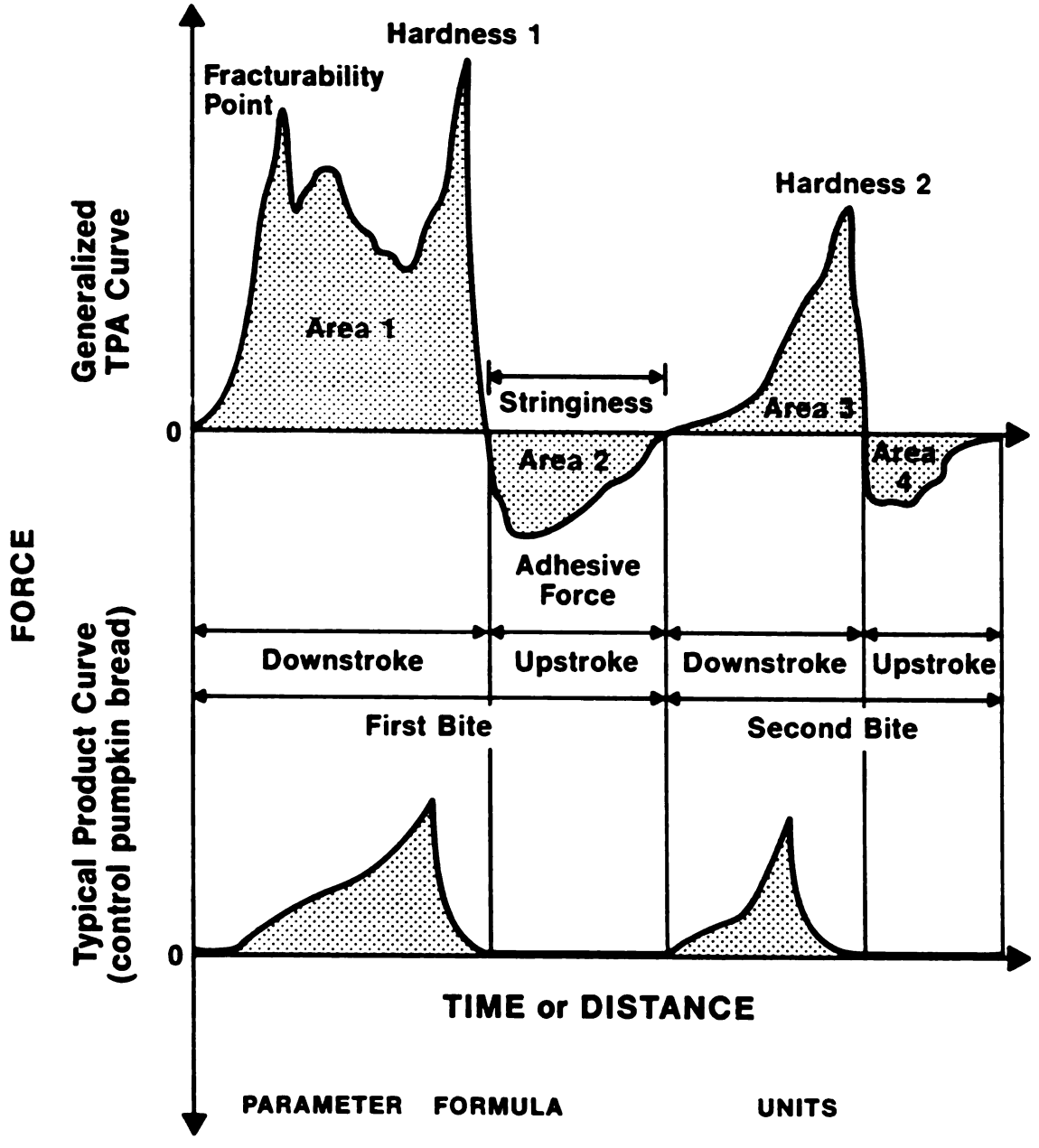
[2] CONTROL, Non-Extracted DDBM; EXTRACT, DDBM leachate removed, fresh formulation water; REFERENCE, 100% wheat flour (0% DDBM)

Table 18. Mean values of physical Texture Profile Analysis (TPA) of drum-dried bean meal pumpkin breads

TREATMENT	PARAMETERS						
	Hardness	Cohesiveness	Adhesiveness	Springiness	Stringiness	Gumminess	Chewiness
Reference(100%)	57.43	0.39	0.24	0.43	0	22.45	9.80
CONTROL							
20%	63.05±15.97a	0.33±0.04a	0.29±0.05a	0.36±0.07a	0	20.85±3.11a	7.49+0.23
40%	83.20±11.63a	0.30±0.01a	0.68±0.18a	0.39±0.03b	0	25.50±3.46a	9.94+0.97
60%	105.15±18.98ab	0.27±0.02a	1.06±0.29a	0.33±0.06a	0	28.51±3.37ab	9.27+0.73
EXTRACT							
20%	73.06±12.90a	0.38±0.00a	0.47±0.29a	0.42±0.04a	0	27.60±4.97a	11.82+3.27
40%	97.04±12.74a	0.34±0.02b	0.54±0.44a	0.45±0.02c	0	32.99±2.97a	14.79+0.64
60%	118.43±14.94ab	0.30±0.01b	0.74±0.03a	0.36±0.05a	0	36.06±4.52b	13.09+3.94
Viscozyme							
20%	72.61±17.24a	0.36±0.01a	0.70±0.35a	0.36±0.08a	0	26.02±5.51a	9.30+1.60
40%	83.46±10.56a	0.31±0.02a	1.30±0.61a	0.37±0.02b	0	25.90±4.31a	9.74+1.93
60%	119.77±10.09b	0.29±0.02ab	0.87±0.33a	0.38±0.06a	0	35.24±5.26b	13.63+3.94
Sprout							
20%	70.80±1.18a	0.36±0.02a	0.38±0.13a	0.36±0.03a	0	25.53±1.79a	9.28+1.24
40%	85.27±24.43a	0.33±0.01b	0.66±0.43a	0.33±0.03a	0	28.06±8.25a	9.34+3.36
60%	94.83±3.58a	0.27±0.01a	0.77±0.16a	0.34±0.05a	0	25.96±1.77a	8.94+1.76

[1] n=3; means followed by different letters are significantly different ($p < 0.05$)

[2] CONTROL, Non-Extracted DDBM; EXTRACT, DDBM leachate removed, fresh formulation water; REFERENCE, 100% wheat flour (0% DDBM)



PARAMETER	FORMULA	UNITS
Hardness	Value of Hardness 1	Lbs. or Newtons
Cohesiveness	Area 2/Area 1	Dimensionless (ratio)
Adhesiveness	Area 3	In.-Lbs. or Newton-Cm
Stringiness	Distance of Area 3	In. or Cm
Gumminess	Hardness x Cohesiveness	Dimensionless (ratio)
Chewiness	Gumminess x Springiness	Dimensionless (ratio)
Springiness	Distance of Area 2	In. or Cm
Fracturability	User Defined	Lbs. or Newtons

Figure 42. A generalized Texture Profile Analysis (TPA) curve and a typical pumpkin bread TPA curve.

substitution levels increased, pumpkin bread volume decreased, with a corresponding increase in crumb density and shear force.

Plate 10 demonstrates the cellular microstructures of the various pumpkin breads substituted with 20% DDBM. The pumpkin bread with all-purpose flour (100%) demonstrated a thick protein matrix enveloping starch granules. The microstructure of the DDBM-substituted pumpkin breads were characterized by a thinner, discontinuous gluten matrix over the flour starch granules. The protein matrix is indicated by the fluorescent bodies. The flour and bean starch granules can not be easily distinguised, but with the low level of substitution, flour starch granules predominate. Microstructure of pumpkin breads with the enzyme pre-digested DDBMs (Viscozyme and Sprout) (Plate 10d and e) demonstrated a more extensive gluten matrix compared to those substituted with CONTROL or EXTRACT DDBMs (Plate 10b and c), and could partly account for the slightly taller height of resultant pumpkin breads.

Weaning Food/Beverage

The beverage concentrations utilized in this study were 5%, 8% and 10% drum-dried bean meal (DDBM) suspensions. Preliminary trials utilizing lower concentrations (2.5% and 4%) of the beverages (Appendix D) demonstrated very limited stability, whereas concentrations higher than 10% exhibited too viscous a consistency for a weaning beverage.

Table 19 summarizes the physical characteristics of the bean beverages. The soybean control and the DDBM beverages all exhibited a neutral color. The extractive pretreatments employed did not have a significant effect ($p < 0.05$) on the color of the weaning beverage. Total solids increased with the increased beverage concentrations, and the enzyme predigested beverage exhibited a higher soluble solids content compared to the product with EXTRACT DDBM.

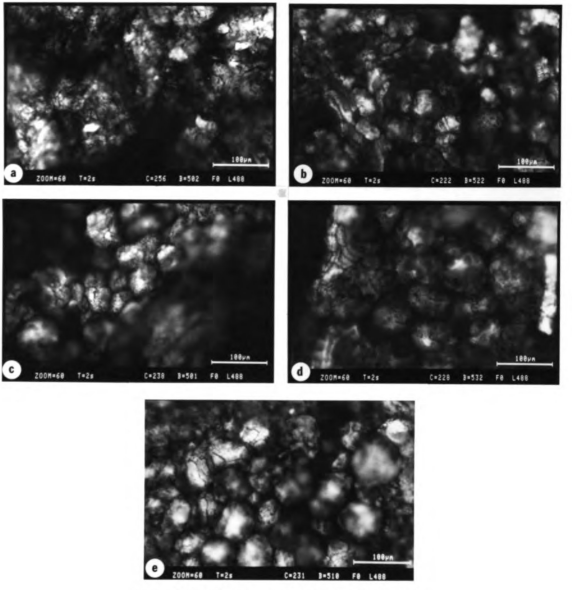


Plate 10. LSM micrographs of pumpkin bread cellular microstructure: a) Reference (100% all-purpose flour) b) CONTROL DDBM c) EXTRACT DDBM d) VISCOZYME DDBM e) SPROUT DDBM

Table 19. Physical characteristics of weaning bean beverages[1]

Sample	Color			pH	Soluble Solids	Total Solids
	L	a_L	b_L		(°Brix)	(%)
5% Bean Beverage						
EXTRACT	49.00±0.01a	-0.40±0.02b	6.0±0.01a	6.60	1.00±0.002a	4.64±0.02a
Viscozyme	49.30±0.01a	-0.10±0.01a	8.3±0.01b	6.90	1.30±0.001a	4.54±0.03a
8% Bean Beverage						
EXTRACT	55.5±0.20a	-0.90±0.01a	5.7±0.02a	6.60	1.90±0.002a	7.08±0.01a
Viscozyme	53.6±0.12a	-1.70±0.02a	9.5±0.01b	6.40	2.50±0.001b	7.52±0.02b
10% Bean Beverage						
EXTRACT	53.7±0.01a	0.40±0.01a	7.90±0.01a	6.80	2.00±0.10a	9.04±0.10a
Viscozyme	53.2±0.12a	0.60±0.15a	10.20±0.10b	6.60	3.00±0.20b	9.00±0.09a

[1]n=2; Mean values and standard deviations (different letters within each treatment group indicate significant differences at $p < 0.05$)

Visual stability of the weaning bean beverages was expressed as the amount of clear, separated supernatant (ml) plotted against time (min), within a 2-hour observation period. A commercially prepared soybean based weaning formula (Isomil Soy Protein Formula, Ross Laboratories, Columbus, OH)(15%) was utilized as a comparative control. Figure 43 demonstrates that for both the EXTRACT and enzyme pre-digested (Viscozyme) bean beverages, suspension stability increased with increased beverage concentration.

The 15% soybean formula was completely stable with time and did not exhibit any settling of solids. With very low concentrations of the beverage (2.5%, Appendix D), settling of solids with a corresponding increase in separated supernatant, occurred almost immediately after homogenization. The 10% Viscozyme sample closely approximated the stability of the soybean control; however, the consistency was highly viscous. In general, the Viscozyme DDBM beverages were relatively more stable than the EXTRACT DDBM suspensions. Plate 11 demonstrates the effect of extractive pretreatments as well as concentration on the relative stabilities of the weaning beverages. After a one hour static holding period, the 8% and 10% concentrations were still stable, particularly the enzyme-predigested (Viscozyme) sample. However, the 10% level might be too viscous a product for a weaning food. A recommended weaning product is one which will produce a low paste viscosity or high calorie density to prevent choking of infants, hence the 8% level was judged to be a more appropriate level.

A micro-quantitative method designed to evaluate beverage stability involved measuring spectrophotometric Absorbance (565 nm) (or % Transmission, Appendix D) of dilute suspensions prepared using differential quantities (ranging from 2.5 to 40 μl) of the weaning beverages prior to the settling of the solids immediately after the homogenization step. Figures 44 to 46 illustrate the effects of the different concentrations (5%, 8% and 10%) of EXTRACT and Viscozyme DDBMs on the stability of the bean beverages. The regression equations derived demonstrated good correlation between the absorbance readings and concentration, and provided a tool to quantitate the influence of DDBM

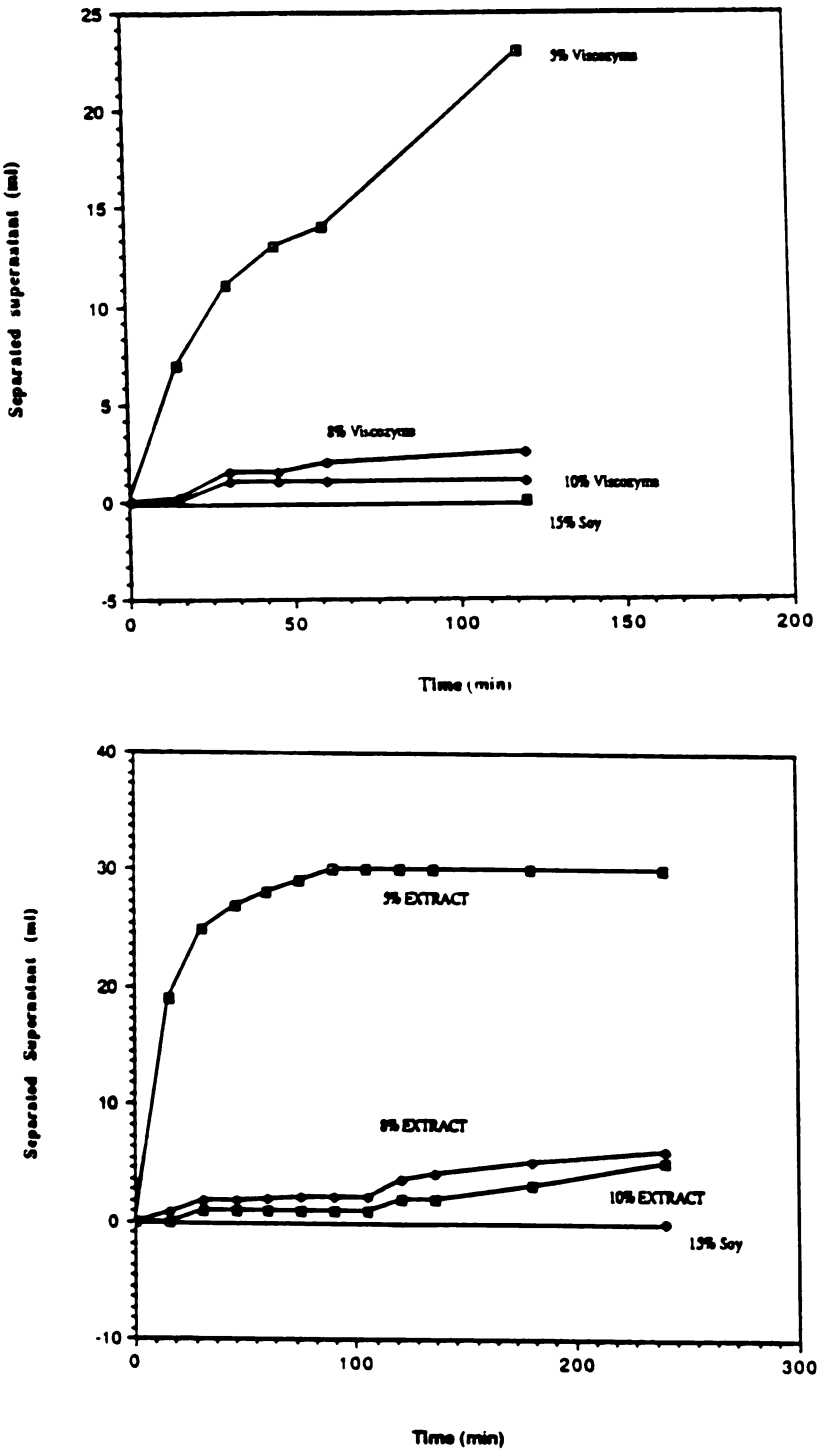


Figure 43. Comparative visual stability of bean beverage from DDBMs and soy formula

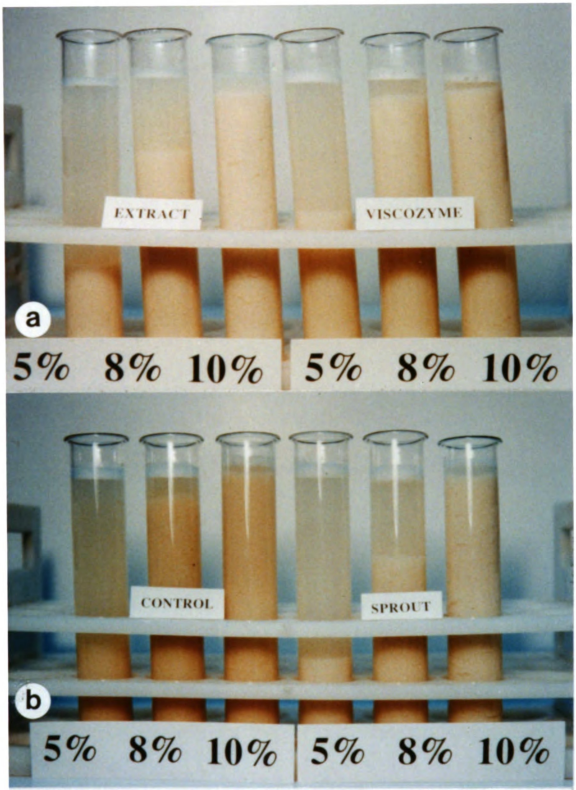


Plate 11. Appearance of weaning beverage prepared with different levels (5%, 8%, 10%) of pretreated (a) EXTRACT and Viscozyme DDBMs and (b) CONTROL and Sprout DDBMs

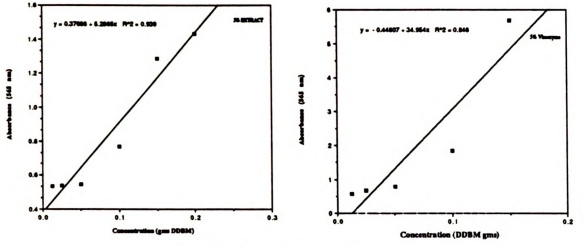


Figure 44. Absorbance vs Concentration for 5% EXTRACT and 5% Viscozyme bean beverages

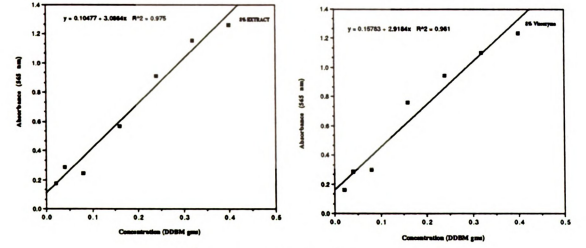


Figure 45. Absorbance vs Concentration of 5% EXTRACT and 5% Viscozyme bean beverages

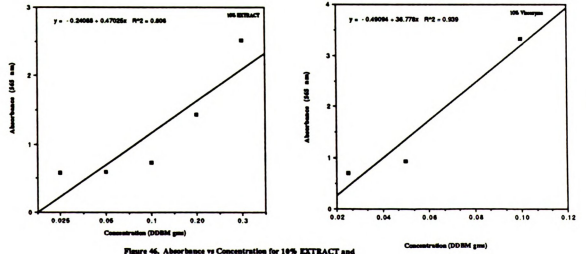


Figure 46. Absorbance vs Concentration for 10% EXTRACT and 10% Viscozyme bean beverages

concentration on stability of the beverage suspensions. As sample concentration was increased, absorbance reading increased (decreased % transmission) which indicated a decrease in settling of solids or a corresponding increase in suspension stability. Figures 47 to 49 demonstrates that at higher concentrations (8% and 10%), the Viscozyme DDBM beverage exhibited higher absorbance readings, indicating a relatively more stable suspension than the EXTRACT DDBM beverage.

Figures 50 and 51 illustrate the comparative transmission (%) of the DDBM beverages over time, another index of stability/settling of solids with time. The higher beverage concentration (10%) exhibited lower transmission (%), indicating decreased settling of solids or increased suspension stability compared to the lower concentration (5%). The 15% soybean control exhibited consistent transmission (%) with time, indicating no separation of solids and therefore complete stability during the period studied.

Figure 52 summarizes the apparent viscosities of the various weaning bean beverages measured using a Brookefield viscometer. The 8% and 10% concentrations of the enzyme pre-digest (Viscozyme) beverage were significantly ($p < 0.05$) higher than the corresponding concentrations of the EXTRACT beverage. The 5% beverages did not differ significantly ($p < 0.05$). Relative viscosities of the 5% beverages are illustrated in Figure 53. Viscozyme was significantly ($p < 0.05$) higher than the 5% EXTRACT beverage, confirming the results for apparent viscosity . The 8% and 10% concentrations produced extremely viscous suspensions which were too thick to pass the Oswald pipet; hence relative viscosities were not measured. Figures 54 and 55 illustrate the apparent and relative viscosities of the bean beverages with the addition of commercial emulsifiers and stabilizers (Methylcellulose, xanthan gum, Carageenan systems - Seakem®, Lactarin MU®, Viscarin GP and SD®; details in Appendix D) obtained from FMC Corporation (Marine Colloids Division, Philadelphia, PA) . Results demonstrate the dramatic increase in apparent viscosity with the addition of 1% levels of carageenan systems (Lactarin®, Viscarin® GP and SD and Seakem®). The emulsified beverages demonstrated greater

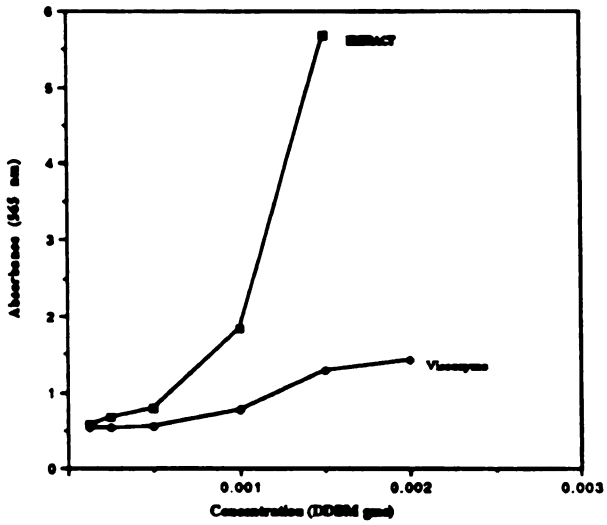


Figure 47. Comparative absorbance of 5% bean beverages

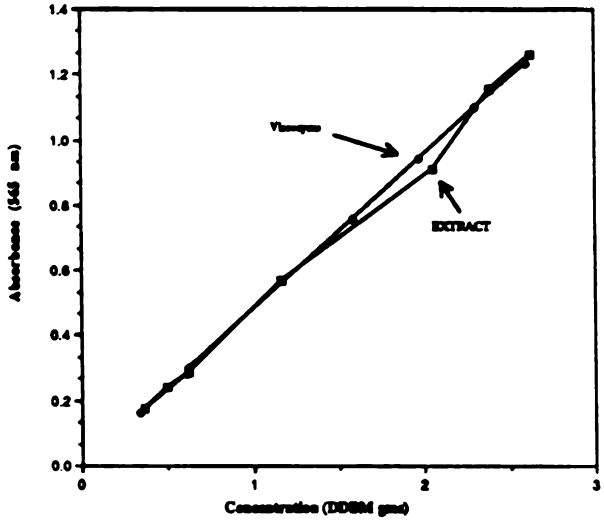


Figure 48. Comparative absorbance of 5% EXTRACT and enzyme pre-digest (Viscozyme) beverages

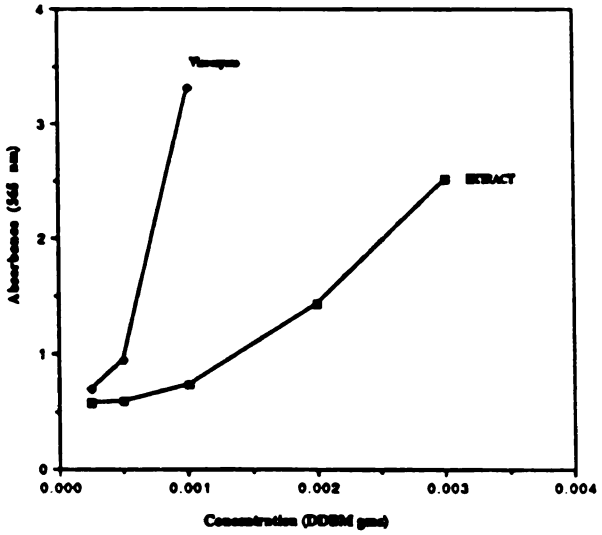


Figure 49. Comparative absorbance of 10% EXTRACT and enzyme pre-digest bean beverages

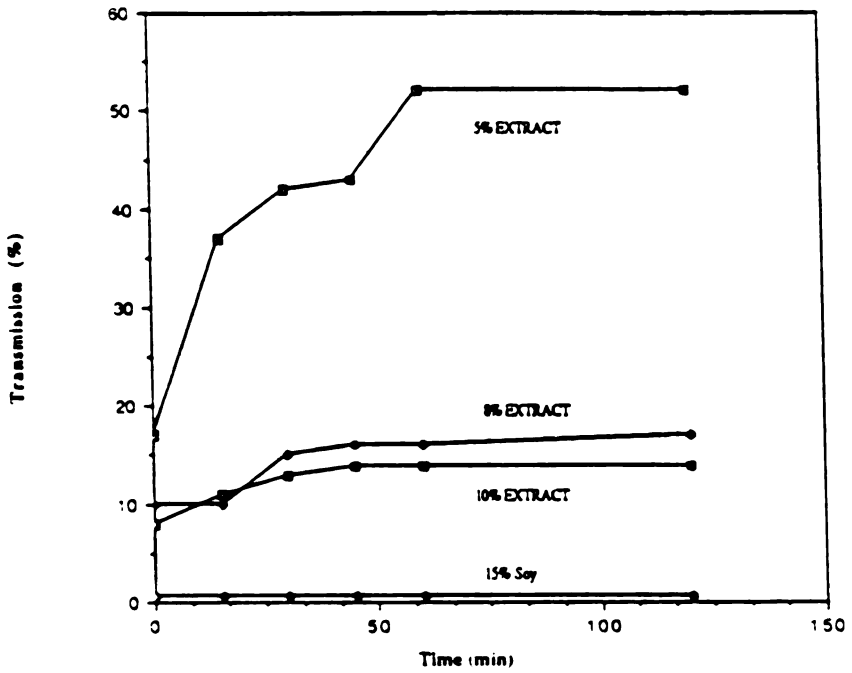


Figure 50. Comparative transmission (%) of bean beverage separated supernate from DDBM EXTRACT and soy formula

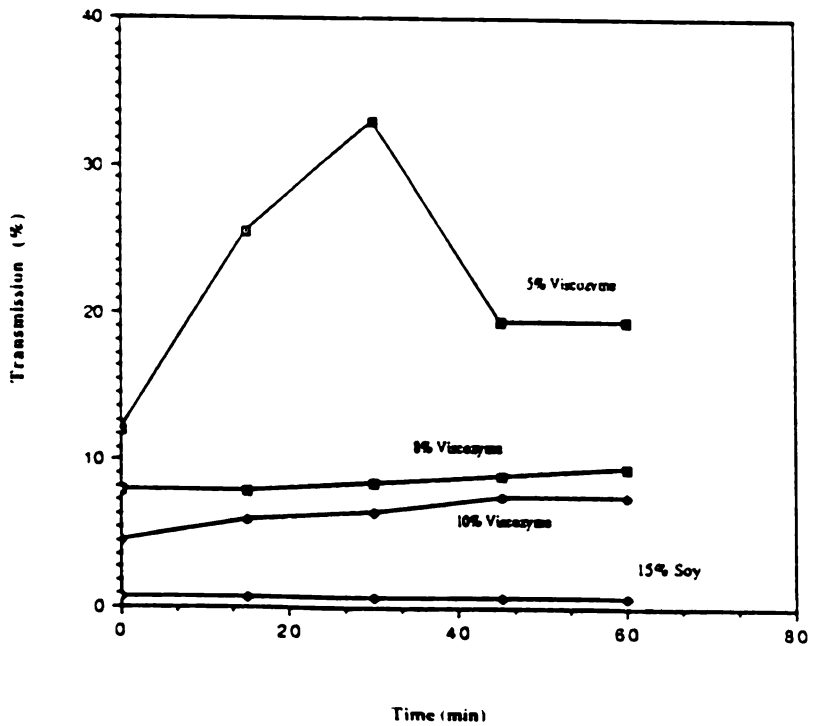


Figure 51. Comparative visual stability of bean beverage from DDBM and soy formula

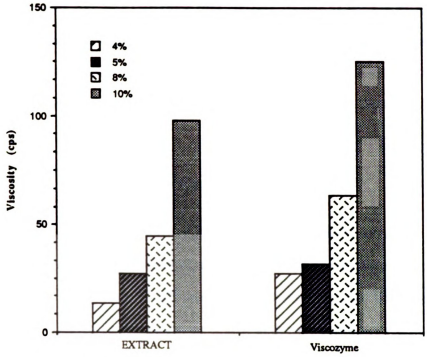


Figure 52. Viscosity of bean beverages from drum-dried bean meals at different concentration levels

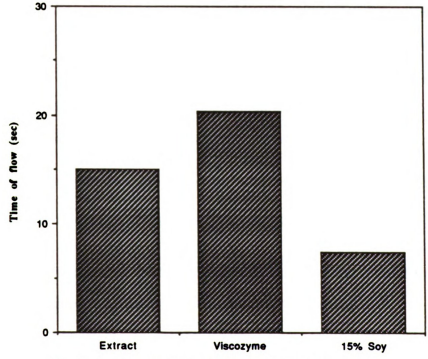


Figure 53. Time for the bean beverage (5%) to flow through the two marks of an Oswald pipette

stability (Figures 56 and 57) by exhibiting less settling of solids over a given period compared to those bean beverages without added emulsifier. The levels of emulsifiers utilized in this study were based on the recommendations of the manufacturer according to the dispersion media (water or milk) and functional categories or applications (gelation, viscosity and cold solubility).

Conclusion

Results demonstrated the feasibility of incorporating drum-dried navy bean meals in pumpkin quick breads and weaning food/beverages. Acceptable quality breads were produced at the 20% and 40% levels of substitution. Enzyme predigested DDBMs possess great potential in weaning food formulations, especially at the 8% concentration level. The use of indigenous enzymes (i.e., sprouting) to modify the functional properties of DDBM used in weaning beverage formulations warrants additional research.

Potential optimization of pretreatments exists to obtain specific functional properties of the meals. High quality products were obtained by the extractive pretreatments and drum-drying process. The functional properties demonstrated through incorporation of DDBM in a quick bread indicated potential use in a broader spectrum of baked products. Potential of the drum-dried bean meals in weaning food formulations to enhance the nutrient density of infant diets also warrants further research.

H_0: Drum-dried navy bean meals do not possess potential for use in food systems.

Reject the Ho and conclude that drum-dried bean meals possess functional properties suitable for incorporation into nutritional food products.

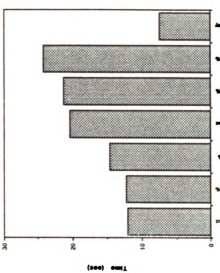


Figure 55. Time for 5% EXTRACT and 5% Viscogum base beverages with carrageenan (0.02 & 0.03%) to flow through the two outlet of an Oswald pipette

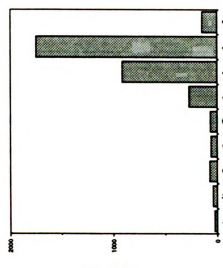


Figure 54. Apparent viscosities of 5% EXTRACT base beverages with different levels of emulsifiers

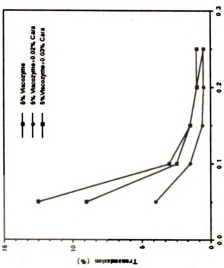


Figure 57. Comparative transmission (%) of 5% Viscogum base beverage with and without carrageenan

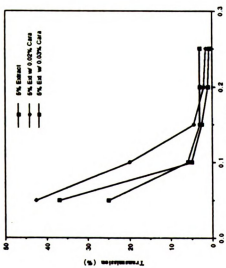


Figure 56. Comparative transmission(%) of 5% EXTRACT with and without carrageenan

CHAPTER III MICROWAVE HEATING CHARACTERISTICS OF WHOLE BEANS and BEAN STARCH

Introduction

A major deterrent to the utilization of dry beans is the long cooking times involved in their preparation. Consumers favor convenience foods which provide consistently high quality with minimum delay. Commodities receiving some pre-preparation which reduce cooking requirements enhancing the potential for microwaveable reheating are sought. Although numerous specialized applications of microwave energy for processing or cooking food products have been developed, little attention has been directed towards its utilization in legumes. Microwave heating has a direct potential for use as a preconditioning treatment for process time reduction of dry beans.

Starch granules undergo physico-chemical changes during thermal processing. Dry bean starch granules are resistant to swelling and rupture; their gelatinization temperatures range from 60°C to over 75°C, relatively high compared to many standard cereal grains (Hahn et al., 1977). Bean starch has a higher percentage of amylose (generally 30-37%) than most plant starches, which increases the potential for formation of indigestible retrograded starch (Hoover and Sosulski, 1985). During cooking, some of the amylose leaches from the starch granule. Since the cell structure in cooked beans remain intact, much of the leached amylose remains within the cell and fills spaces between protein bodies and starch granules. The unique properties of bean starch can have practical applications in food systems, particularly in modifying product textural quality.

The Null Hypothesis of this study is stated as

H_0: Microwave energy will not adequately disrupt starch granules and facilitate gelatinization *in situ* to improve whole seed cooking, functional properties and digestibility.

The objectives of the studies were : 1) to evaluate microwave heating as a pretreatment to dry beans prior to processing, storage and final preparation and 2) to compare the effects of microwave and conventional heating on the physical and functional properties of navy bean starch.

This chapter is comprised of two parts : Study I - the microwave preconditioning of whole dry navy beans and evaluation of resultant bean characteristics; and Study II - determination and comparison of the effects of conventional and microwave heating on the physical and functional properties of isolated navy bean starch were compared.

Materials and Methods

Microwave Preconditioning of Whole Dry Navy Beans

The experimental plan for this Chapter is presented in Figure 58. Standard (C-20) whole dry navy beans were ambient soaked for different time periods and allowed to equilibrate at 4°C for 2 days. The beans were thus rehydrated to different moisture contents (range 47% to 54%) and subjected to microwave heating at three microwave energy levels (440, 590 and 740 watts) for 2 minutes in a Radarange microwave oven (Model - RS458P, Amana Refrigeration, Inc.). A microwavable pressure cooker (Nordicware) was used to contain the beans and reduce dessication during heating. The heated beans were subsequently packaged in polyethylene ziplock bags and stored at (-)18°C.

The frozen preconditioned beans were heated in the microwave oven for 30 seconds. Microwave heating time was established after a come-up time ranging from 3 to 7 minutes. Control beans were conventionally cooked in an open kettle for 15, 30 and 45 minutes.

Texture of microwave and conventionally cooked beans were evaluated using a Kramer Texture Test System (Model TMS-90) equipped with the FT 3000 transducer and

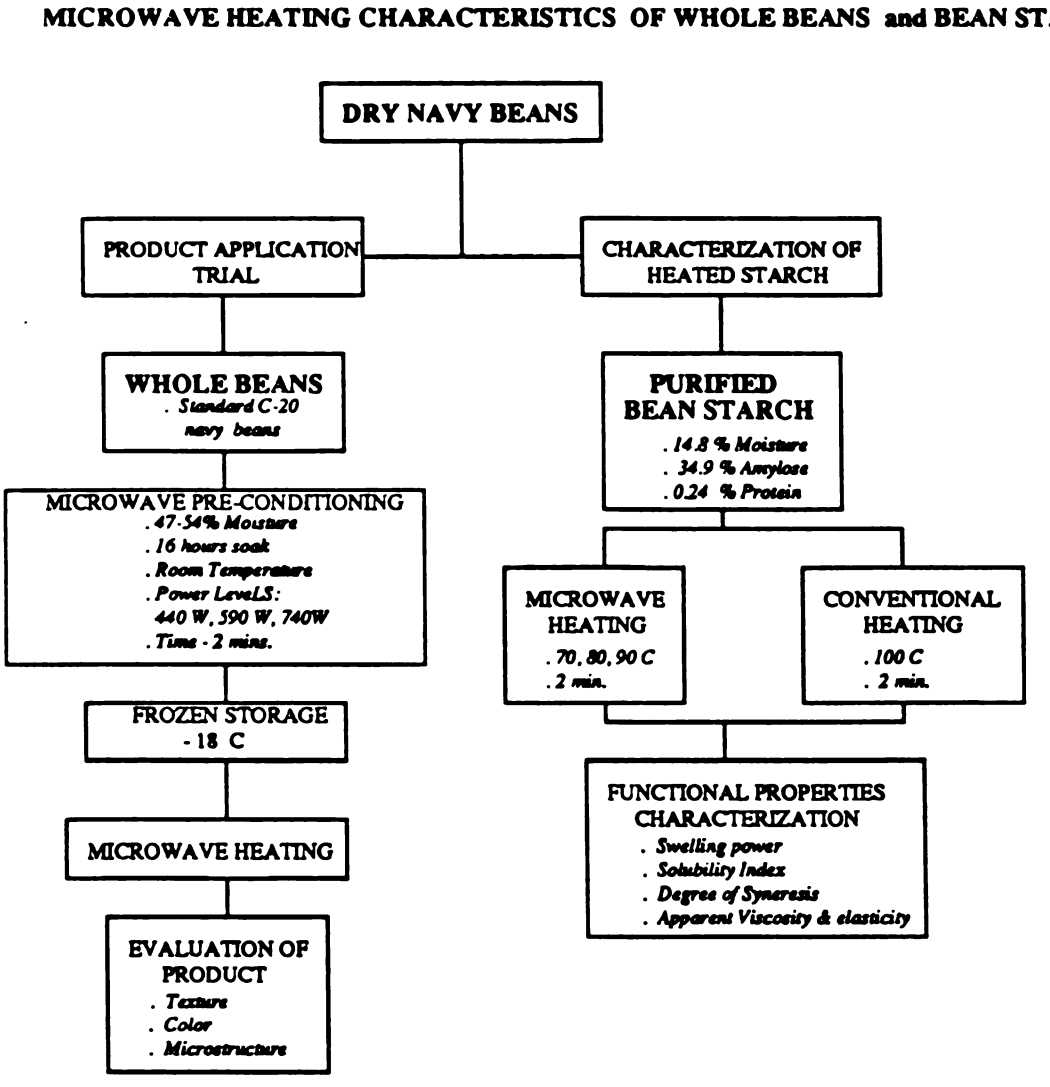


Figure 58. The experimental flowchart for Chapter III: Microwave preconditioning of dry beans (Study I) and microwave heating of bean starch (Study II)

the No. C-15 standard multiple blade shear compression cell. Color of beans was obtained using a Hunter Lab Model D25-PC2 (Hunter Associates Laboratory, Inc., Reston, Va.).

Cellular structure and starch granule appearance of microwave pre-conditioned beans was observed under SEM (Model JSM 35CF, Japan Electron Optics Limited, Tokyo, Japan) and LSM (Carle Zeiss, Inc., West Germany).

Microwave Heating of Bean Starch

Navy beans (Fleetwood cultivar /firm texture) grown during the 1987 crop year in research plots in Saginaw, Michigan, were used in this study. Bean starch was isolated using the method described by Naivikul and D'Appolonia (1979) with slight modifications. Commercial corn starch (Argo R Pure Corn Starch, CPC International Inc., Englewood Cliffs, NJ) was used as a comparative control. Proximate Analyses were conducted using the standard methods (AACC, 1983 and AOAC, 1984). The amylose content was determined by a modification of the colorimetric procedure of Morrison and Laignelet (1983).

Starch slurries were heated in screw-cap culture tubes (14 mm I.D.) using a Radarange microwave oven (Model - RS458P; Amana Refrigeration, Inc.) with a 700 watt rated power. Temperatures were monitored using a Luxtron 755 unit with MIW-02-10740 fiber optic probes. For conventional heating, starch slurries were heated in screw-cap test tubes (14 mm I.D.) placed in a thermostat-controlled water bath.

Bean starch physical properties including swelling power, solubility, degree of syneresis, gel rheological properties and examination of cellular structure and starch granule appearance using SEM were evaluated.

Results and Discussion

Microwave Preconditioning of Whole Dry Navy Beans

Figure 59 illustates the moisture gain in dry beans during soaking, yielding a typical water uptake response which plateaued after 5 hours of soaking at room temperature. Moisture content of the soaked beans ranged from 50 to 55% during an overnight soak period (16 hours). These results are consistent with numerous previous reports (Uebersax, M., 1985; Tittiranonda, A., 1984; Burr, et. al., 1968).

Significant differences in bean texture ($p < 0.05$) were observed after microwave preconditioning (Figure 60). Preconditioned samples with higher initial moisture content (54%) possessed a softer texture (required a lower shear force) compared to samples with lower initial moisture content (47%). Figure 61 demonstrates that texture of the frozen preconditioned samples was significantly softer ($p < 0.05$) after the 30-second cook. Lower microwave energy level (440W) was associated with longer come-up times which resulted in significantly higher weight gains (Figure 62) ($p < 0.05$) during cooking due partly to longer sample/water contact. Figure 63 illustrates that as weight gain (% above soak weight) increased, the texture of microwave cooked bean samples approached that of the conventionally retorted 115°C (240°F) canned bean samples, regardless of the preconditioning microwave power levels used. Initial bean hydration level and the maintenance of moisture during microwave heating are critical factors which affect final product texture. Sufficient thermal energy and adequate moisture are needed to produce a desirable and palatable texture. A desirable texture of cooked beans was associated with a weight gain of approximately 30% above the initial bean soak weight. Figure 64 demonstrates that color of the final product (expressed as lightness, L) was a function of both initial moisture content of the bean and microwave processing power level. In general, beans of softer texture, darker color and higher yield were produced using the

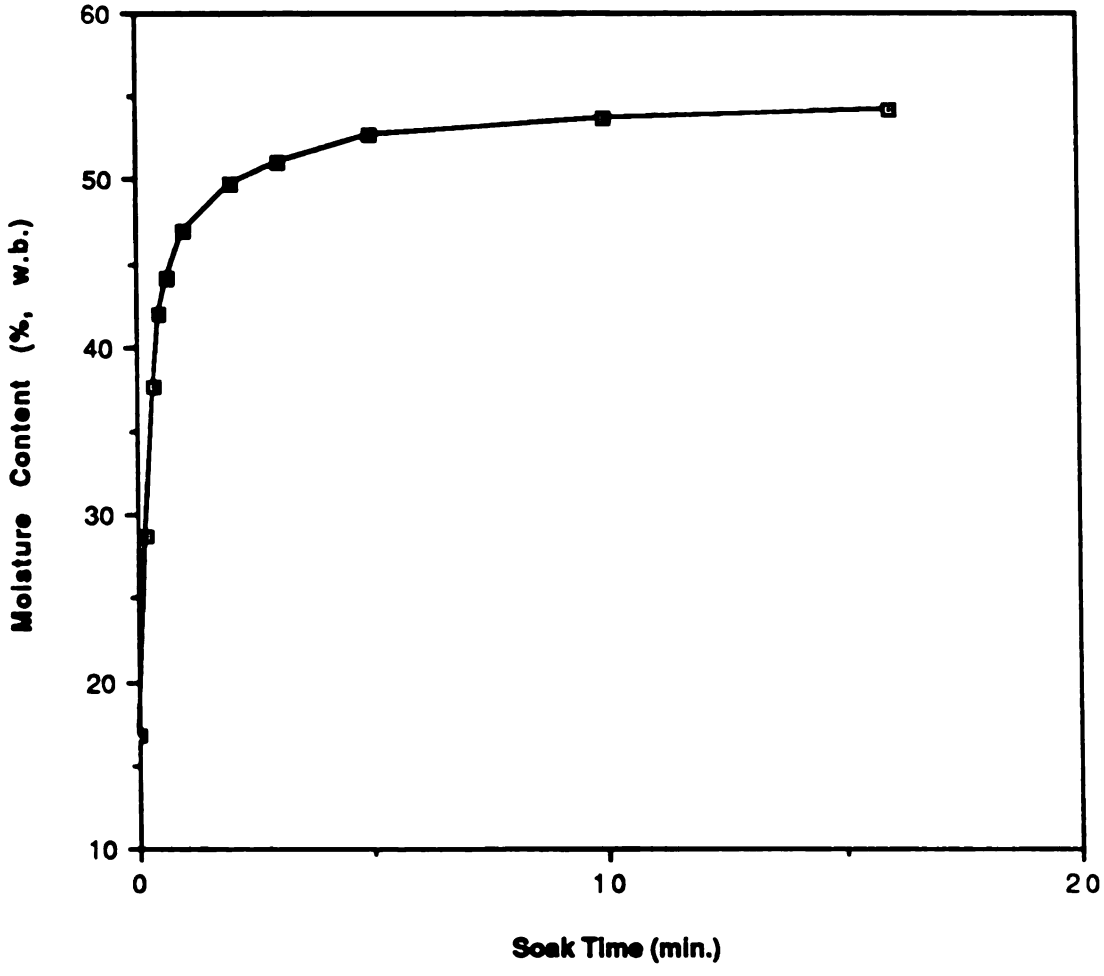


Figure 59. Moisture content of dry navy beans soaked at 25 C for various time periods

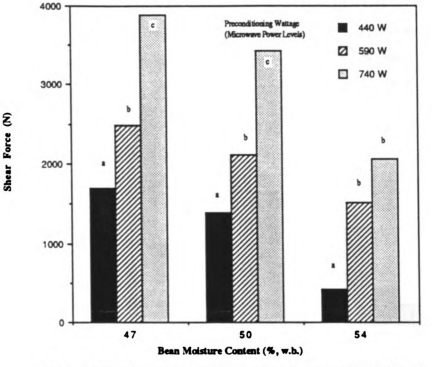


Figure 60. Maximum force required to shear navy beans preconditioned by microwaves in a Nordicware Pressure Cooker

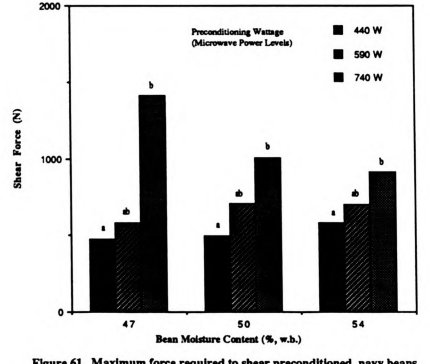


Figure 61. Maximum force required to shear preconditioned navy beans after reheating (0.5 min., 740 W) in a Nordicware pressure cooker

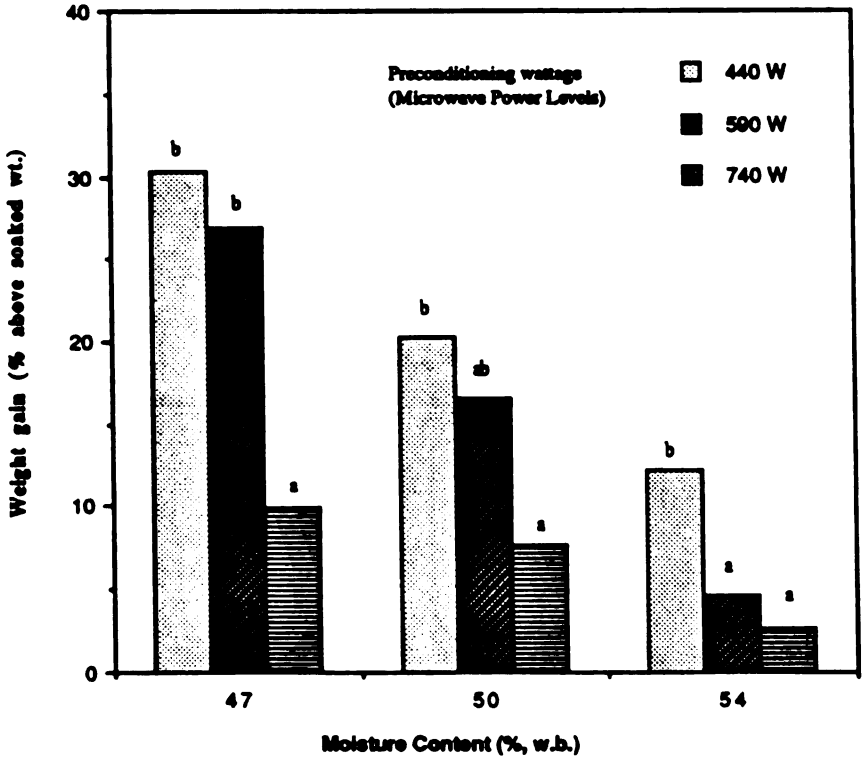


Figure 62. Total increase (% above soak weight) in weight of preconditioned navy beans after reheating (0.5 min., Nordicware pressure cooker)

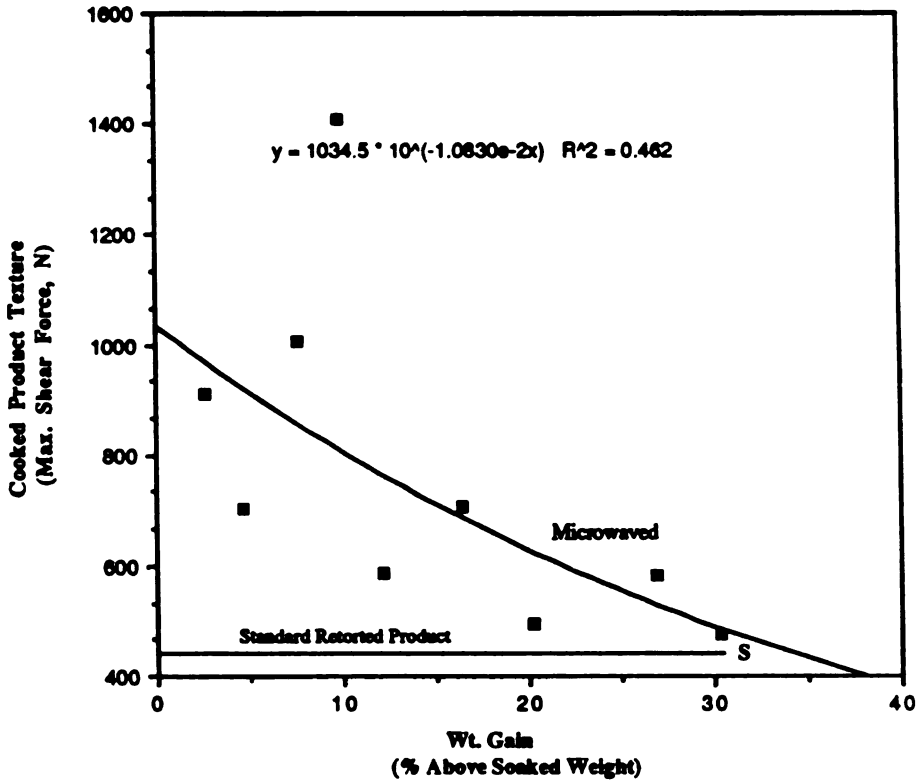


Figure 63. Maximum force required to shear navy beans cooked in a conventional retort (45 minutes, 116 C) or subjected to microwave energy levels in a Nordicware Pressure Cooker

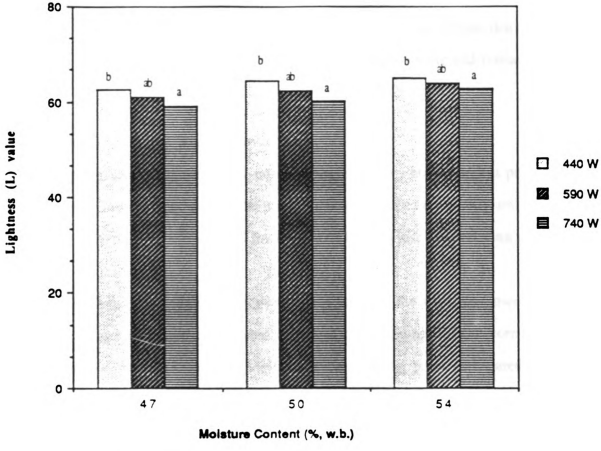


Figure 64. Color of final product as affected by post-soaking moisture content and microwave energy levels used for preconditioning (Nordicware pressure cooker)

pressure cooker at lower microwave energy levels with subsequent longer total cooking times (Table 20).

Plate 12 demonstrates that the average diameter of starch granules in soaked beans was approximately twice as large as that in dry seeds as a result of water uptake during the 16 hours of ambient soaking. Microstructural changes including cellular disruption and separation were observed as a result of microwave preconditioning and reheating (Plate 13).

Microwave Heating of Bean Starch

The chemical composition of corn and isolated navy bean starch is presented in Table 21. The moisture and protein contents of the two starches are significantly different ($p < 0.05$). Corn starch has a much lower ($p < 0.05$) amylose content than navy bean starch.

There was a significant difference ($p < 0.05$) in the swelling power between microwave and conventionally heated corn and bean starches. Both corn and bean starches microwave heated at 70°C exhibited a higher swelling power compared to those conventionally heated (Figures 65 and 66). With microwave heating, the characteristic 2-stage pattern of swelling of corn starch was not observed. For both methods heat treatments, corn starch granules exhibited a much higher degree of swelling compared to bean starch. Bean starch demonstrated the characteristic restricted swelling pattern under microwave heating. Swelling power of conventionally heated bean starch more than doubled when heated within the range of 70-80°C. Microwave heating did not have a significant effect ($p < 0.05$) on swelling power at the same temperature range. Microwave heating at 70°C had a significant effect ($p < 0.05$) on swelling potential of bean starch which was observed to be more than double that of starch heated using the conventional heating method.

Table 20. Microwave preconditioning and reheating times (min.) using a Nordicware microwave pressure cooker

Power Wattage	Preconditioning		Reheating		Total Period
	Come-up (min.)	Period (min.)	Come-up (min.)	Period (min.)	
440	7	2	7	0.5	16.5
590	5	2	5	0.5	12.5
740	3	2	3	0.5	8.5

Table 21. Chemical Characteristics of Corn and Isolated Bean Starches[1]

Starch Source	Moisture (%)	Ash (%, db)	Protein (%, db)	Amylose in Starch (%)
Corn	4.69 ± 0.12a	0.09 ± 0.02b	0.39 ± 0.02b	20.43 ± 0.39a
Fleetwood	14.77 ± 0.21b	0.07 ± 0.00a	0.24 ± 0.03a	34.88 ± 0.60b

[1] n=3, Means in a column followed by different letters are significantly different ($p<0.005$)

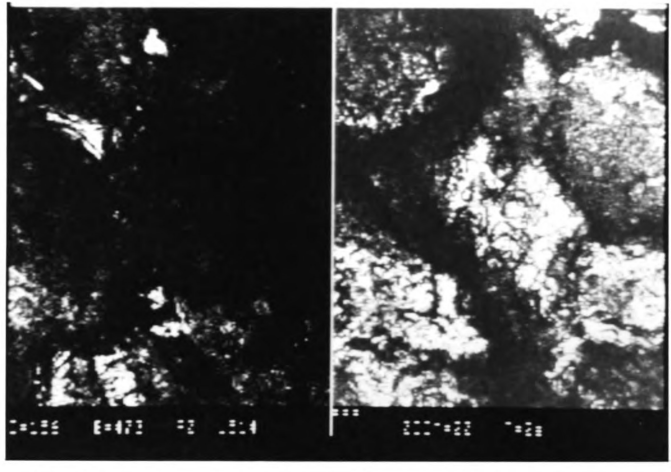


Plate 12. Structural components of dry (a) and soaked (b)(25^{O}C, 16 hours) navy beans. Pictures were taken from cross-section of the seeds using LSM

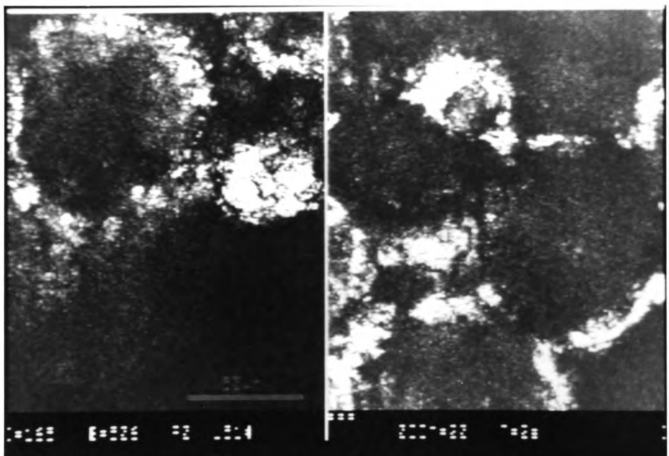


Plate 13. Cellular structure of navy beans preconditioned (a) at 590 watts for 5 min. and subsequently reheated (b) at 740 watts for 3.5 min. from frozen state. Pictures were taken from a cross-section of the whole beans using LSM

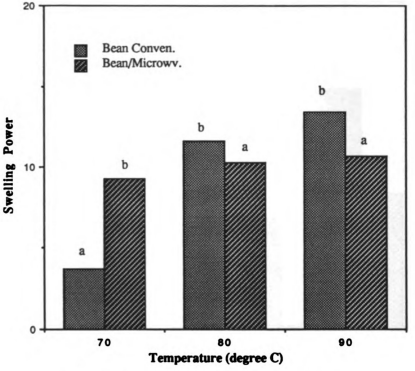


Figure 65. Swelling power pattern of bean starch with conventional and microwave heating

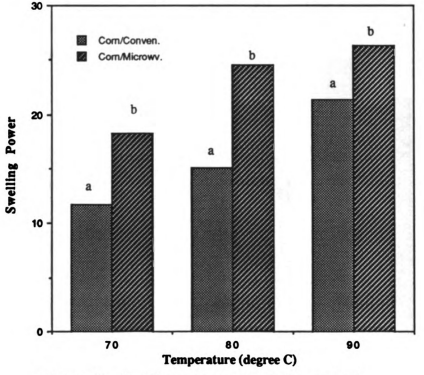


Figure 66. Swelling power pattern of corn starch with conventional and microwave heating

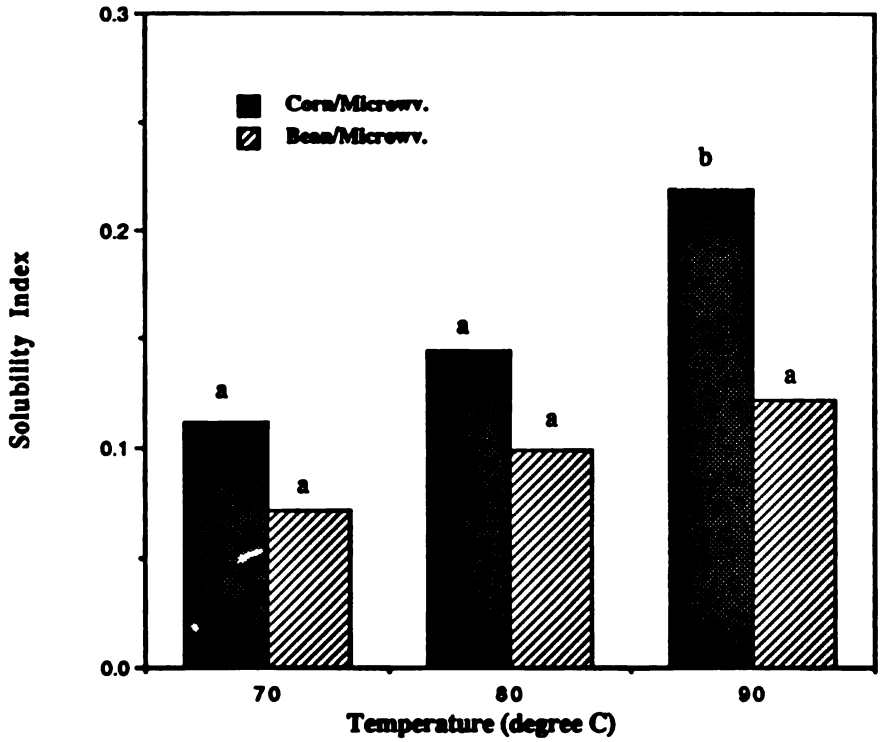


Figure 67. Solubility Index (SI) of corn and bean starches during microwave heating

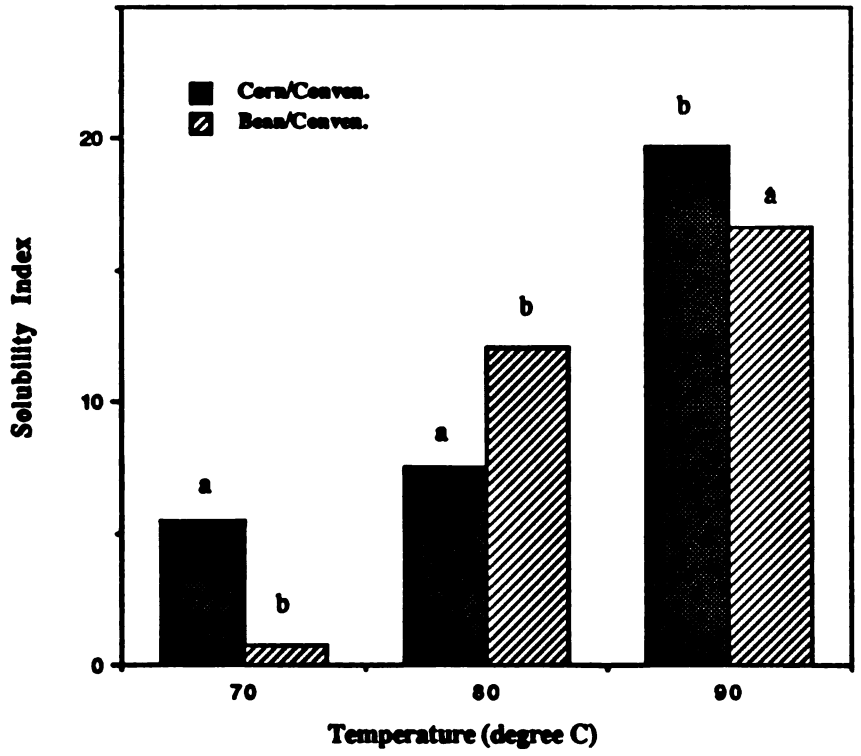


Figure 68. Solubility Index (SI) of corn and bean starches during conventional heating

Solubility of both starches was low with microwave treatment, indicating some leaching of amylose molecules out of the starch granules (Figures 67 and 68). Corn starch solubility was higher than bean starches throughout the temperature range 70-90°C for microwave heating. Increased solubility of starches with increased temperature was observed for conventional heating. The restricted swelling power and solubility patterns of the bean starch indicates the existence of strong bonding forces within the granules, allowing the starch to gradually relax when heated over the temperature range 70-90°C.

Conventional heating resulted in a significantly higher degree of syneresis for both corn and bean starches ($p < 0.05$) than observed for microwave heating, as demonstrated by a greater amount of exudate. Corn starch gels have a well recognized ability to hold liquid, and are expected to have only slight exudate, as illustrated by the microwave heated sample (Figure 69). However, the conventionally heated corn starch sample exhibited a very low liquid holding capacity, as demonstrated by the greater gel shrinkage and higher percentage of exudate. The conventionally heated bean starch sample exhibited a significantly higher ($p < 0.05$) degree of syneresis and percentage of exudate compared to microwave-heated bean starch, as shown in Plate 14 This observation was consistent with results which demonstrated relatively low swelling power at 70°C (Figure 65).

According to Ring (1985), starch gel rigidity is affected by granule deformability and the strength and stability of matrix amylose gel. Results demonstrated conventional heating had a significant increase ($p < 0.05$) on the rigidity of heated corn starch gels compared to microwave heating, while bean starch gels exhibited a slightly softer gel when microwaved (Figures 70-71). Corn starch has been previously reported to have a low amylose content and weak bonding strength within its starch granules, which could partly account for its ease of deformation. Bean starch possesses a higher amylose content ($p < 0.05$) and a higher tendency for retrogradation than corn starch.

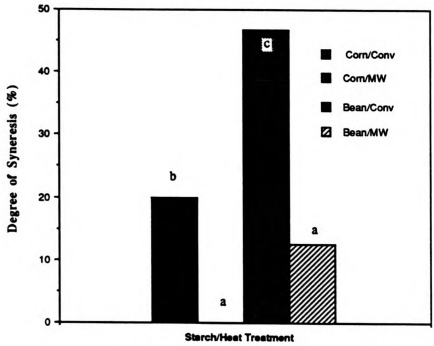


Figure 69. Degree of syneresis(%) of 8% (w/w) starch gels stored at room temperature for 1 week

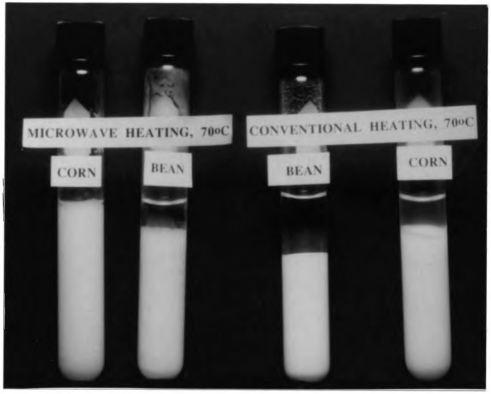


Plate 14. 8% starch gels stored at room temperature for 7 days

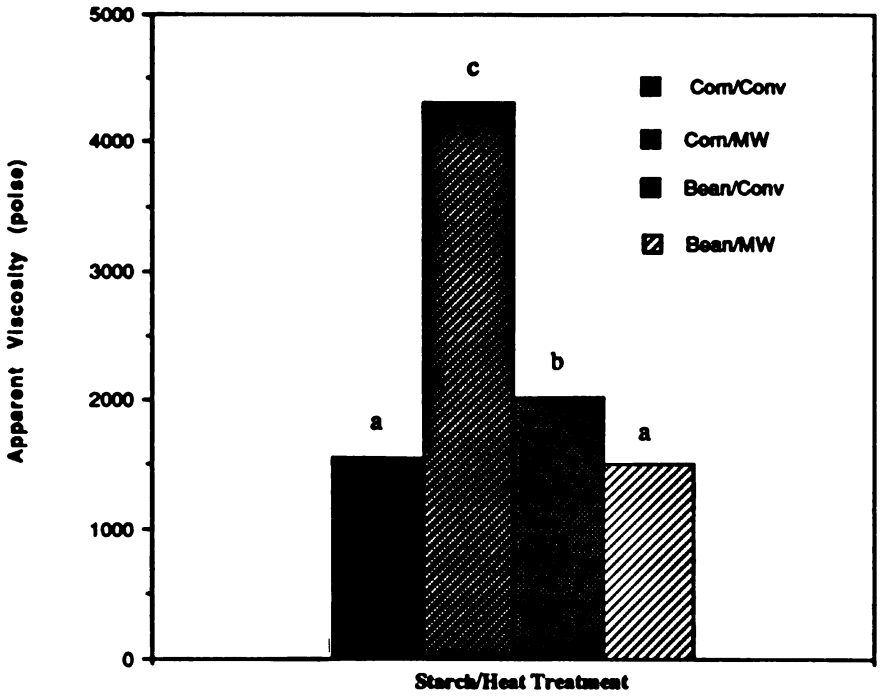


Figure 70. Apparent viscosity (poise) of 8% (w/w) starch gels stored at room temperature for 7 days

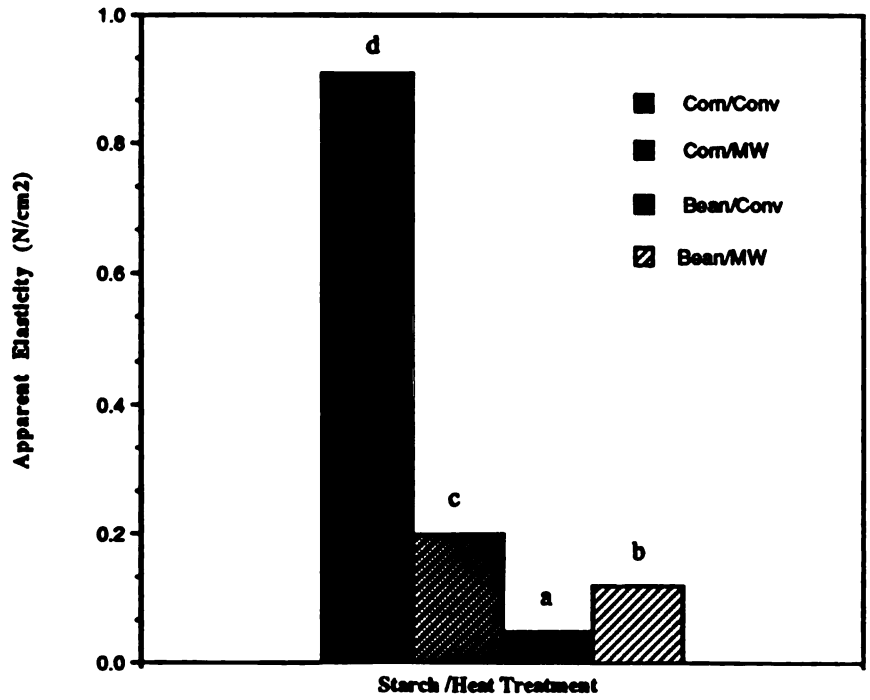


Figure 71. Apparent Elasticity of 8% (w/w) starch gels stored at room temperature for 7 days

The Scanning Electron Micrographs (SEM) of corn and bean starches microwave and conventionally heated at 70°C for 2 minutes are shown in Plates 15 and 16, respectively. Corn starch granules appeared to have collapsed and melted under both methods of heat treatment, possibly as a result of solubilization of the amylose. However, for the microwave heated corn starch, disintegration of the starch granule was more pronounced with matrix formation of the leached materials. These data for corn starch coincide with the very high swelling power, without the characteristic 2-stage pattern, observed at 70°C .

The more resistant bean starch granules still appeared swollen under both methods of heating. Fibrous-like exudates, generally recognized to be amylose, leached out of the granules. However, the microwave-heated starch appeared to have a greater quantity of fibrous-like exudate leaching , with some evidence of matrix formation. The bean starch granules also exhibited shrinkage and deformation within the central region, indicating a weak bonding region.

Conclusion

Microwave preconditioning of dry beans demonstrated the potential of microwave energy to improve whole bean seed cooking, ensure rapid processing of beans by disrupting starch granules and facilitating gelatinization. A number of factors must be controlled in microwave processing of beans to assure appropriate quality. These factors include: a) bean initial moisture, b) the water to bean (liquid/solids) ratio, c) microwave power level (wattage) and d) heating period. Palatable products may be obtained upon application of a suitable combination of these factors.

The physical and functional properties of pure bean starch were significantly affected by microwave heating. Microwave heated starch gels exhibited a significantly lower degree of syneresis and a slightly softer texture than did conventional heated gels. Amylose molecules leached out from the bean starch granules when microwave heated at

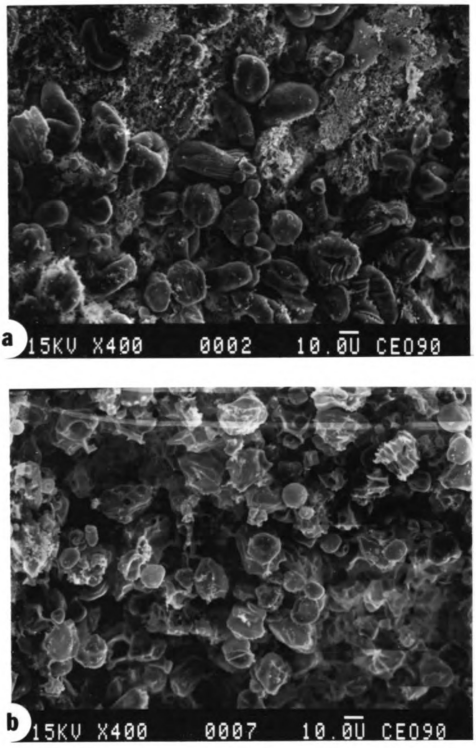


Plate 15. Scanning Electron Micrographs of (a) Bean and (b) Corn starch granules microwave-heated at 70°C for 2 minutes

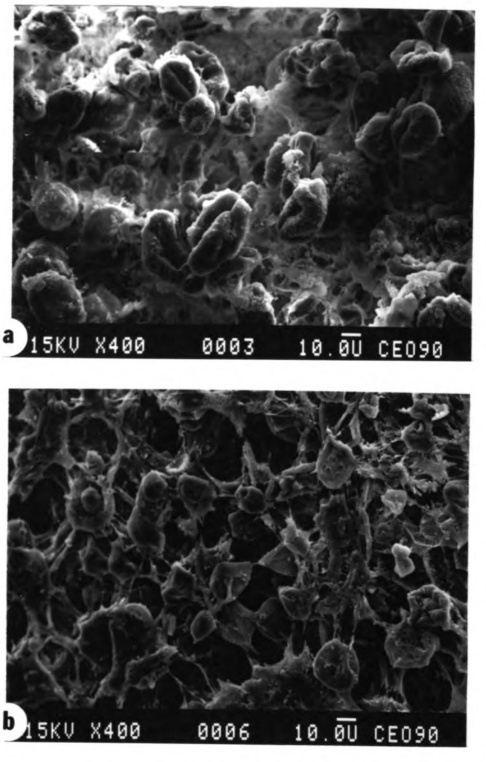


Plate 16. Scanning Electron Micrographs of (a) Bean and (b) Corn starch granules with conventional heating at 70°C for 2 minutes

70°C for 2 minutes, compared to conventionally heated bean starch. Microwave heating of isolated bean starch slurries confirmed the potential for the use of microwave preconditioning of dry beans *in situ* to facilitate subsequent cooking. Further work to provide continuous in-line process treatments which could facilitate improved ease of cookability in bean is warranted.

H_0: Microwave energy will not adequately disrupt starch granules and facilitate gelatinization *in situ* to improve whole seed cooking and functional properties.

Reject the Ho as stated and conclude that microwave energy pretreatments dramatically influence cooking properties of dry beans.

SUMMARY and RECOMMENDATIONS

The research conducted in the course of this dissertation has been directed towards the improved utilization of dry edible beans. The hypothesis and objectives have been focused on the development of processing methodologies which may be adaptable to centralized small and intermediate-scale processing operations and provide products of utility for feeding of young children. All of the studies incorporated practical applications of fundamental technologies and have provided results which indicate that improved functional and nutritional properties may be obtained through process manipulation.

The process pretreatments employed in this research influenced the composition of the drum-dried bean meals. CONTROL and EXTRACT DDBMs significantly differed in their *in-vitro* starch digestibilities. Oligosaccharide content was reduced by the aqueous and enzymatic pretreatments. The use of enzymatic predigestions was conducted with commercially available enzyme preparations to provide fundamental information regarding the changes in intrinsic properties of bean meals. The use of germinated seed as a resource for development of indigenous enzymes was provided to assure linkage to appropriate village-based technology . The enzymatic pretreatments of DDBMs significantly increased starch digestibility by disruption of intact cellular structures and denaturation of protein matrix, thereby increasing accessibility of the amylases to starch. The results reported indicated that the use of enzymatic digests derived from indigenous enzyme sources has potential for immediate adoption. Further research is warranted in the development of selected enzymes from native plant materials.

The drum-drying technology utilized in this research has direct application for village-level and cooperative centralized processing. Such a facility would enable the production of nutritious food ingredients derived from dry edible beans for use in a variety of products. Protein digestibility of DDBMs was significantly improved by the drum-drying process. Trypsin activiity was significantly reduced, and phytohemagglutinin was inactivated by the high heat treatment utilized during the drum-drying process.

The feasibility of incorporating DDBMs in pumpkin quick breads and weaning food/beverages was demonstrated. Acceptable breads were produced at the 20% and 40% levels of substitution. Enzyme pre-digested DDBMs possess great potential in weaning food formulations, particularly at the 8% concentration level. The research conducted on the functional properties of prepared bean meals has demonstrated that these products are functionally suitable for use in standardized foods. Further research is warranted to develop specific recipes for use in regionalized traditional foods.

The potential for development of weaning beverages with reduced viscosity and improved nutritional value has a significant potential for improving the nutritional status of young children. Ingredients for these foods have been prepared and provide a readily accessible option. The bean ingredient could be blended with other appropriate ingredients mixed with water and available without additional cooking. Work is warranted to develop additional native products suitable for blending with the DDBM to enhance and optimize the formulation.

The use of microwave energy although appearing as an advanced technology, was demonstrated to have potential as a pretreatment for enhancing the cookability of dry beans, and for modifiying physical and functional propterties of bean starch for applications in food systems. Such a protocol could be established in a centralized and public sector framework to facilitate pre-processing of dry beans. Whole dry beans could be prepared and pretreated with microwave energy to enable enhanced cookability and storage of dry, shelf-stable bean products with improved cooking potential. This methodology could be established as a continuous operation and provide sound whole seed for consumer use. Such a technology would reduce the requirements for long term cooking and the associated requirements for fuel gathering and use.

The development and use of technologies to enhance the use of dry beans must be pursued within the context which is sensitive to the endproduct outcome. The products derived under a village-level organizational scheme could benefit families, especially those

with young children, and the overall economic development within the region through the utilizationn of women in the development and processing of value-added nutritious food products. Additional processing methodologies could be incorporated into this central pre-processing regime. These enterprises would enable women to serve in managerial, supervisory and labor roles.

The research conducted in this dissertation has provided technological methodologies to improve the utilization of dry edible beans. This work provides a framework for further research in this area. It is essential that additional research be focused on village-level adaptation of these technologies in the framework of the Bean Cowpea-CRSP to assure the sociological integration of technologies designed to enhance the use of dry beans throughout the world.

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