Effects of Pretreatment and Micronization on the Cookability, Chemical Components and Physical Structure of Navy and Black Beans (*Phaseolus vulgaris* L.)

BY

Guillermo Guido Bellido

A Thesis Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements for the Degree of

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Department of Food Science
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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EFFECTS OF PRETREATMENT AND MICRONIZATION ON THE COOKABILITY, CHEMICAL COMPONENTS AND PHYSICAL STRUCTURE OF NAVY AND BLACK BEANS (*PHASEOLUS VULGARIS L.*)

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GUILLERMO GUIDO BELLIDO

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

Master of Science

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LIST OF ABBREVIATIONS

Abbreviation	Description
FAO	Food and Agriculture Organization of the United Nations
EDTA	Ethylene Diamine Tetra Acetic Acid
SEM	Scanning Electron Microscopy
IR	Infrared
SL	Slope of the micronization conveyer trough
GL	Instrumental setting of a gas-air mixture valve
DL	Distance between the conveyer trough and the IR radiation source
VFA	Vibratory Feeder Adjuster
SC	Slope adjuster
GLM	General Linear Model
SAS	Statistical Analysis Software
RSM	Response Surface Methodology
CPE	Combined Percentage Error
ρ_{ap}	Apparent density
ρ_B	Bulk density
ρ_P	Particle density
ε_{op}	Open pore porosity
η_T	Dynamic viscosity at temperature T
D	Overall diffusivity coefficient

Effects of Pretreatment and Micronization on the Cookability, Chemical Components and Physical Structure of Navy and Black Beans

ABSTRACT

Micronization is a process that can be used to improve the marketability of pulses by reducing their cooking times. It is well accepted that the cooking time of beans is one of their most important quality attributes in addition to size and colour. The present investigation was undertaken to study the effects of pretreatments and micronization on the cookability and the physical chemical properties of both navy and black beans. The physicochemical properties of beans were characterized in terms of the degree of gelatinized starch, the percentage of protein solubility, hardness, firmness, colour, size, physical appearance, apparent density, bulk density, particle density, porosity and water imbibition characteristics. Hardness or the maximum force to compress a set amount (30 g) of cooked beans was used as the cooking quality criterion for assessing the effectiveness of micronization processing pretreatments.

Results from an investigation of operational parameters indicated that the slope of the conveyer bed (SL) and the instrumental setting of a gas-air mixture valve (GL), but not the distance (11-11.4 cm) between the conveyer trough and the infrared radiation source (DL), made significant ($P \leq 0.05$) contributions to the optimization of micronization processing conditions by controlling the rate and extent of the micronization process as evidenced by higher final temperatures and lower moisture contents. Optimum micronization operational conditions occurred at GL=9 and SL=5.

Using distilled water to temper the beans, optimum tempering conditions for the micronization of navy and black beans occurred at 28% moisture for 11.5h and 25.8%

moisture for 32h, respectively based on the degree of starch gelatinization. Using these conditions, it was found that a mixture of salts at an alkaline pH (0.2% sodium tripolyphosphate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate) was more effective than a mix of acids (1% citric acid and 2% ascorbic acid) and 150 ppm of sodium EDTA in reducing the hardness of micronized beans. Compared to unprocessed beans, micronization significantly ($P \leq 0.05$) reduced the hardness value of both navy and black beans by 28.7 and 13.7 % when tempered with distilled water and the mix of salts, respectively. Lower hardness was accompanied by a decrease in the percentage of soluble protein and an increase in the degree of gelatinized starch ($P \leq 0.05$).

Although the micronization process influenced the physical properties of both navy and black beans, a tempering pretreatment containing a mixture of salts improved the water imbibition characteristics of black beans only. Results indicated that during the first 30 min of water-soaking (at 21 °C), micronized navy beans that had been tempered with distilled water absorbed significantly ($P \leq 0.05$) more water than both unprocessed and alkaline-treated beans. Micronized black beans tempered with distilled water or the alkaline salt solution imbibed significantly ($P \leq 0.05$) more water than unprocessed beans during the first 60 min of soaking. SEM microphotographs revealed that the micronization process can generate bubbles in both navy and black beans. The pore sizes of intercellular spaces emphasised the limitations of porosity measurements in that they accounted for physically open air-bubbles only. The use of the alkaline salt solution as a tempering pretreatment did not influence the physical properties of either navy or black beans. Porosity studies indicated that micronized black beans tempered with water had statistically ($P \leq 0.05$) higher porosity values than both unprocessed and salt-treated black beans. The higher transportation rates of water through the air phase were thought

to be responsible, at least in part, for the improved water imbibition characteristics of micronized navy (water-treated) and black (water-treated and salt-treated) beans. This study demonstrated that the degree of starch gelatinization, percentage of soluble proteins, open pore porosity, and apparent density are good indicators of the textural quality of micronized beans.

FOREWORD

The following publications or presentations have resulted from the studies reported in this thesis:

Publications

1. Bellido, G.G., Arntfield, S.D., Scanlon, M.G., Cenkowski, S. 2002. The Effect of the micronization operational conditions on the physicochemical properties of navy beans (*Phaseolus vulgaris* L.), submitted.

2. Bellido, G.G., Arntfield, S.D., Scanlon, M.G., Cenkowski, S., Watts, B.M. 2002. Effects of processing conditions and the effects of tempering solutions of the physicochemical properties of micronized beans (*Phaseolus vulgaris* L.), in preparation.

3. Bellido, G.G., Arntfield, S.D., Scanlon, M.G., Cenkowski, S., Watts, B.M. 2002. Effects of micronization on the water imbibition rates of two cultivars of *Phaseolus* beans, in preparation.

Poster Presentations

1. Bellido, G.G., Arntfield, S.D., Scanlon, M.G., Cenkowski, S., Watts, B.M. 2001. The Effect of the micronization operational conditions on the physicochemical properties of navy beans (*Phaseolus vulgaris* L.). Poster presented at the annual meeting of the CIFST, Toronto, ON, 2001. *Outstanding poster presented by an MSc student.*

2. Bellido, G.G., Arntfield, S.D., Scanlon, M.G., Cenkowski, S., Watts, B.M. 2002. Effects of processing conditions and the effects of tempering solutions of the physicochemical properties of micronized beans (*Phaseolus vulgaris* L.), in preparation. Poster presented at the annual meeting of the CIFST, Edmonton, AB, 2002.

INTRODUCTION

Among the world's pulses, dry beans rank first in production and consumption (FAO, 2000). In Canada, beans from the genus *Phaseolus* are becoming an increasingly important commercial crop. Beans are an excellent source of proteins, B-complex vitamins and several minerals. Beans are therefore an excellent food choice, and they are considered a staple food in many countries. However, North American consumption of beans is still limited, in part due to the long cooking times required for their preparation. Micronization technology has been shown to reduce the cooking time of lentils by one third (Zhao, 2000), and of field peas by a half (Toews, 2000). It was expected that micronization could also reduce the cooking time of beans. It is therefore not surprising that the concept underlying the micronization process opens a broad spectrum of opportunities for expanding the pulse crop industry both domestically and internationally.

In previous studies with lentils (Zhao, 2000) and peas (McCurdy, 1992, Toews, 2001) the operational parameters of a gas-fired pilot scale micronizer unit were manipulated during processing to achieve desirable ranges of final surface temperature in the micronized product. This approach, however, would not be appropriate for large-scale operations since it would not result in a product of consistent quality. As with other pieces of equipment, a micronizer has several operational parameters that could have different degrees of impact on the quality of the final product. After preliminary experimentation, the slope of the conveyer bed (SL), the instrumental setting of a gas-air mixture valve (GL) and the distance between the conveyer bed and the infrared radiation source (DL) were selected as the operational conditions that had a greater impact on the quality of micronized beans. The objective of the first experiment was therefore to study

the effect of micronization operating conditions on the physicochemical properties of navy beans.

The effectiveness of the micronization process in reducing the cooking time of pulses has been found to depend upon several factors including physical structure and chemical composition. Several investigations carried out at the University of Manitoba have also indicated that the moisture content of pulses should be adjusted prior to micronization processing or the process can undermine the quality of the final product (Scanlon *et al.*, 1998; Zhao, 2000; Toews, 2001). As well, availability of water in the seed was particularly important to improve the cooking quality of pulses tempered with solutions containing distilled water alone or dilute solutions of salts, acidulates, or alkali. The second experiment of this investigation was undertaken to optimize the use of tempering conditions (tempering time and tempering level) and to evaluate the use of several tempering solutions as pretreatments for reducing the cooking time of both navy and black beans. It is hypothesised that the micronization process affects the cooking quality of beans, and thereby could be optimized, by particular tempering conditions and using certain aqueous solutions containing salts, acidulates or alkali. Using the literature, several tempering solutions were identified and prepared. These included distilled water alone, a mixture of salts (0.2% sodium bicarbonate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate), a mixture of acids (1% citric acid and 2% ascorbic acid), and 150 ppm of sodium EDTA.

The last experiment was conducted to determine the effect of the micronization process on physical properties such as water imbibition rates of both navy and black beans (*Phaseolus vulgaris* L.). The tempering solutions (micronization pretreatments) included distilled water and the alkaline salt solution due to their effectiveness for

improving the cooking quality of micronized both navy and black beans, compared to unprocessed beans. Changes in the degree of gelatinized starch and the percentage of soluble protein during micronization processing were also included in this chapter in order to broaden the scope of the discussions.

Chapter 1: Literature Review

1.1. Background

Common beans, *Phaseolus vulgaris* L., are plants from the family Leguminosae (Papilionaceae), also known as legumes. Despite the 13,000 or more species of legumes known, only 20 are commonly consumed by humans (Deshpande *et al.*, 1984). *Phaseolus vulgaris* is the most widely distributed species of the *Phaseolus*, and is thought to have originated in the western Mexico-Guatemala area (Kay, 1979).

Food legumes may be divided into pulses and oilseeds. Pulses or grain legumes are the dried edible seeds of a cultivated legume, whereas oilseeds are legumes used primarily for their oil content (Aguilera and Stanley, 1999). Legumes may also be consumed as immature green seeds, or as green pods with the immature seeds enclosed (Kay, 1979; Salunkhe *et al.*, 1989). In Java, young leaves of *Phaseolus vulgaris* are eaten as a salad (Salunkhe *et al.*, 1989).

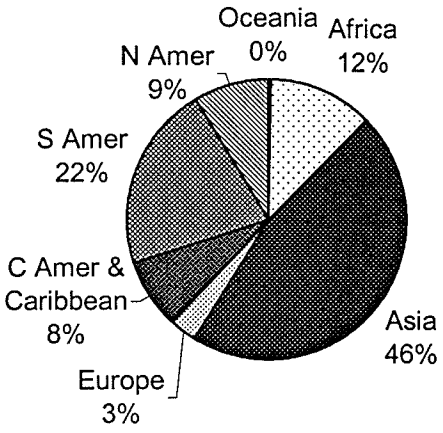
Several attempts have been made to divide *Phaseolus vulgaris* into subspecies based on features such as dwarf versus tall habit size, shape and colour of the seed. However, the creation of subspecies has been limited due to the existence of several hundred cultivars within species (Kay, 1979). The commercial classification of grain legumes is primarily based on the colour and size of the seeds, and this classification seems to be more prevalent in international trading. Hussain *et al.* (1988) indicated that lustre of the seed coat could be used as an additional classification criterion since it is related to genotype. Dry beans from the *Phaseolus vulgaris* species include varieties commercially known as great northern, navy, black, white kidney, light red kidney, dark red kidney, pinto, dutch brown, small red, and cranberry beans (Pulse Canada, 2000).

The Canadian Food Inspection Agency though, acknowledges that only the following varieties are registered in Canada: pea (navy), black, cranberry, great northern, marrow, manteca, pink, pinto, red kidney, red mexican, white kidney, and yellow eye beans (Canadian Food Inspection Agency, 2002). In international trading, navy beans are also known as Michigan, alubias chicas, pea or small white beans, whereas black beans may also be known, especially in Latin American and the Caribbean, as turtle or preto beans (Kay, 1979; Pulse Canada, 2000).

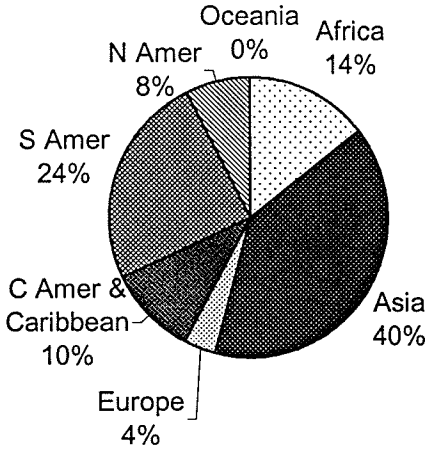
The physical characteristics of seeds such as size, shape or colour play an important role in food processing, especially in micronization processing (Ginzburg, 1969). Even though navy and black bean seeds have relatively the same size and shape (Salunkhe *et al.*, 1989), the micronization process should have a different impact on the light-coloured navy beans versus the dark-coloured black beans since the amount of infrared radiation energy absorbed by a material is related to the material's light reflectance characteristics (Ginzburg, 1969). As such, navy and black beans were therefore used in the present study due to their potential as a means to compare the effect of micronization on physically and chemically-alike materials and due to their importance as major pulse crops in Western Canada (Pulse Canada, 2000).

1.1.1. Production and consumption of dry beans

In 2001, the world production of legumes was estimated to be 52.3 million metric tonnes, and dry beans accounted for 32 % (16.8 million metric tonnes) of this total production (FAO, 2002). India ranked first in production of dry beans (2.6 million metric tonnes), followed by Brazil (2.5 million metric tonnes), China (1.5 million metric tonnes) and Mexico (1.1 million metric tonnes). In Canada, pulses are also an important commercial crop. Canada's pulse production for the year 2001 was 3,558,700 metric



A. Production of dry beans



B. World distribution of dry beans

Figure 1. The production (A) and world distribution (B) of dry beans by continent (FAO, 2002).

tonnes, 261,400 of which were dry beans (FAO, 2002). In Canada, the seeded area of navy and black beans constitutes more than 53 % of the total seeded area for dry beans (Pulse Canada, 2000). Canada's production of dry beans has increased by almost 100% in the past 5 years, and more than 81% of Canada's annual production in the year 1999 went to the export market (FAO, 2002). To illustrate net pulse production and exports, Figure 1 shows the production and world distribution of dry beans for the year 2001. It can be deduced from Figure 1 that Asia, South America, and Africa are the main producers and consumers of dry beans worldwide. However, note that both their internal demand and supply was relatively equilibrated in the year 2001. Asia is still the largest producer and the main destination for most of the world's dry bean production. Conversely, North America's demand for dry beans is still relatively low, making North America a net exporter. In total, 51% of dry beans are consumed in developing countries (FAO, 2002). Overall, these figures indicate that North America's production of dry beans, especially Canada's, is steadily growing, but if following today's trends less than 20% of this production should go to the local markets. Current food trends in North America, however, suggest that demand for healthy foods like dry beans should also increase.

Food legumes, particularly grain legumes or pulses, are considered staple foods in many countries, while in others are considered second after cereals as a source of energy and plant proteins. Dry beans are a good source of proteins, complex carbohydrates, vitamin C, complex-B vitamins, folic acid, and minerals (Salunkhe *et al.*, 1989). Although pulses are a good source of amino acids, they are deficient in methionine. However, they provide a good amino acid balance when combined with lysine-deficient but methionine rich cereals. Furthermore, dry beans are palatable after

cooking and have the potential to meet the needs of consumers seeking both economical and nutritionally healthy foods.

1.2. Seed structure

The structure of legume seeds is composed of two major parts: the embryo, and the seed coat or testa. The embryo of mature seeds of *Phaseolus vulgaris* consists of the embryonic axis and the cotyledons. The embryo axis in turn is composed of the hypocotyl (a stem-like embryonic axis below the cotyledons), and the radicle (an embryonic root) (Koning, 2002). The embryo axis and the seed coat constitute about 1-2% and 8-15% of the weight of beans, respectively. As a result, the nutritional contribution of the embryo axis to the whole seed is almost negligible despite being a rich source of nutrients (Kadam *et al.*, 1989).

The seed coat is chemically made up of cellulose, hemicellulose, lignin, pectin and calcium (Kadam *et al.*, 1989, Aguilera and Stanley, 1999). These components provide structure and support to cells (Hincks and Stanley, 1987). Since the seed coat constitutes 80-90% of the total dietary fibre, removal of the seed coat is undesirable (Kadam *et al.*, 1989). As well, the seed coat acts as an outer protective layer, and is an effective barrier to fungi under normal storage conditions (Wolf *et al.*, 1981; Swanson *et al.*, 1985). Sefa-Dedeh and Stanley (1979) indicated that the seed coat characteristics of cowpeas influenced their water absorption characteristics, which in turn was related to their textural characteristics.

Both the external palisade layer and the hourglass cells characterize the seed coat microstructure (Swanson *et al.*, 1985). Agbo *et al.* (1987) reported that the thickness of the seed coat palisade cell layer was responsible, at least in part, for differences in water uptake in isogenic lines of dry beans. They found that the 'sanilac' cultivar had an

opened micropyle and prominent seed coat pores that favoured rapid water uptake, as compared to the isogenic cultivar "nep-2' that had a partially opened micropyle and only a few prominent seed coat pores.

The outermost portion of the seed coat is the waxy cuticle layer. This layer is composed of a cuticular membrane containing embedded wax, over which is deposited a layer of epicutelar wax (Swanson *et al.*, 1985). This layer serves as the prime barrier against penetration of water. To illustrate, soybeans that were grown at high temperature and high humidity conditions developed an unusual thick waxy cuticle layer that caused impermeability to water (Arechavaleta-Medina and Snyder, 1981). As well, dark-coloured seed coats were reported to imbibe less water than light-coloured seed coats, probably due to a denser arrangement of seed coat cells in dark-coloured seeds (Powell, 1989) or as a result of oxidative reactions of phenolic substrates resulting in hydrophobic substances (Hincks and Stanley, 1986; Hohlberg and Stanley, 1987; del Valle *et al.*, 1992b; Stanley, 1992).

The anatomical seed structures raphe, hilum and micropyle have also been put forward as responsible for the water imbibition capacity of legume seeds. Specifically, the raphe is a ridge on the seed coat formed from adnate funiculus, the hilum is a funicular scar on the seed coat, and the micropyle is a hole that goes through the seed coat (Koning, 2002). Hyde (1954) theorized that the hilum acts as a hygroscopic valve, opening to absorb moisture during times of low environmental humidity, and closing to eliminate moisture during high environmental humidity. Alternatively, Powrie *et al.* (1960) hypothesised that water migrates principally through the seed coat, and then hydrates the bean cotyledons during soaking. Later, Sefa-Dedeh and Stanley (1979) reported that during soaking of cowpeas, the raphe, hilum and micropyle together form

an integral water absorption/removal system. Microstructure therefore plays an important role in water imbibition characteristics of pulses during soaking and cooking. The function/importance of the seed coat and seed coat components in water uptake will be discussed later.

Unlike cereals, dry beans are non-endospermic seeds, which means that the endosperm is almost completely absorbed and almost degraded by the developing embryo, especially by the cotyledon (Aguilera and Stanley, 1999). Thus, the cotyledons serve as the main food storage organs in mature seeds. The fleshy storage cotyledons make up most of the seed's volume and weight. The cotyledon (84-90% of the seed) is composed of storage parenchyma cells, with an average size of 60 to 120 μm, enclosing starch granules embedded in a protein matrix. Parenchyma cells are large, thin walled and polygonal in shape (Mohsenin, 1970). The parenchyma cells are not tightly packed and are separated by intercellular spaces, which may be filled with air or water (Mohsenin, 1970).

The mechanical properties of cell walls in plant materials reflect the mechanical properties of the plant tissues (Mohsenin, 1970). Therefore, the elasticity, strength and rigidity of plant tissues are the result of the rheological properties of the cell wall (Mohsenin, 1970). The plant cell wall has many functions. These include: the provision of strength and shape to the cell, and rigidity to the whole plant; the control of cell growth by selective weakening of the wall; protection against other organism; participation in cell-cell communication; and storage of food reserves (Brett and Waldron, 1990). The cell wall is comprised of several layers that are deposited at different stages during cell division and have different thickness. Cell wall layers include the middle lamella (earliest-formed cell wall layer), the primary cell wall, and the

secondary cell wall (Brett and Waldron, 1990). Sefa-Dedeh *et al.* (1978) indicated that the middle lamella is mainly composed of calcium salts of polymers of galacturonic acid that have been partially esterified with methanol. It has been reported that the primary cell wall consists of cellulose microfibrils that are loosely attached together in an irregular pattern and embedded in an amorphous matrix composed mainly of pectic substances and hemicellulose (Sefa-Dedeh *et al.*, 1978). Brett and Waldron (1990) indicated that the plant cell wall consists of two phases, a microfibrillar phase and the matrix phase. The microfibrillar phase is comprised of cellulose (β1,4-glucan), and the matrix phase is comprised of pectins (i.e. rhamnogalacturonan I, arabinan, galactan, arabinogalactan I, homogalacturonan, and rhamnogalacturonan II), hemicelluloses (i.e. xylan, glucomannan, mannan, galactomannan, glucuronomannan, xyloglucan, callose, β1,3-,β1,4-glucan, and arabinogalactan II), proteins (i.e. extensin, arabinogalactan-protein, others including enzymes), and phenolics (i.e. lignin, ferulic acid, others including coumaric acid and truxullic acid) (Brett and Waldron, 1990). In beans, a protein matrix is more predominant in the outer parenchyma cells, while starch granules are more predominant in the centre (Kadam *et al.*, 1989).

1.3. Composition of dry beans

The chemical composition of dry beans, as for most legumes, is dependent upon cultivar, as well as agronomic and environmental growing conditions (Hile, 1976; Evans and Boulter, 1980; Hussain *et al.*, 1988; Kadam *et al.*, 1989; Singh *et al.*, 1991; Periago *et al.*, 1996; Casanas *et al.*, 1999). Singh *et al.* (1991), for example, studied the genetic diversity of 306 landraces of cultivated common beans from Latin America in the year 1987-1988. When phaseolin seed proteins were used as a classification criterion, they

found the existence of subgroups within the major Andean and Mesoamerican groups, with distinctive morphology, adaptation, and disease resistance.

In another attempt to classify beans by composition, Casanas *et al.* (1999) studied the nutritional properties of several varieties of *Phaseolus vulgaris*. They found that 'ganxet' bean germplasm had higher levels of protein, digestible dietary fibre and glucose, but less total dietary fibre and starch than white kidney, navy, faba asturiana and tolosa beans. They also found a sufficient degree of morphological, agronomic and nutritional variability among seven inbred lines of 'ganxet' bean, of which only two inbred lines were chosen for further commercialization.

In a further exploration of dry bean variety diversity, Agbo *et al.* (1987) studied the relation between the microstructure of isogenic dry bean lines (Nep-2, San Fernando and Sanilac) and their water uptake capacity. They found differences in the water hydration capacity of Nept-2 and San Fernando, two genetically similar varieties differing by only a single Mendelian factor that imparts colour to the seed coat. Since Nep-2 was derived from San Fernando, they suspected that the gene for seed coat colour in Nept-2 exhibited pleitropy-the property of a gene having more than one phenotypic effect on an organism.

Casanas *et al.* (1999) and Agbo *et al.* (1987) studies, among many other studies, suggested that the physical and chemical characteristics of *Phaseolus vulgaris* depends upon genetic and environmental factors, and that they should not be overlooked during interpretation of experimental data.

1.3.1. Carbohydrates

Legume carbohydrates form 50 to 60%, on a dry weight basis, of the seed (Salunkhe *et al.*, 1989) and include starch, reducing and non-reducing sugars, oligosaccharides of the raffinose family and other polysaccharides such as pectin (El-

Shimi *et al.*, 1980; Sathe and Salunkhe, 1981a). Total sugars, including oligosaccharides, represent only a small portion of total carbohydrates in dry beans. Reddy *et al.* (1989) reported that soluble sugars (including oligosaccharides) in navy and black-eyed beans represented 5.6-6.2% and 3.4-4.5% of total carbohydrates, respectively. Table 1 indicates the typical chemical composition of navy and black beans (*Phaseolus vulgaris* L.).

1.3.1.1. Soluble sugars

Soluble sugars in beans include reducing sugars, such as glucose and fructose, as well as oligosaccharides from the raffinose family, namely raffinose, stachyose,

Table 1. Chemical composition of *Phaseolus vulgaris* L. (var. navy and black).

	Amount (%) [1]	Reference
Carbohydrate	67- 74.4	Deshpande *et al.*, 1984 Salunkhe *et al.*, 1989
Sugars	3.4 - 6.2	Labaneiah and Luh, 1981 Salunkhe *et al.*, 1989 Reddy *et al.*, 1989
Starch	54.5 – 60.2	El-Shimi *et al.*, 1980 Labaneiah and Luh, 1981 Reddy *et al.*, 1989
Dietary fibre	3.8-8.1	Salunkhe *et al.*, 1989
Protein	20.4 - 27.6	Salunkhe *et al.*, 1989 Deshpande *et al.*, 1984
Lipid	1.5 - 4.3	Salunkhe *et al.*, 1989 Deshpande *et al.*, 1984
Ash	3.5-4.5	Salunkhe *et al.*, 1989

[1] Dry weight basis

verbascose, and ajugose. It was found that during seed germination, raffinose and stachyose were hydrolysed by endogenous α-galactosidase into sucrose and galactose, and the latter further metabolized through a galactose-utilization system (Labaneiah and Luh, 1981). These oligosaccharides are therefore metabolized during germination to meet the requirement of the developing plant tissue. The human digestive system, however, does not produce α-galactosidase, an enzyme responsible for breaking down oligosaccharides. After ingestion, oligosaccharides are metabolized by anaerobic microflora in the human colon. As a result, carbon dioxide, hydrogen and methane are released. This mechanism of oligosaccharide digestion is responsible for flatulence, and being a social discomfort to many people, often leads to a limited consumption of beans (Sathe and Salunkhe, 1981 c).

1.3.1.2. Starch

Starch is an omnipresent substance in plant seeds. As well, starch is the main source of energy for many humans, and constitutes the major portion of legume carbohydrates (Sathe and Salunkhe, 1981a). Starch is a glucan composed of amylose and amylopectin. The macromolecular arrangements of the starch subunits, amylose and amylopectin, are known as starch granules. Amylose is essentially a linear chain of (1→ 4)-linked α-D-glucopyranosol units with a few α-D-(1→ 6)-linked units (9-20 per molecule), while an amylopectin molecule is made up of (1→ 4)-linked α-D-glucopyranosol units that are highly branched with α-D-(1→ 6)-linked units (Aguilera and Stanley, 1999).

In plants, starch is found in the form of granules of irregular rounded shapes ranging in size from 2 to 100 μm (Kulp, 1973; Duffus and Murdoch, 1979; Lii and Chang, 1981; Reddy *et al.*, 1989; Aguilera and Stanley, 1999). The use of light

microscopy and scanning electron microscopy (SEM) to study the surface of legume starches has facilitated their characterization in relation to their morphology, physicochemical, and functional property changes during processing treatments. Early studies described cotyledonary bean cells as collapsed oblate-shaped plastic bags containing large, almost spherical, starch granules (Rockland and Jones, 1974). Rockland and Jones (1974) reported that starch granules in large lima beans had nearly spherical shape with an average size of 25 μm. However, Sathe and Salunkhe (1981b) reported that the starch granule size of great northern beans ranges from 12 x 12 to 58 x 40 μm (length x width).

Most starch granules have irregular shapes, although, generally, the length is greater than the width. As well, more defined shapes have been reported including round, oval, and elliptical (Colonna *et al.*, 1981; Reddy *et al.*, 1989; Saio and Monma, 1993). Varriano-Marston and Jackson (1981) defined navy bean starch granule shape as reniform with size ranging from 10 to 60 μm. Gujska *et al.* (1994) reported that the mean starch granule size (length x width) for navy and pinto beans was 21 x 20 μm and 22 x 19 μm, respectively. Hoover and Manuel (1996) reported that the granule sizes of green arrow pea, black bean, othello pinto bean, express field pea, and eston lentil were in the range of 10-15, 6-22, 8-18, 6-29, and 4-20 μm, respectively. Overall, the wide variation in granule size and shape is attributed to genetic variability and seed maturity (Reddy *et al.*, 1989).

In legumes, amylose may constitute the major part of starch, the range being from 10 to 66% of the starch content (Reddy *et al.*, 1989). Yet most importantly, the amylose content in the starch influences starch solubility, lipid binding, and other functional properties (Reddy *et al.*, 1989). Labaneiah and Luh (1981) reported that the

amylose to amylopectin ratio for red kidney, gloria pink, and black eye beans was 0.59, 0.55, and 0.62, respectively. These results were slightly different from the amylose to amylopectin ratio of 0.41 and 0.45 for black and othello pinto beans respectively, found by Hoover and Manuel (1996). It is interesting to note that the amylose to amylopectin ratio for dry beans differs greatly from that of corn (0.28), potato (0.21), tapioca (0.17), and wheat (0.28) (Hoover and Manuel, 1996). The differences in amylose to amylopectin ratios in plant material explain, in part, the differences in the physicochemical properties of their starches.

Starch granules contain both amorphous (unordered) and crystalline (ordered) regions. The crystalline portion is responsible for the Maltese cross (birefringence) appearing when the native granules are seen under polarized light. Hahn *et al.* (1977) observed, using SEM, that birefringence was eliminated in water-soaked beans boiled for 90 min, and showed that each stage of starch gelatinization had a characteristic distribution of granule configurations. When starch granules are heated in the presence of water, several structural changes take place. For example, it is known that starch granules swell in the presence of water. In general, the hydration capacity of legume starches has been reported to be 10g/g (Sathe and Salunkhe, 1981 a, 1981 b; Hoover and Manuel, 1996). The swelling capacity of starch granules increases as the temperature is increased, although both the starch source and pH have also been reported to influence swelling (Reddy *et al.*, 1989, Hoover and Manuel, 1996). After granules swell to a point where the internal forces can no longer maintain their integrity, they collapse and lose their configuration (Hood and Liboff, 1982). Hood and Liboff (1982) reported that as the starch granule of corn swells, a filamentous material was leached out. The extra-granular material was then identified as amylose since it stained blue with iodine, a

reaction that does not appear in starch solutions that contain no amylose (Hood, 1980). It has also been observed that the water absorption capacity of bean starches is inversely related to solubility and directly to swelling (Reddy *et al.*, 1989).

As temperature exceeds a value typical for each plant species, the crystal structure of the starch granule is disrupted by gelatinization (Aguilera and Stanley, 1999). It was also reported that chemical crosslinking occurs, which not only minimizes the expulsion of amylose from the granule but also prevents the granule from collapsing (Hood, 1980). The gelatinization temperature of starch granules increases as the amount of water is reduced or solutes are added (Fujimura and Kugimiya, 1994; Aguilera and Stanley, 1999). Hoover and Manuel (1996) studied the physicochemical properties of isolated legume starches heated at 100 °C for 16 h at moisture content of 30%. They found that bonding forces within the amorphous region of the granules were disrupted and that the crystallite orientation and the granule surface were altered during heat treatment. Furthermore, the extent of these changes was dependent upon the starch source since amylose leaching and swelling of cotyledons was relatively the same in all varieties. The degree of starch gelatinization is a good indicator of the extent of cooking in legumes as it has been correlated with the texture of micronized lentils (Arntfield *et al.*, 1997).

1.3.2. Protein

Phaseolus beans are an excellent source of dietary protein, the content of which ranges from 20 to 28% of the total bean weight (Table 1). Proteins are polymers of amino acids linked by peptide bonds, and are formed by condensation during synthesis in the ribosome (Aguilera and Stanley, 1999). The criteria for protein classification are numerous in the literature, and at times confusing. This investigation attempts to

incorporate literature pertinent to the study of legume proteins, more specifically bean proteins. The reproducibility of the seed protein content reported of *Phaseolus* beans is subject to cultivar, agronomic and growing conditions, and even location within the plant (Adsule and Kadam, 1989).

The Osborne classification (1907) is widely used to classify seed proteins according to their solubility in different solvents. The fraction extracted by water is defined as albumins, the fraction extracted by dilute salts as globulins, the fraction extracted by ethanol as prolamines, and the fraction extracted by acid or base as glutelins. Protein solubility is affected by several factors including pH, temperature, processing conditions, ionic strength, presence/absence of other components (which are capable of binding with proteins), and solvents (Salunkhe *et al.*, 1989).

Salunkhe *et al.* (1989) reported that dry bean proteins could also be classified into two types, namely metabolic and storage proteins. Metabolic proteins include both the enzymatic and structural proteins, which are responsible for normal cellular activities and the synthesis of storage proteins. Storage proteins are synthesised during seed development along with reserves of carbohydrates (mainly starch for beans) and oils. More than 95% of the extractable proteins in dry beans are considered storage proteins. In beans, these proteins have been identified as albumin and globulin units (Sathe and Salunkhe, 1981a). Storage proteins in beans supply the developing seeds with nitrogen and carbon during germination. This could explain the high levels of nitrogen-containing amino acids in dry beans including asparagine (12.2 g/16g N), glutamine (16.3 g/16g N), and arginine (9.2g/16g N) (Adsule and Kadam, 1989). Dry beans, as with other legumes, are an excellent source of lysine (6.8 g/16g N), but are deficient in the sulphur containing amino acids methionine (1 g/16g N) and cystine (0.8 g/16g N) (Adsule and

Kadam, 1989). High protein content tends to be associated with small, spherical, black or white seeds, and from plants grown in tropical areas, while low protein content tends to be associated with elongated seeds from bush-type plants grown in temperate areas (Kay, 1979).

Although, there does not appear to be consensus in the literature regarding the nomenclature of *Phaseolus* storage bean proteins, it is well accepted that globulins are the major protein subunits (Sathe and Salunkhe, 1981a; Adsule and Kadam, 1989; Salunkhe *et al.*, 1989; Deshpande and Damodaran, 1990). Salunkhe *et al.* (1989), for example, summarised the albumin and globulin contents of 15 varieties of *Phaseolus* beans. They indicated that the range for albumins was 12 to 31% and that for globulins was 46 to 81% of the total crude proteins. Legume proteins can be further classified into other groups, principally based on their sedimentation coefficients. The main protein subunits were identified as vicilin or glycoprotein II or globulin G1 (6.9S or 7S), phytohemagglutinin or globulin G2, or lectins (6.4S), and legumin (11S). Salunkhe *et al.* (1989) reported that vicilin and phytohemagglutinin were the major protein subunits accounting for 50 and 10% of the total seed protein. They also recommended that the presence of other protein subunits such as the 11S (legumin) and 2S proteins should not be disregarded. Though Kay (1979) reported that dry bean proteins are mainly composed of phaseolin (20%), phaselin (2%), and conphaseolin (0.35-0.4%), the criterion for this classification was not addressed in the publication. Further, Bollini and Chrispeels (1978) reported that vicilin (6.9S) was made up of three non-identical subunits with molecular weights (MW) of 52,000, 49,000, and 46,000. Phytohemagglutinin or lectin was reported to be a 6.4S protein with a leukoagglutinating subunit (MW 34,000) and an erythroagglutinating subunit (MW 36,000).

Looking at specific bean varieties, Hussain *et al.* (1989) found between 12 and 20 protein subunits in field-dry and heated-dry black beans. They suggested that this variation in the number of protein subunits was due to compositional changes occurring in the samples during a 9-month storage period. Since the concentration of amino acids in the seed is a function of the amino acids in the storage proteins, the concentration of amino acids in dry beans is largely dependent upon the concentration of albumin and globulin proteins (Sathe and Salunkhe, 1981b). For example, in kidney and great northern beans the percentage of albumins and globulins together was reported to vary from 67.9% to 99.97% of the total crude proteins (Salunkhe *et al.*, 1989).

1.3.3. Antinutritional factors

The availability and utilization of proteins is a function of the amino acid composition and digestibility of the proteins and can be restricted by the presence of antinutritional factors (Adsule and Kadam, 1989; Van der Poel, 1991). Despite the high protein content of legume seeds (20-40%), their utilization for human food purposes is still limited. Restricted utilization of bean proteins may be attributed to the presence of enzyme inhibitors (trypsin, chymotrypsin, α-amylase inhibitors) and toxins like phytohemagglutinin, as well as to their deficiency of sulphur-containing amino acids (Sathe and Salunkhe, 1981c; Adsule and Kadam, 1989; Proulx *et al.*, 1993; Barampama and Simard, 1994).

Of the antinutritional factors in legumes, hemagglutinins or lectins are particularly problematic as they are proteinaceous toxic compounds that reduce the bioavailability of nutrients by combining with cells lining the intestinal wall (Adsule and Kadam 1989). Other notable antinutritional factors are polyphenols (tannins), which are compounds that impart disease-resistance properties in legume seeds. Antinutritional

effects of bean tannins include depression of food/feed intake, formation of tannin complexes with dietary protein and other food components, inhibition of digestive enzymes, and increased excretion of endogenous proteins (Jadhav *et al.*, 1989). Phytic acid has also been reported to lower bean nutritional quality by reducing bioavailability of minerals and proteolytic enzymes (Reddy *et al.*, 1989; Uzogara *et al.*, 1990). As well, phytic acid has been implicated in the hard-to-cook phenomenon of beans. It has been hypothesised that when phytate is hydrolysed enzymatically by phytase, its chelation potential is reduced, allowing enhanced cross-linking of pectic substances with divalent cations in the middle lamella. The resultant magnesium and calcium pectates do not solubilize upon cooking in water, which in turn restricts cell separation and thus results in the hardening of beans (Moscoso *et al.*, 1984; Aguilera and Stanley, 1985; Hincks and Stanley, 1987).

1.4. Physical properties of beans

1.4.1. Size and Shape

Shape and size are inseparable physical properties, and they are generally used to describe an object (Mohsenin, 1970). Seeds and grains are irregular in shape and a complete specification of their form, in theory, requires an infinite number of measurements (Mohsenin, 1970). Since the seed coat of beans encloses the seed contents, the surface area of beans is approximately equivalent to the surface area of the seed coat. The surface area and volume of many plant materials can be described by using Euclidian geometry (Rahman, 1995). While the shape of beans can be approximated to that of a prolate or an oblate spheroid (Mohsenin, 1970), their surface area and volume need to be calculated by other means. The measurement of the axial dimensions of seeds, although not very accurate, is a common method to calculate seed

size and volume (Mohsenin, 1970; Deshpande *et al.*, 1984; Salunkhe *et al.*, 1989; Fasina *et al.*, 1997). The axial dimensions of a seed could be simply measured by using callipers or calculated from orthogonal views obtained by projecting a seed in a photographic enlarger (Mohsenin, 1970). Both the size and shape of seeds are important quality attributes when grading pulses. The Canadian Grain Commission (2002), for example, established that Canada Grade Extra No.1 beans should have a 'uniform size'. Their report indicates that pulse quality determinations (i.e. shape and colour) are, to a large extent, based on subjective assessments. Nevertheless, objective methods for calculating the physical characteristics of beans provide important data for predicting physical or mechanical phenomena such as water diffusivity during water hydration or cooking.

1.4.2. Colour

Colour is one of the major quality attributes that is used to grade grains and pulses (Canadian Grain Commission, 2002). The Canadian Grain Commission (2002) stipulates that the colour of pulses is assessed against the colour standards for a set of grades. Beans, for example, have to have a reasonably good colour before they are graded as Canada Grade No. 1. There is no numeric tolerance for colour. Table 2 indicates the standard of quality to grade beans including a brief description of the standard.

It is known that heating foods changes their physical and chemical qualities. Consequently, colour also changes during micronization processing of pulses (McCurdy, 1992, Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998; Arntfield *et al.*, 2001; Toews, 2001). Colour can be assessed using a sensory test (subjective method) or using a colour measuring system (objective method). Several colour-measuring systems

Table 2. Canadian standards of quality for evaluation of colour in dry beans

Standard of quality	Description (for grading)
Good natural colour	Beans may be slightly dull, slightly immature or have lightly adhered soil.
Reasonably good colour	Beans are moderately immature, with lightly adhered soil, or are lightly stained, or are lightly discoloured from storage
Fairly good colour	Beans have moderately adhered soil or are stained, or moderately discoloured from storage.
Off colour	Beans cannot be considered of fairly good colour.

Source: Official Grain Grading Guide, The Canadian Grain Commission (2002)

have been developed (Hunter, 1975; Little, 1976; Hunt, 1991). The colour-measuring system based on the *L*, a, and *b* values, however, is a reliable and commonly used method to objectively assess colour changes of legumes due to micronization processing (McCurdy, 1992; Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998; Arntfield *et al.*, 2001; Zhao, 2000; Toews, 2001). The *L* value measures lightness, the *a* value measures the degree of redness (+) or greenness (-), and the *b* value measures yellowness (+) or blueness (-). Arntfield *et al.* (1997) reported that although the texture of cooked lentils softened as tempering moisture increased, this occurred concomitantly with a darkening of the lentils. They reported lower colour scores for the Hunterlab *L* and *b* values and higher colour scores for the Hunterlab *a* value of micronized lentils. Micronized yellow peas also exhibited a strong yellow (Hunterlab b value) colour that decreased the brightness (Hunterlab L value) score (Cenkowski and Sosulski, 1998).

1.4.3. Density and porosity

Density is an important physical characteristic of pulses that influences heat and mass transportation phenomena during cooking, and could also be used as a quality parameter in grading beans (Pulse Canada, 2000). In food engineering, for example, density is used for determining Reynolds number in pneumatic and hydraulic handling of grains, for determining the purity of seeds, for separating the product from undesirable material, and even for correlating physical structure to chemical composition (Mohsenin, 1970; Deshpande *et al.*, 1984).

Specific gravity of a plant material is the ratio of the density of a material to the density of fresh water at 4 °C, where density is defined as the ratio of mass to volume. Density may exclude the volume of occluded air-gas spaces in plant materials, termed particle or solid density, or include them, which has been described as bulk density (Mohsenin, 1970). Rahman (1995) reported the existence of up to five different ways to measure density, which include true density, substance density, particle density, apparent density, and bulk density. The particle density, for example, is the density of a sample which has not been structurally modified, so will include the volume of all enclosed pores but not the externally connected spaces. The apparent density is the density of a substance including all pores remaining in the material. Bulk density, on the other hand, was defined as the density of a material when packed or stacked in bulk.

Because of the irregular shapes of a seed, density data is measured by different methods. Such methods include the platform scale, specific gravity balance, specific gravity gradient tube, specific gravity bottle, the gas comparison pycnometer, and the radiation method (Mohsenin, 1970; Choi and Okos, 1986). From all of them, the gas compression pycnometer has proven to be an accurate and reliable device to measure

density changes in micronized cereals and pulses (Scanlon *et al.*, 1999; Fasina *et al.*, 1997).

The seeds of dry beans exhibit significant differences in length, breadth, and thickness. Geometrical shapes of known objects can also be used to approximate the volume of seeds (Mohsenin, 1970). For example, Fasina *et al.* (1997) used a triaxial ellipsoid to calculate the volume of kidney, black, and pinto beans. They reported length/breadth ratios for kidney, black and navy beans of 1.86, 1.39, and 1.51, respectively. Deshpande *et al.* (1984) reported similar L/B ratios (1.37-2.14) in dry beans. Further, they found a significant ($P \leq 0.10$) linear relationship between shape and size, more specifically between the L/B ratio and the 100-bean weights ($r=0.57$) of beans. Though relationships between bean physical properties and their chemical composition were not statistically significant in beans (Deshpande *et al.*, 1984), shape and size of beans are important quality attributes. In commercial trading, for example, the number of seeds per 100g is used a quality index (Pulse Canada, 2000). Quality navy and black beans have 450-525 and 500-550 seeds per100g, respectively.

Conversely to density, porosity indicates the volume fraction of void space or air. It is defined as the ratio of air or void volume to the total volume of a substance (Rahman, 1995). Scanlon *et al.* (1999) found that micronization caused a porosity increase in durum wheat of up to 11%. They reported that the increase in porosity occurred concomitant with a decrease in stiffness and an increase in water absorption of durum wheat. The porosity changes in a highly vitreous material like durum wheat could be calculated from their changes in particle density (Scanlon *et al.*, 1999).

Providing that the porosity of a vitreous kernel is close to zero, a change in the seed's porosity because of micronization can be calculated using the following equation:

$\Delta E=[1-(\rho_m/\rho_r)]*100$. Where '$\Delta E$' is the change in porosity (percentage), 'ρ_m' is the density of micronized seeds (kg/m^3), and 'ρ_r' is the density of the raw sample (kg/m^3) (Scanlon *et al.*, 1999). In another study, micronization processing changed the structure of beans, lentils, and peas in terms of increased volume, lower rupture point and toughness, higher water uptake, and higher leaching losses (Fasina *et al.*, 1997). In addition to the reported changes in the seed's density, they indicated that the use of a gas compression pycnometer facilitated quantification of slight changes in particle density, and therefore, changes in porosity. Gas pycnometers have also been used for studying the density and porosity of several cereals (Chang, 1988). The use of pressurized low molecular weight gas (e.g. helium) in a gas compression pycnometer allows a better approximation of the seed's particle density since they penetrate even the smallest pores and surface irregularities.

1.4.4. Water imbibition

Although dry seeds when placed in a moist environment begin to absorb water, some legumes will resist the penetration of water and remain unchanged. Impermeable seeds may remain dormant in soils for many years, which may reduce their effectiveness in propagation. In addition, seeds that fail to imbibe water during processing of legumes for human consumption are referred to as 'hard' seeds (Deshpande and Cheryan, 1986).

Many studies have reported a possible relationship between seed microstructure and water absorption in legume seeds (Powrie *et al.*, 1960, Sefa-Dedeh and Stanley, 1979; Rockland, 1978; Paredes-Lopez *et al.*, 1991; Black *et al.*, 1998a). However, the precise role of microstructural constituents of the seed in water absorption is still not known (Deshpande and Cheryan, 1986). The seed coat (Powrie *et al.*, 1960, Sefa-Dedeh

and Stanley, 1979), hilum (Hyde, 1954), and micropyle (Kyle and Randall, 1963) have each been related to water permeability in legume seeds.

Sefa-Dedeh and Stanley (1979) studied the relation of microstructure of cowpeas to water absorption properties. The eight varieties of cowpeas studied had similar cotyledon structure but different seed coat structure and thickness, micropyle and hilum size. Using a stepwise multiple regression analysis they proposed that the water capacity of the seed during a 24 h period (1, 3, 6, 12, 18, and 24 h) could be explained by the individual contribution of the seed components (initial water content, protein content, seed coat thickness and hilum size). They concluded that water was absorbed in three distinct stages. In the first stage (0-3 h), the seed coat contributed to over 40% of the total variability of the water absorbed. During the second stage (3-12h), data indicated that 85-92% of the variation was explained by hilum size. In the third stage (after 12 h), the protein content of the seed accounted for most of the variations in cowpea water absorption capacity. Sefa-Dedeh and Stanley (1979) concluded that all three constituents (seed coat, hilum, and micropyle) form an integral water absorption/removal system.

Once the water has entered the seed, other factors influence the water absorption rate, such as the type of protein, and the ratio of amylose to amylopectin in the seed (Deshpande *et al.*, 1984). Sefa-Dedeh and Stanley (1979) and Deshpande and Cheryan (1986) found that water absorption characteristics of cowpeas, *Phaseolus* and winged beans were related to their seed coat characteristics. The rate of water absorption was slow in cowpeas with a thick and smooth seed coat, whereas the rate of water uptake was greater in samples with thin and irregular seed coats. Overall, many studies have reported the effect of water absorption in legumes on their cooking time, appearance and palatability. In general, it is well accepted that good water absorption is one of the most

important factors in producing good-quality products (Varriano-Marston and Jackson, 1981).

In seeds, several forces at various tissues levels drive water uptake. Del Valle *et al.* (1992 a) reported that during soaking of beans, the rate of water uptake is determined by the product of two quantities, namely a proportionality factor and a driving force. They also described the imbibition process using Darcy's law of hydraulic flow, in which the proportionality factor was the hydraulic conductivity coefficient, and the driving force was the difference in water potential between the soaking media and the cotyledon cells. The osmotic potential and matrix potential contribute to the total water potential of a system by the presence of dissolved solids and hydrocolloids, respectively (Hyde, 1954; Plhak *et al.*, 1989). This would therefore suggest that the water potential of a soaking solution decreases as the concentration of added solutes increase.

Deshpande and Cheryan (1986) also studied the role of microstructure in initial water uptake of *Phaseolus* and winged beans. They found that varieties with high initial water uptake had thin seed coats, a loosely arranged cell structure on the raphe-side of the hilum, a deeply grooved hilar fissure, and a narrow tracheid bar. The studies of Deshpande and Cheryan (1986) indicated that both hilum and micropyle are the most important anatomical structures affecting the initial water uptake, whereas seed coat influenced water uptake only after 30 to 60 minutes of the initial soaking period.

1.5. Processing of beans

The conventional modes of bean preparation include cooking, germination, fermentation, and roasting/frying (Salunkhe *et al.*, 1989). Cooking, by far, is the most common method of preparation of dry beans prior to consumption. The traditional preparation of beans and the commercial procedure for canning dry beans includes a

soaking pretreatment followed by cooking. Alternatively, the beans can be blanched in order to improve product firmness (Lawande and Kadam, 1989). Similarly, micronization processing may also include a tempering pretreatment prior to cooking with radiant energy principally in the infrared region of the spectrum (Ginzburg, 1969; Arntfield *et al.*, 1997; Scanlon *et al.*, 1999; Cenkowski and Sosulski, 1998; Arntfield *et al.*, 2001).

1.5.1. Soaking and tempering

The processing of legumes usually requires a water-conditioning step that tenderises the seed and facilitates further processing such as cooking. The pre-treatment may include tempering, where a predetermined amount of water is added to achieve a desired moisture in the seed, or soaking, where the seed is placed in an excess of water and the final moisture is high and unevenly distributed (Scanlon *et al.*, 1998). Water imbibition forms an integral part of bean processing methods such as milling (Kadam and Salunkhe, 1989), germination (Labaneiah and Luh, 1981; Spaeth and Hughes, 1987; Ghorpade and Kadam, 1989), fermentation (Reddy and Salunkhe, 1989), toasting (Salunkhe *et al.*, 1989), and micronization (Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998; Scanlon *et al.*, 1998, 1999; Arntfield *et al.*, 2001).

Traditional soaking and the use of soaking salt solutions to reduce the cooking time of legumes have been broadly studied in several investigations (Rockland and Metzler, 1967; Al-Nouri and Siddiqi, 1982; Uzogara *et al.*, 1988; Buckle and Sambudi, 1990; Garcia-Vela *et al.*, 1991, Black *et al.*, 1998b). Beans are traditionally soaked to increase availability of water in the seed prior to cooking and thus to shorten cooking times. For the same reason, soaking of beans is also common practice used by the canning industry (Van Loey *et al.*, 1994). Soaking of beans can be accomplished by

using aqueous media consisting of water alone or dilute solutions of salts, acidulates, or alkali.

Rockland and Metzler (1967) were able to reduce the cooking time of various legume varieties by 80% or more by using intermittent vacuum infiltration technology (Hydravac process) in a solution of inorganic salts (1.0% sodium chloride, 0.5% sodium tripolyphosphate, 0.75% sodium bicarbonate and 0.25% sodium carbonate) followed by drying. The hydration media was selected based on both its ability to solubilize protein and to chelate divalent cations. The mechanisms by which the beans were rendered quick cooking were not explained by Rockland and Metzler (1967) but were proposed later by Rockland and Jones (1974) in an investigation that used SEM micrographs as supporting evidence. Rockland and Jones (1974) found that during normal cooking of beans in boiling water, intercellular material within the middle lamella softens and permits cell separation. They hypothesised that separation of bean cells during cooking was related to the transposition or removal of divalent cations, particularly of calcium and magnesium from bridge positions within the proteinaceous matrix of the middle lamella. As a result, the hypothesis of Rockland and Jones (1974) was the corner stone for numerous studies including those regarding the storage-induced textural defect hard-to-cook phenomenon in beans.

Hardening of legumes, also known as the hard-to-cook phenomenon, is the failure of improperly stored seeds to soften sufficiently after cooking for a reasonable time (Aguilera and Rivera, 1992). The hard-to-cook phenomenon also reduces nutritional quality, as well as increases energy requirements for the preparation of beans (Aguilera and Stanley, 1985). It has been reported that the temperature and humidity of storage conditions control the rate and extent of hardening (Jones and Boulter, 1983;

Moscoso *et al.*, 1984; Dos Santos Garruti and Bourne, 1985; Aguilera and Ballivian, 1987; Hincks *et al.*, 1987; Hincks and Stanley, 1987; Hohlberg and Stanley, 1987; Garcia-Vela and Stanley, 1989; Hentges *et al.*, 1990; Aguilera and Rivera, 1992; del Valle and Stanley, 1995). Although many hypotheses have been put forward to explain the hard-to-cook phenomenon in beans, the exact physicochemical mechanisms that cause the deterioration are still not well understood.

Numerous studies have reported the use of tempering/soaking solution pre-treatments for improving the final quality of processed legumes. These tempering/soaking solutions include EDTA (Aguilera and Rivera, 1992, Scanlon *et al.*, 1998), sodium bicarbonate (Al-Nouri and Siddiqi, 1982; Buckle and Sambudi, 1990; de Leon *et al.*, 1992; Black *et al.*, 1998 b), sodium tripolyphosphate (Al-Nouri and Siddiqi, 1982; Scanlon et al, 1997; Black *et al.*, 1998 b), sodium chloride (Ros and Rincon, 1991; Black *et al.*, 1998 b), and calcium chloride (Drake and Muehlbauer, 1985). Generally, it has been found that adding sodium salts to the soaking media increases the water absorption rate of legumes. Toews (2001) evaluated 10 different tempering solutions as potential pre-treatments for the micronization of field peas. She found that a solution containing 0.2% sodium bicarbonate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate was the most effective in reducing the cooking time of field peas. Scanlon *et al.* (1997) indicated that 2% sodium tripolyphosphate, mixtures of 1% citric and 2% ascorbic acid, and 150-ppm disodium EDTA could also be used as successful micronization pretreaments. However, it was found that reagents were only effective in reducing the cooking of lentils when added at 40% moisture. Scanlon *et al.* (1998) suggested that availability of free water to the lentil constituents was necessary for the

reagents in the tempering solution to reduce cooking time further than the reduction caused by water alone.

Spaeth and Hughes (1987), reported that water imbibition causes cellular disruption of cotyledonary surfaces of beans including ruptures and fractures. Materials released from cotyledonary tissues were identified as both dense aggregates of protein bodies and a dispersed phase of protoplasm. Soaking of legumes resulted in similar losses of water-soluble compounds including oligosaccharides, saponins, pectin, amino acids, calcium, potassium, magnesium, phosphorus, reducing sugars, phytate, and tannins (Kon 1979, Iyer *et al.*, 1980; Honig and Wolf, 1987; Hincks *et al.*, 1987; Uzogara *et al.*, 1990; Buckle and Sambudi, 1990; Plhak *et al.*, 1989; de Leon *et al.*, 1992; Ruiz *et al.*, 1996). Leaching losses have been reported to be greater at higher soaking temperatures (Kon, 1979; Iyer *et al.*, 1980). Although leaching losses of antinutritional factors is a desirable event, the loss of soluble nutritional factors, i.e., soluble vitamins and minerals, may be detrimental to the nutritional quality of legumes. Yet overall, leaching losses during soaking appears to be a desirable event since most antinutritional factors are reduced and a concomitant softening of the packed cell structure takes place.

1.5.2. Cooking

Cooking of legumes is a process by which the seed coat and cotyledon soften and develop an acceptable texture and flavour (Rockland and Jones, 1974). Proctor and Watts (1987) reported that growing location and cultivar influence the cooking time of *Phaseolus vulgaris* beans. Important nutritional changes have also been reported to take place during cooking, including an improvement in protein availability.

Aguilera and Stanley (1985) described cooking as a two-phase process. During the first phase the middle lamella breaks down and the cells separate. During the second phase, the predominant phenomenon is starch gelatinization. Aguilera and Stanley (1985) suggested that by promoting either of these phases, as is done when salts are added to soaking solutions; it is possible to achieve a reduction in the cooking time. During normal cooking in boiling water, intercellular material within the middle lamella softens and permits separation of adjacent whole cells (Rockland and Jones, 1974). Mechanical stresses, as a result of starch gelatinization, protein denaturation, swelling, and may further facilitate cell separation and the development of the uniform, smooth texture in fully cooked beans (Rockland and Jones, 1974). The cooking time of beans therefore appears to be controlled by the degree to which one or more of the following physicochemical events take place: protein denaturation (Garcia-Vela and Stanley, 1989; Garcia-Vela et al., 1991; del Valle *et al.*, 1992 b; Arntfield *et al.*, 1997), starch gelatinization (Aguilera and Stanley 1985; Garcia-Vela and Stanley, 1989; Arntfield *et al.*, 1997), air-bubble formation (Scanlon *et al.*, 1999), and pectate solubilization (Jones and Boulter, 1983; Stanley and Aguilera, 1985; Deshpande and Damodaran, 1990; Hentges *et al.*, 1991; Aguilera and Rivera, 1992). In addition, the cooking process may be related to the ability of the seed components to facilitate cell separation during cooking through an ion exchange and chelation mechanism (pectin-phytate reaction) (Rockland and Metzler, 1967; Varriano-Marston and de Omana, 1979; Al-Nouri and Siddiqi, 1982; Moscoso *et al.*, 1984; Hincks and Stanley, 1986; de Leon *et al.*, 1992; del Valle and Stanley, 1995).

1.6. Micronization

1.6.1. Principles

Micronization, an intensive heat process, involves the exposure of an absorbent material for a short time to electromagnetic radiation in the infrared (IR) region of the spectrum (Ginzburg, 1969, Scanlon *et al.*, 1999). Micronization is an efficient method to dry products since infrared rays cannot release their energy unless they strike an absorbent material. Energy losses during micronization may occur due to an absorbent medium between the radiator source and the drying material. Air, for example, absorbs insignificant amounts of infrared radiation at all wavelengths, whereas carbon dioxide and water vapor may absorb a considerable amount of energy at wavelengths greater than 2.24 μm. Below this wavelength, however, carbon dioxide and water vapor are essentially transparent to infrared radiation (Ginzburg, 1969, Sakai and Hanzawa, 1994).

In radiative heat transfer, energy is transported by electromagnetic waves from a body of high temperature (IR radiation source) to one of a low temperature (absorbent material, food). Infrared radiation is favored over conduction and convection heat transfer because there is a good understanding of the physics of radiative heat transfer. Of the total IR radiation emitted by a radiation source during food processing, a portion is reflected back, and the rest is absorbed into the food. A part of this absorbed energy is then transmitted into the next section of the product. The efficiency of the infrared radiation to heat and dry plant materials depends on several factors including: assembly of the material's microstructural components, the distribution of the emitted energy (infrared radiation emissivity), the optical (reflectivity, absorptivity, and transmissivity) and physical (size and shape) characteristics of the sample, and the geometrical parameters that describe the relative position of the radiation source and the material

being treated (Ginzburg, 1969). The depth of penetration of the infrared rays depends on the properties of the heated material and the temperature of the radiator, which depends on the radiation wavelength (Ginzburg, 1969).

The IR region of the electromagnetic spectrum contains wavelengths from 0.75 to 1000 μm, which can be further divided into three regions: near IR (0.75-1.4 μm), mid-IR (1.4-3 μm) and far-IR (3-1000 μm) (Ginzburg, 1969; Sakai and Hanzama, 1994). IR radiation can be produced artificially with electrical and gas-fired generators (Ginzburg, 1969). Electric generators of IR heat are easy to use and control, and are ideal for small-scale laboratory studies. However, the low operational cost associated with a gas-fired micronizer unit makes it more appropriate for large-scale operations. There are some fundamental differences in the way electric and gas-fired generators work. For example, sufficient IR energy to micronize foods in electric generators is obtained at temperatures around 2200 °C, with a peak wavelength of 1150 nm (Cenkowski and Sosulski, 1998), while gas-fired micronizing units have working temperatures between 400 and 750°C, with radiation wavelengths in the range of 1800 to 3400 nm. The shorter-wavelength radiation harbours greater thermal energy, but the higher degree of penetrability of longer-wavelength radiation suggests that it would be more suitable for large-seeded legumes (Cenkowski and Sosulski, 1998).

The electrical generators of infrared radiation are based on a spiral tungsten filament in a quartz envelope (Cenkowski and Sosulski, 1998). The tungsten wire filament generates infrared radiation as a result of electrical resistance and the quartz allows good transmission of radiation from the ultraviolet to microwave range. On the other hand, the gas generators of infrared radiation may comprise panels that are not

permeable to gas, and porous ceramic panels or metallic perforated surfaces in which flameless combustion of gas takes place (Ginzburg, 1969).

During micronization, infrared rays strike an absorbent material (i.e., food) and cause the molecules within to vibrate at a frequency of 80-170 million megacycles per second (Ginzburg, 1969; Lawrence, 1973 a, Micronizing Co. (U.K.) Ltd., no date). As a result of such rotation, there is a rapid internal frictional heating and a rise in water pressure vapor (Lawrence, 1973a). Products such as cereal grains and pulses can reach internal temperatures of 90-124 °C after 50-60 seconds of processing (Lawrence 1975, Rusnak *et al.*, 1980; Kouzeh-Kanani *et al.*, 1981; Kouzeh-Kanani *et al.*, 1982; Kouzeh-Kanani *et al.*, 1984; McCurdy, 1992; Sarantinos and Black, 1996; Cenkowski and Sosulski, 1998).

1.6.2. Food Applications

In the early 1970's, micronization started to be used by the cocoa bean industry for facilitating bean shell separation and reducing yeast, mold, insect, and microbial counts (Anonymous, 1981). Currently, the micronization process is also being used in the food and feed industry for improving the nutritional quality of food and animal feed. These effects were most marked when flaking followed the micronization process (Lawrence 1973a, 1973b; 1975; Blendford, 1980). Micronization has been applied to cereal and pulses including full-fat soybeans (Kouzeh-Kanani *et al.*, 1981), maize (Lawrence 1973a, 1973b), maize germ (Kouzeh-Kanani *et al.*, 1984), barley (Lawrence 1973a, 1973b), lentils (Zhao, 2000), field peas (McCurdy, 1992; Igbasan and Guenter, 1996; Toews, 2001), and sorghum (Shiau and Yang, 1982) for improving palatability and digestibility in animal feeding. As well, micronization has been used to pre-cook rice and pearl barley (Blendford, 1980), to dry dough-rice (Abe and Afzal, 1997), to

caramelize sugars in malted wheat flakes for producing specialty breads (Chubb, 1982; Micronizing Co. (U.K.) Limited, no date), and to improve nutritional quality of soymilk (Metussin *et al.*, 1992). Micronization has been proven to reduce the level of antinutritional factors in cereals and pulses, such as myrosinase in canola seeds and canola screenings (McCurdy, 1992), lipoxygenase (Kouzeh-Kanani *et al.*, 1982) and trypsin inhibitors (Chubb, 1982; Kouzeh-Kanani *et al.*, 1981) in soybeans.

More recently, micronization has been proven to reduce the cooking time of pulses (Scanlon *et al.*, 1997; Arntfield *et al.*, 1997; Zhao, 2000; Toews, 2001; Arntfield *et al.*, 2001). The cooking time of lentils, for example, decreased by one-third, and for field peas by a half because of the micronization treatment (Arntfield *et al.*, 1997; Toews, 2001). Micronization treatment, however, increased the cooking time of kabuli chickpeas (Sarantinos and Black, 1996), and pinto beans (Abdul-Kadir *et al.*, 1990). Yet overall, micronization was reported to improve the water absorption capacity of cereal and pulses (Kouzeh-Kanani *et al.* 1984; Abdul-Kadir *et al.*, 1990; Cenkowski and Sosulski, 1998; Scanlon *et al.*, 1999).

During micronization, starch granules undergo partial gelatinization as a result of water availability and the high processing temperatures, and this in turn translates into an increase of starch availability (Lawrence, 1973a; 1973b; Croka and Wagner, 1975). The degree of starch gelatinization increases with higher processing temperatures (Croka and Wagner, 1975; Kouzeh-Kanani *et al.*, 1984). For example, Lawrence (1973a) found that micronization increased starch availability of barley and maize, and when flaking followed micronization, starch availability was further improved. Micronization, however, was found to have little potential as a processing technique for improving the nutritional value of wheat for the growing pig (Lawrence, 1975). He suggested that the

difference in response by wheat, compared to barley and maize, was due to differences in starch content and in the arrangement of starch and proteins within the cereal grain structure. Waxy sorghum was also found to be more susceptible to starch gelatinization after micronization than heterowaxy and nonwaxy sorghum strains (Rusnak *et al.*, 1980). Amylopectin starch content and intrinsic kernel structure differences were thought to be responsible for such changes.

Infrared heat treatment has also been shown to affect protein availability and the structural conformation of cereals and pulses. For example, micronization was found to improve the digestibility of nitrogen in barley (Lawrence, 1973b), maize (Lawrence, 1973b) and sorghum (Savage and Clark, 1988). In 1992, Metussin *et al.* found that micronization did not affect the protein composition of soymilk proteins. Arntfield *et al.* (1997; 2001), however, found that protein solubility in micronized lentils decreased as the temperature at the end of the micronization process increased. Similar results were reported for peas (McCurdy, 1992; Cenkowski and Sosulski, 1998), soybeans (Kouzeh-Kanani *et al.*, 1981), and maize germ (Kouzeh-Kanani *et al.*, 1984). Zheng *et al.* (1998) also reported that because of micronization, nitrogen solubility decreased in six different kinds of cereals (wheat, barley, rye, triticale, millet, and wildrice) and six legume groups (green, yellow pea, lentil, black, kidney bean and pinto bean).

Even though micronization has proven to induce several beneficial changes, excessive heat treatment has been reported to result in losses of heat-labile nutrients such as lysine, cystine, methionine and thiamine (Kouzeh-Kanani *et al.*, 1981). In animal studies using pigs, Lawrence (1975) reported that wheat micronized to high temperatures (220 °C) caused a significant depression in growth rate of pigs and in the efficiency of conversion of dietary dry matter, as compared with treatments at 155 and

190 °C. Overheated micronized soybean flour also developed rancid odors and off flavors (Kouzeh-Kanani *et al.*, 1982). The micronization processing conditions should be therefore carefully regulated to avoid undesirable quality changes in IR treated biological materials.

Chapter 2. The Effect of Micronization Operational Conditions on the Physicochemical Properties of Navy Beans (*Phaseolus vulgaris L.*)

ABSTRACT

A full factorial experiment was used to optimize the effect of processing conditions of a pilot-scale MR2 micronizer unit on micronized product attributes of navy beans. These attributes were product temperature at micronizer outlet, hardness, final moisture content, percent of soluble protein, hydration capacity, and density. Processing conditions included the slope of the conveyer bed (SL), instrumental setting of a gas-air mixture valve (GL), and the distance between the conveyer bed and the infrared radiation source (DL). The SL angle was increased from -0.0236 to +0.0236 rad, with respect to the horizontal plane. As the extent to which SL and GL increased, so did the final temperature of the micronized navy beans. Bean moisture content was reduced as the SL and GL increased. The percent of soluble protein and hydration capacity of the micronized navy beans was reduced as SL increased. Overall, results indicated that SL and GL, but not DL, made significant ($P \leq 0.05$) contributions to the optimization of micronization processing conditions by controlling the rate and extent of the micronization process.

2.1. INTRODUCTION

Among the world's pulses, dry beans rank first in production and consumption (FAO, 2000). Dry beans are an excellent low-fat source of proteins and dietary fiber, containing significant amounts of B-complex vitamins, folic acid, potassium, calcium and iron (Salunkhe *et al.*, 1989). In many countries, beans are considered a staple food. Today, North America consumption of pulses is steadily growing in response to current

food trends. Canada's pulse production alone has increased by almost 100% in the past 5 years (FAO, 2000). Despite this, the consumption of dry beans in North America is still limited, in part due to the long cooking times required in their preparation.

Cooking time has been identified as one of the most important quality attributes of edible dry beans in addition to size and colour (Proctor and Watts, 1987). There have been several attempts to develop a process for preparing both quick-cooking and pre-cooked dry beans. Numerous studies have investigated the effect of soaking or the use of tempering solutions to obtain a reduction in cooking time (Rockland and Metzler, 1967; Rockland *et al.* 1977; Al-Nouri and Siddiqi, 1982; Uzogara *et al.*, 1988; Buckle and Sambudi, 1990; Garcia-Vela *et al.*, 1991). However, difficulties in handling and storage of wet seeds have restricted the use of these approaches to the consumer, where they can be employed immediately prior to cooking. Pulses have a great potential to meet the desires of consumers for natural and healthy foods, but the lack of adequate technology to improve their cooking quality has restricted this potential until very recently.

Micronization technology has been proven to have the potential to increase the marketability of Canadian pulse crops by reducing their cooking time (Abdul-Kadir *et al.*, 1990; Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998; Arntfield *et al.*, 2001). Micronization of water-conditioned beans may result in a pre-cooked dry product, which is shelf-stable and therefore suitable for wide-range distribution.

IR radiation can be produced artificially with electrical and gas-fired generators (Ginzburg, 1969). Electric generators of IR heat are easy to use and control, and are ideal for small-scale laboratory studies. However, the low operational cost associated with a gas-fired micronizer unit makes it more appropriate for large-scale operations. There are some fundamental differences in the way electric and gas-fired generators

work. For example, sufficient IR energy to micronize foods in electric generators is obtained at temperatures around 2200 °C, with a peak wavelength of 1150 nm (Cenkowski and Sosulski, 1998), while gas-fired micronizing units have working temperatures between 400 and 750 °C, with radiation wavelengths in the range of 1800 to 3400 nm. The shorter-wavelength radiation harbours greater thermal energy, but the high degree of penetrability of longer-wavelength radiation suggests that it would be more suitable for large-seeded legumes (Cenkowski and Sosulski, 1998).

Previous studies have addressed the fact that micronization operational conditions must be adjusted during processing in both electric and gas-fired micronization units (McCurdy, 1992; Toews, 2001). The aim in the studies of McCurdy (1992) and Toews (2001) was to expose dry yellow and green peas, and canola and canola screenings, respectively, to a specific degree of IR radiation intensity as measured by the temperature of the product exiting the micronizer. Setting adjustments made during micronization to achieve a desired range of final product temperature, however, would not result in a product with consistent quality. To date, there has been no report in the literature of studies looking at the effect of the operational conditions of a gas-fired micronizer unit on the quality of processed pulses.

In this study, a pilot-scale gas-fired micronizer unit (MR2, Micronizer Ltd. (UK) Co., FramLingham) was used to micronize water-conditioned beans. The instrumental setting of a gas-air mixture valve (GL), the slope of the micronization conveyor trough (SL), and the distance between the conveyor trough and the infrared radiation source (DL) allowed for manipulation of the rate and duration of the micronization process. The objective of this study was to evaluate the effect of micronization operational conditions

on the physicochemical properties of navy beans, specifically final surface temperature, final moisture content, percent of soluble protein, bean density, and hydration capacity.

2.2. MATERIALS AND METHODS

2.2.1. Materials

The navy beans *(Phaseolus vulgaris)* used in this investigation were grown in Manitoba during the 1999 crop year and were purchased from Roy Legumex Ltd. (St. Jean-Baptiste, MB, Canada). Upon arrival, beans were stored at 5°C in closed plastic containers (Trimeld Rubbermaid®). Appendix I indicates the chemical composition of both navy and black beans.

2.2.2. Tempering

Based on previous studies, a fixed tempering level of 25% m.c. was chosen. Beans (7.5 kg) were divided into three equal parts and mixed with a predetermined amount of water (Scanlon *et al.*, 1998) in 4.5 L Rubbermaid® containers. Uniformity of water absorption among beans was assured by tumbling the sample-containers every 10 min for the first hour, and then once every hour for the next three hours. Moisture content after tempering was 25±2 % of the desired level.

2.2.3. Micronization process

A pilot-scale MR2 micronizer unit (Micronizing Company Ltd., UK) fitted with a gas-fired infrared manifold and equipped with a vibrating stainless-steel conveyer trough and four ceramic IR burners was used to process the tempered beans. Using natural gas as a fuel, the MR2 micronizer is able to generate between 8.8 and 29.3 kW of power as specified by its manufacturer.

At the beginning of each run, the distance between the conveyer bed and the infrared

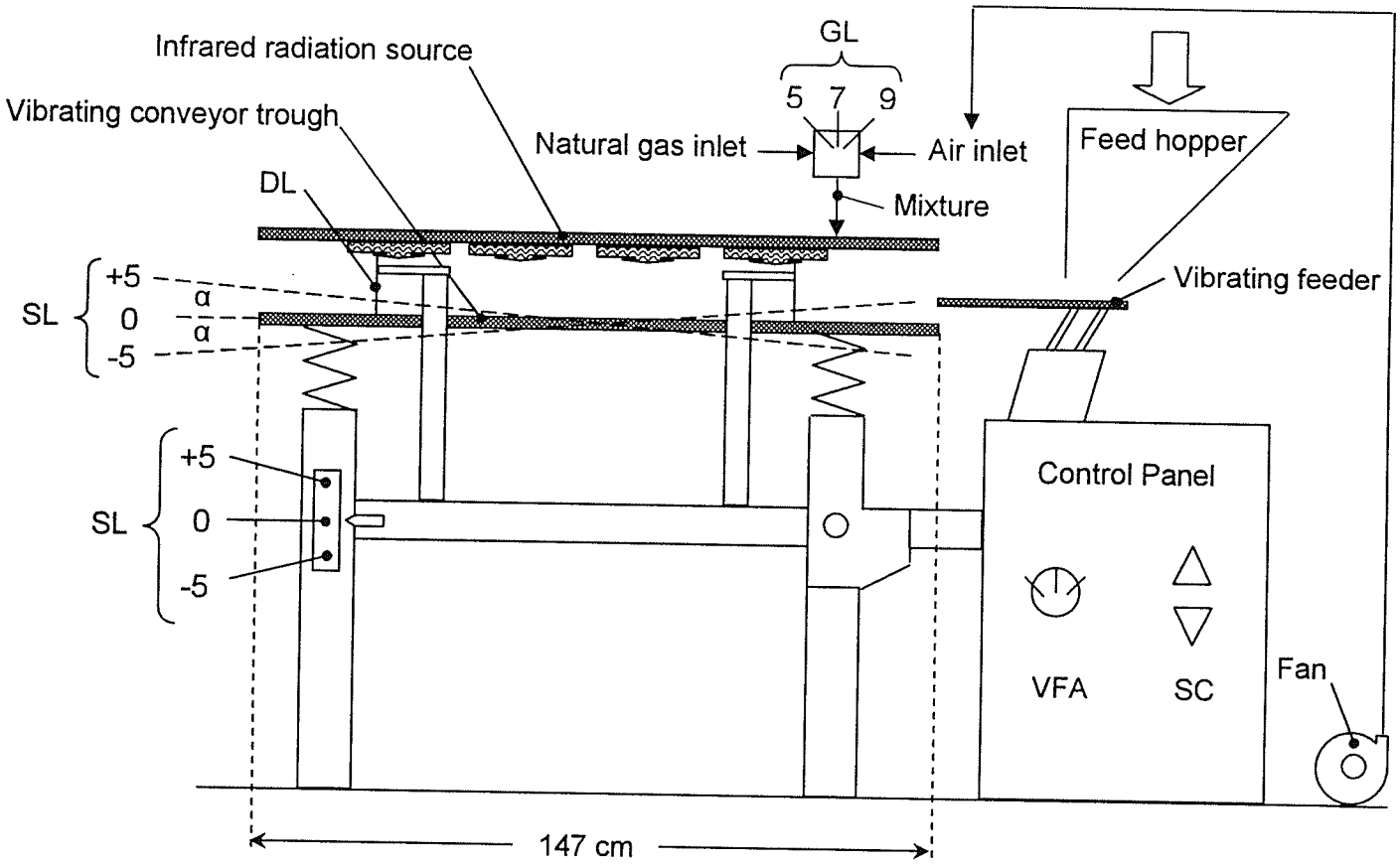


Figure 2. Schematic diagram of the pilot-scale gas-fired micronizer unit (MR2, Micronizing Company Ltd., FramLingham, U.K.). The keys to the symbols are: (SL) slope of the conveyor trough, (DL) distance between the conveyor trough and the infrared radiation source (11 to 12.4 cm), (GL) instrumental setting of a gas-air mixture valve, (VFA) vibratory feeder adjuster, and (SC) slope adjuster. Values included in brackets represent the experimental range for that operational setting. $\alpha = 0.0236$ rad.

radiation source DL (11-12.4 cm) was adjusted to meet the treatment settings by loosening or tightening four screws holding the radiator source to the top of the conveyor bed (Figure 2). The micronizer was warmed up for 15 min using a warm-up batch of beans prior to processing the tempered experimental sample. At this point, a constant flow rate of beans was observed and the temperature of the exiting beans was approximately 100 °C. The warm-up batch was allowed to exit the micronizer. Experimental samples were then placed into the hopper that fed a single layer of beans onto the 0.26 m wide x 1.47 m long vibrating conveyor trough (Figure 2). Vibration of the conveyor trough assured a constant rotation of individual beans, thus ensuring that beans received uniform IR energy on all surfaces. The feed rate was kept constant at 0.67 ± 0.03 kg/min using a setting of 60 in the vibratory feeder (Figure 2).

Before sample loading, the conveyor bed was lowered to the zero mark on the slope setting in order to quickly fill up the conveyor trough with the sample and thus reduce the risk of overheating the conveyor trough. Pressing the upward and downward arrow keys in the control panel adjusted the slope (SL) of the conveyer bed between settings of –10 to 10 on an arbitrary scale. In this experiment, SL was limited to a range of –5 to +5 on the basis of preliminary experiments (Figure 2). Approximately six minutes after the start of processing of the experimental samples, micronized product samples were collected. Micronized beans were collected in a plastic pail, at which point the average surface temperature was measured using an infrared thermometer gun (Cole-Palmer Instruments Co., Niles, IL).

The amount of natural gas drawn into the gas-air mixer varied in proportion to the amount of air going through the mixer. Natural gas was supplied to the mixer at an operating pressure of 3.4 - 4.8 kPa, whereas the amount of incoming air was adjusted by

changing the instrumental setting of a gas-air mixture valve or GL (Figure 2). The gas level (GL) had a range of values from zero to ten on an arbitrary scale, and changes in the gas-air ratio affected the intensity of radiated energy. In our experimental design, GL was limited to values from 5 to 9 (Figure 2) to prevent burning system failure on processing (based on preliminary testing).

2.2.4. Moisture content

The moisture content of processed beans was measured by an air-oven method according to the AACC (1995) approved method 44-15A.

2.2.5. Hydration capacity

Beans (20 g) were poured into a 300 mL beaker containing 100 mL of distilled water, and covered with a paper towel for 16 h. After the soaking time, the beans were drained for 5 min on a 1410 μm sieve (14 mesh US Standard Sieve Series) at a 45-degree angle. The beans were then blotted dry, and their weight recorded as 'hydrated wt'. The drained water was collected in the beaker with the remaining soak water. The weight of leached matter in 2 mL of this collected water was found after evaporating to dryness at 100 °C for 24 h. Total leaching losses for 20 g of sample or 'leached solids wt' was then calculated by multiplying the dry matter weight in 2 mL of collected water by the total remaining water volume. The water hydration capacity of whole beans was expressed as percent of solids remaining after soaking (corrected for solids loss) using the following formula:

$$\frac{(\text{hydrated wt} - \text{dry wt} + \text{leached solids wt}) \times 100}{(\text{dry wt} - \text{leached solids wt})}$$

2.2.6. Bean density

A triplicate of approximately 30 g of micronized beans was used for volume determinations using an air comparison pycnometer (Model 930, Beckman Instruments, Inc.). The micronized samples were retrieved from storage conditions (5 °C), and were equilibrated to room temperature (21 °C). The equipment was calibrated using 3 stainless steel balls of 3.81, 2.54, and 1.74 cm diameter before volume measurements were made. Volume and weights were then used to calculate particle density.

2.2.7. Soluble protein

The soluble protein level was determined by extracting 5 g of sample in 50 mL of 0.5 M NaCl (pH= 7) for 30 min and centrifuging at 17,300 x ***g*** for 10 min. The protein content in the supernatant was analysed spectrophometrically using the Coomasie® Blue Protein Assay Reagent G-250 (Pierce Chem. Co., Rockford, IL).

2.2.8. Texture analysis

During preliminary experimentation, a sensory panel was used to identify the maximum force value at which cooked navy beans were considered acceptable for eating. The sensory panel found that the optimum texture of navy beans corresponded to a hardness-force value of 325 ± 17 N (Appendices II and III). To reach this value and yet allow for a range of hardness values within the limits of the texture analyzer (load limit = 1000 N) that would result from extreme data points in our experimental design the cooking time was then set to 1 h.

Sixty-five grams of micronized beans were placed in 975 mL of boiling deionized water (ratio 1:15 beans to water) and boiled for 1 h. Cooked beans were then drained thoroughly for about 3 min, and placed in 250 mL covered plastic containers to

cool down for 1 h. Texture measurements were taken within 2 h. The Lloyd Materials Testing Machine Model L1000R (Lloyd Instruments Ltd., Fareham, UK) was used to analyze texture, as described by Arntfield *et al.* (1997).

2.2.9. Experimental design

A full factorial design that included 11 treatment combinations GL (5, 9), SL (-5, +5), and DL (11.0, 12.4) was used and samples were prepared and evaluated. The 11 treatments included three repetitions of the center design point (GL 7, SL 0, DL 11.7). The factorial experiment boundary was represented in coded units by –1 and +1, and the center design point by 0 (Table 3). Design-Expert® software was used to optimize operational conditions with respect to the outlet temperature, hardness and moisture content of processed navy beans.

Table 3. Actual and coded levels for factorial analysis

Variable	Code	Coded levels		
		-1	0	+1
Gas-Air level	GL	5	7	9
Slope level	SL	-5	0	+5
Distance level (cm)	DL	11	11.7	12.4

Two-dimensional contour plots were generated from the fitted model using Design-Expert® software. Correlation analysis of the data was performed using Pearson correlation coefficient analysis of SAS (version 8.02, SAS Institute Inc, 2001, Cary, NC).

2.3. RESULTS AND DISCUSSION

2.3.1. Outlet temperature

Results indicated that GL and SL, but not DL, had significant ($P \leq 0.05$) effects on the final temperature of micronized navy beans (Table 4). Moreover, final temperature was well correlated (R^2=0.8914) with the main effects of GL and SL (Table 5), although the linear model indicates that a change in SL causes a greater effect on outlet temperature than a similar change in GL for the tested range (Table 5). In general,

Table 4. Variables and their significance (probability) in factorial analysis

Independent variable[1]	Dependent variable					
	Final temperature (°C)	Soluble protein (%)	Moisture content (%)	Hydration capacity (%)	Hardness (N)	Bean density (kg/m^3)
Linear:						
GL	0.04	n.s.	0.03	n.s.	<0.01	n.s.
SL	<0.01	<0.01	<0.01	0.04	n.s.	n.s.
DL	n.s.	n.s.	n.s.	n.s.	n.s.	0.04
Interaction						
GL*SL	n.s.	n.s.	0.08	n.s.	n.s.	n.s.
SL*DL	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
GL*DL	n.s	n.s.	n.s.	n.s.	n.s.	0.02

[1] Note: GL =Gas-Air level, SL =Slope level, DL =Distance level.
n.s. = not significant ($P > 0.05$).

Table 5. Regression equations with independent variables in coded units

Product properties	Regression Equation[1]	R^2
Outlet temperature (°C)	Y=75.88+5.75GL+16.38SL	0.8914
Soluble protein (%)	Y=10.27-2.05SL	0.8264
Moisture content (%)	Y=21.65-0.81GL-1.12SL-0.59GL*SL	0.7586
Hydration capacity (%)	Y=124.75 – 1.22SL	0.4210
Hardness (N)	Y=248.62-25.28GL	0.8105
Bean density (kg/m^3)	Y=1376-4GL+6.75DL-7.75GL*DL	0.7586

[1] Note: GL =Gas-air level, SL =Slope level, and DL =Distance level

as SL was more positive and GL increased so did the final outlet temperature of micronized navy beans (Figure 3).

The feed gate gap and the velocity of the incoming feed are also known to affect the duration of the micronization process and thus the temperature of product exiting the micronizer. In a previous study of the micronization of yellow and green peas, SL and the velocity of the feeder were adjusted during processing to collect samples at specific temperatures (Toews, 2001). McCurdy (1992) included the feed gate gap, SL, and DL as operational parameters to manipulate the end temperature of micronized dry peas, canola, and canola screenings. In the present study, the feed gate gap was set in such a way (aprox. 5 mm) that a single layer of tempered beans fed into the vibratory conveyor trough, and the feed rate was adjusted by setting the controller to 60.

Arntfield *et al.* (1997) and Cenkowski and Sosulski (1998), using an electric generator of IR radiation, also addressed the high degree of dependence between

radiation heat intensity and DL. The present study, however, suggested that the change of DL by 1.4 cm (11 to 12.4 cm) did not make a significant contribution to the optimization of the micronization process. Ginzburg (1969), using an infrared electrical lamp, found similar results for IR treated carrots placed at 7 and 9 cm away from the radiation generator (2 cm distance range), where they did not exhibit significant differences in their maximum final temperatures (about 115 °C in both cases).

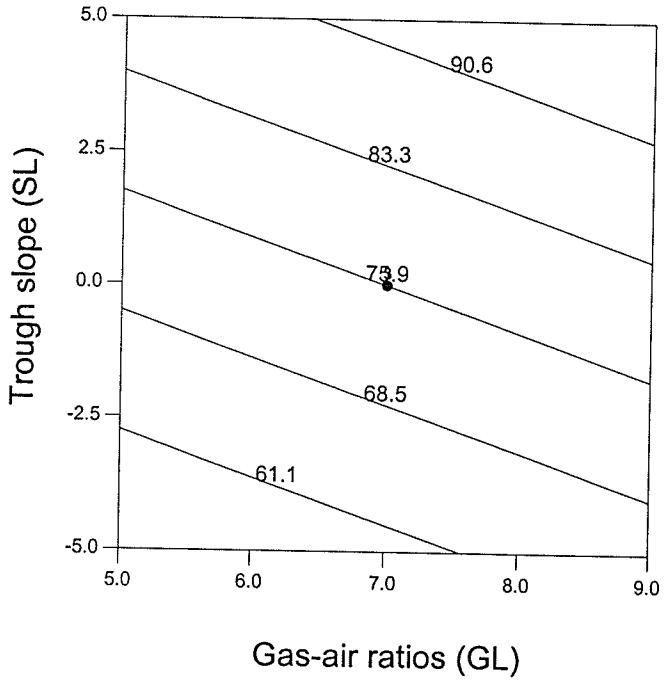


Figure 3. Contour plot of outlet temperature (°C) of micronized navy beans as a function of GL and SL. DL=14 cm.

The correlation analysis shows that an increase in the outlet temperature of micronized beans would reduce hardness, moisture content, percent of soluble protein and hydration capacity of the final product (Table 6) indicating that high temperatures during processing would lead to desirable structural and physicochemical changes in the

Table 6. Correlations between characteristics of micronized navy beans (n=11)

	Outlet temperature	Soluble protein	Moisture content	Hydration capacity	Hardness	Bean Density
Outlet Temperature	X	-0.62 [b]	-0.85 [c]	-0.70 [b]	-0.54 [a]	0.29
Soluble Protein		X	0.49	0.24	0.11	-0.33
Moisture Content			X	0.62 [b]	0.59 [b]	-0.17
Hydration Capacity				X	0.43	-0.62 [b]
Hardness					X	0.12
Bean density						X

[a] Significant at $p \leq 0.10$.
[b] Significant at $p \leq 0.05$.
[c] Significant at $p \leq 0.01$.

product. Figure 3 suggests that the highest outlet temperature is reached at the highest combination of GL and SL. See Appendix IV for observed experimental data.

2.3.2. Soluble protein

This study indicates that SL alone had a significant effect ($p \leq 0.05$) on the protein solubility of micronized beans (Table 4). As SL increased, the protein solubility in the micronized beans decreased, to 8.2 % at a SL of 5 (Table 5). This suggests that SL, and not GL, plays a major role in attaining desirable chemical effects in the micronized product. It is possible that the effect of GL was overshadowed by the effect of SL, and thus the model will not pick up significant effects of GL.

The present study indicates that positive slopes result in longer residence times and thus extends the exposure of beans to high intensities of IR radiation. Arntfield *et al.* (1997, 2001) found that protein solubility in micronized lentils decreased as the temperature at the end of the micronization process increased. Similar results were found in peas (McCurdy, 1992; Cenkowski and Sosulski, 1998), soybeans (Kouzeh-Kanani *et al.*, 1981), and maize germ (Kouzeh-Kanani *et al.*, 1984). Zheng *et al.* (1998) also observed that nitrogen solubility decreased in six cereals (wheat, barley, rye, triticale, millet, and wildrice) and six legumes (green pea, yellow pea, lentil, black bean, kidney bean, and pinto bean) after completion of the micronization processing. Limitation of water during the micronization process could also be responsible for mediating the extent of protein change (Scanlon *et al.*, 1997; Zheng *et al.*, 1998). Zheng *et al.* (1998) found that lower percentages of protein solubility were inversely correlated ($P \leq 0.01$) with the hardness of micronized lentils. Our study, however, did not find significant correlation between bean hardness (maximum force after cooking) and the percent of soluble protein in micronized navy beans (Table 6). The percent of soluble protein in the micronized navy beans was negatively ($P \leq 0.05$) correlated with outlet temperature (Table 6). This confirms previous findings indicating that as the severity of the heat treatment increases, there is a significant decrease in the percent of soluble proteins due to protein aggregation (Zheng *et al.*, 1998) or protein denaturation (Arntfield *et al.*, 1997). Protein denaturation in beans may be a desirable effect since it reduces the level of anti-nutritional factors such as trypsin inhibitors (Arntfield *et al.*, 1997). See Appendix IV for observed experimental data.

2.3.3. Moisture content

Tables 4 and 5 show that SL, GL, and the interaction between SL and GL significantly influenced the moisture content. The nature of this interaction is depicted in Figure 4. It is clear that increases in either SL (more positive slopes) or GL resulted in lower moisture contents. The influence of slope is more noticeable at higher gas-air

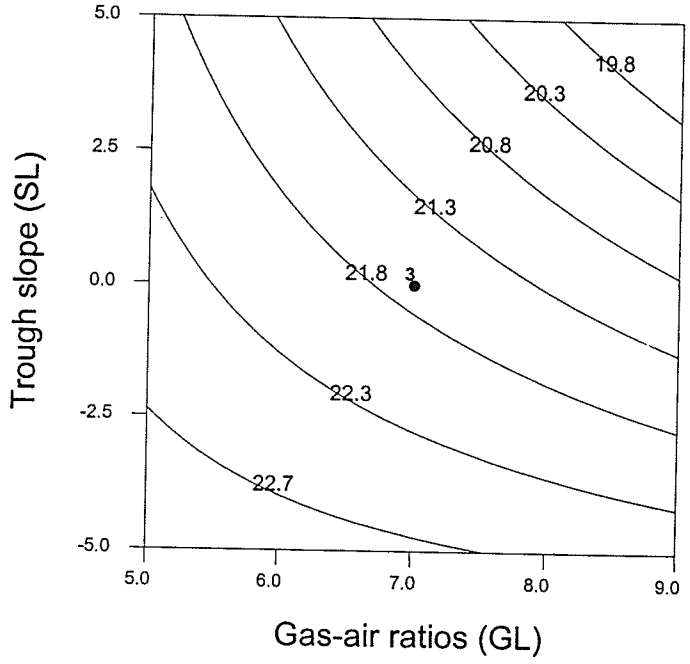


Figure 4. Contour plot of moisture content (%) of micronized navy beans as a function of GL and SL. DL=14 cm.

ratios. A more positive slope (upward slope) leads to longer residence times due to greater influence of the gravitational force. The larger slopes therefore increased the extent of moisture loss due to longer exposure times. Similarly, higher temperatures resulting from a higher setting in the gas-air mixtures would also be likely to evaporate more water thus reducing moisture content in the processed beans. The combination of SL and GL at high levels further accentuated these effects. The Pearson correlation

coefficient analysis (Table 6) shows that the moisture content in the micronized product decreased as the outlet temperature increased ($P \leq 0.01$). In this study, micronization processing was optimized in terms of bean outlet temperature. As a result, moisture content resulted in a final product that was not shelf stable, and a further drying step would be required to extend the shelf life of micronized beans. See Appendix IV for observed experimental data.

2.3.4. Hydration capacity

Table 4 shows that only SL had a significant ($P \leq 0.05$) effect on the hydration capacity of micronized beans. It should be noted that all micronization treatments produced beans with similar hydration capacities (121-126%) (Appendix IV). Hydration capacity has previously been significantly and negatively correlated with cooked bean hardness (Plhak *et al.*, 1989); but Abdel-Kader (1995) found that the rate of water absorption of faba beans did not correlate well with protein content, size or density. Hard-to-cook beans were found to bind 25 % less water during soaking than control beans (Plhak *et al.*, 1989). In this study, the linear equation for hydration capacity (R^2=0.4210) suggests that as SL increases, the hydration capacity of navy beans decreases (Table 5). It should be noted that an increase in SL leads to a longer processing time, which causes greater chemical changes in the seed such as a higher degree of protein denaturation or starch gelatinization (Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998). Although starch gelatinization was not controlled in this experiment, protein denaturation was accentuated as the processing times increased due to the higher SL (Table 5). The hydration capacity was also negatively correlated to both outlet temperature and bean density (Table 6). In general, as the micronization process continues, there is a reduction in the hydration capacity of navy beans. The hydration

capacity is defined as the degree to which the cotyledon and cell contents (starch, protein and cell wall components) become fully saturated with water. A decrease in the hydration capacity may be the result of hydrophobic aggregation in micronized legume proteins (Zheng *et al.*, 1998), which could reduce the number of water binding sites available.

2.3.5. Force after cooking

The force after cooking was reduced as GL increased. This suggests that higher GLs contribute to the optimization of the processing conditions. As it was surmised, different gas-air mixtures do indeed translate to different intensities of IR radiation and cause a reduction in the maximum force required to compress cooked beans. In this study, the lowest maximum force values were achieved at the highest GL (Table 5). Nevertheless, the maximum force values for all cooked micronized beans in these experiments (206-281 N) were lower values than for non-micronized beans cooked after the same cooking time. It should be noted that unlike traditionally cooked beans, micronized beans were not subjected to the eighteen-hour soaking pre-step. See Appendix IV for observed experimental data. High hardness values were significantly correlated with samples with high final moisture contents (Table 6). This confirms previously discussed results indicating that the higher the moisture loss, the more severe the heat treatment was and therefore the lower the hardness values are expected to be.

2.3.6. Bean density

DL and the GL*DL interaction significantly affected the particle density of micronized beans, while GL alone did not (Table 4). In addition, the linear model for particle density in Table 5 indicates that the effect of the GL and DL interaction is greater than the effect of DL alone. Although the effect of GL was not significant ($P > 0.05$), the

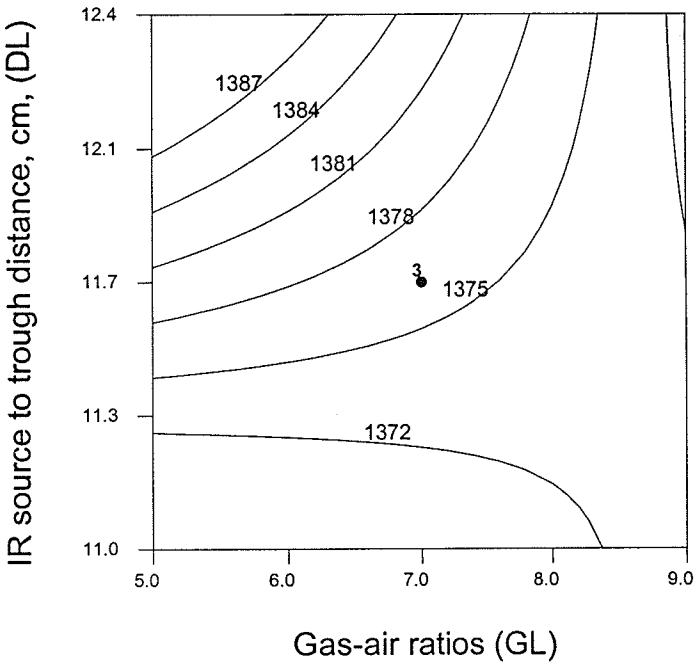


Figure 5. Contour plot of density (kg/m^3) of micronized navy beans as a function of GL and DL. SL= 3.

linear model for density includes the effects of GL (Table 5). The inclusion of a not significant factor (GL) was necessary to meet the requirements of a hierarchical model that would include the effects of such factor in a significant interaction (GL*DL).

Changes in density indicate that the micronization process affected seed structure. Figure 5 indicates that at a DL lower than 11.4 cm, micronized beans increase their volume as GL increases. An increase in the bulk volume has previously been reported in a wide variety of micronized pulses (Fasina *et al.*, 1997). Air-bubbles created in micronized lentils have been hypothesized to be responsible for their reduced cooking time (Scanlon *et al.*, 1997; Cenkowski and Solsulski, 1998). This could explain, at least in part, the significant differences found in the cooking times of samples receiving different micronization treatments.

In the present study, no significant correlation was found between the density of micronized beans and their hardness, although there was a negative correlation ($P \leq 0.05$) with hydration capacity (Table 4). Scanlon *et al.* (1999) reported that for micronized durum wheat, large increases in porosity were observed as tempering level increased up to 32 % (11% porosity). However, a highly vitreous material such as durum wheat is expected to originate greater porosity changes after micronization than beans due to its more compact structure. Perhaps it is necessary to optimize the tempering times and/or tempering levels of navy beans in order to achieve significant volumetric changes in the seed. Air bubbles in micronized beans are desirable since they act as a separate phase and increase water transportation rates during cooking, thus accelerating the overall cooking process (Scanlon *et al.*, 1999).

Overall, results indicated that SL and GL, but not DL, made significant ($P \leq 0.05$) contributions to the optimization of micronization processing conditions by controlling the rate and extent of the micronization process. Optimum micronization operational conditions occurred at GL=9 and SL=5. It is noteworthy that other factors were kept constant during micronization processing. For example, the feed rate controller was set to 60 (approx. 0.67 ± 0.03 kg/min) and the distance of the feed gate gap was set in such a way that allowed a single layer of water-conditioned beans to be fed into the vibratory conveyer trough (approx. 5 mm).

Chapter 3: Optimization of Processing Conditions and the Effect of Tempering Solutions on the Physicochemical Properties of Micronized Navy and Black Beans (*Phaseolus Vulgaris L.*)

ABSTRACT

The objectives of this study were (1) to identify optimal tempering conditions and (2) to evaluate the use of tempering solutions for reducing the cooking time of both navy and black beans. Using distilled water, a response surface methodology analysis indicated that optimum tempering conditions (tempering time and tempering level) for micronization of navy and black beans, based on the degree of starch gelatinization, occurred at 28% moisture for 11.5h and 25.8% moisture for 32h, respectively. In addition, the Hunterlab colour *a* value of navy beans was significantly influenced by the extent to which the tempering time changed. Using the optimum processing conditions, it was found that a mix of salts (0.2% sodium tripolyphosphate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate) was more effective than a mix of acids (1% citric acid and 2% ascorbic acid) and 150 ppm of sodium EDTA in reducing the hardness of micronized beans. Generally, a decrease in the percentage of soluble protein and an increase in the degree of gelatinized starch were significantly ($P \leq 0.05$) correlated with lower hardness scores in micronized beans. Nevertheless, these changes were not able to fully explain textural changes in micronized beans suggesting that other *in situ* changes (i.e., pectin solubilization, porosity changes) must not be disregarded. In conclusion, the micronization process significantly ($P \leq 0.05$) reduced the hardness value of both navy and black beans by 28.7 and 13.7% when tempered with distilled water and the mix of salts, respectively.

3.1. INTRODUCTION

Micronization, an infrared heat treatment, has been shown to have the potential for improving the cooking quality of cereals and pulses (Arntfield *et al.*, 1997; Scanlon *et al.*, 1998, 1999; Cenkowski and Sosulski, 1998). During micronization, products such as cereal grains and pulses can reach internal temperatures of 90-95 °C in only 50 seconds (Lawrence 1975, Kouzeh-Kanani *et al.*, 1982; Kouzeh-Kanani *et al.*, 1984; McCurdy, 1992; Cenkowski and Sosulski, 1998). Micronization can be an attractive processing method to improve the utilization of beans since it only uses water, high temperature and mechanical pressure to reduce their cooking times.

Physicochemical changes that have been put forward as responsible for reduced cooking time in pulses include partial gelatinization of starch (Arntfield *et al.*, 1997), reduced protein solubility (Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998), increased pectin solubility (Toews, 2001), and a more open structure (Scanlon *et al.*, 1999; Arntfield *et al.*, 2001). Nevertheless, available data is consistent in demonstrating that moisture of cereal grains is paramount in determining the extent of physicochemical changes resulting from micronization processing. The cooking times of pinto beans (Abdul-Kadir *et al.*, 1990), chickpeas (Sarantinos and Black, 1996) and yellow peas (Tyler and Karoutis, 1993) were larger after micronization than before, possibly because of their low moisture contents (<18%) prior to processing (Tyler and Karoutis, 1993; Scanlon *et al.*, 1998).

The processing of legumes usually requires a water-conditioning step that tenderizes the seed and facilitates further processing such as cooking. The pre-treatment may include tempering, where a predetermined amount of water is added to achieve a desired moisture in the seed, or soaking, where the seed is placed in an excess of water

and the final moisture is high and unevenly distributed (Scanlon *et al.*, 1997). Water imbibition forms an integral part of bean processing methods such as milling (Kadam and Salunkhe, 1989), germination (Labaneiah and Luh, 1981; Spaeth, and Hughes, 1987; Ghorpade and Kadam, 1989), fermentation (Reddy and Salunkhe, 1989), toasting (Deshpande *et al.*, 1990), and micronization (Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998; Scanlon *et al.*, 1998, 1999; Arntfield *et al.*, 2001).

Traditional soaking and the use of soaking salt solutions to reduce the cooking time of legumes have been broadly studied in several investigations (Rockland and Metzler, 1967; Al-Nouri and Siddiqi, 1982; Uzogara *et al.*, 1988; Buckle and Sambudi, 1990; Garcia-Vela *et al.*, 1991). Beans are traditionally soaked to increase availability of water in the seed prior to cooking and thus to shorten cooking times. For the same reason, soaking of beans is also a common practice used by the canning industry. Soaking of beans can be accomplished by using aqueous media consisting of water alone or dilute solutions of salts, acidulates, or alkali.

Effective tempering/soaking solutions to reduce cooking time in beans may include EDTA (Aguilera and Rivera, 1992, Scanlon *et al.*, 1998), sodium bicarbonate (Buckle and Sambudi, 1990; de Leon *et al.*, 1992), sodium tripolyphosphate (Scanlon *et al.*, 1999), a mix of carbonates and phosphates (Al-Nouri and Siddiqi, 1982; Scanlon *et al.*, 1998; Zhao, 2000; Toews, 2001), sodium chloride (Ros and Rincon, 1991), and calcium chloride (Drake and Muehlbauer, 1985). The mechanisms by which salt solutions soften the texture of beans are yet not well understood. Nevertheless, it is well accepted that the addition of chelating agents (i.e., EDTA) softens the texture of beans by facilitating cell wall separation during cooking through ion exchange and chelation mechanism between monovalent cations (Na^+, K^+) in solution and divalent cations

(Ca^{+2}, Mg^{+2}) in the middle lamella (Rockland and Metzler, 1967; Varriano-Marston and de Omana, 1979; Hincks and Stanley, 1986; de Leon *et al.*, 1987; Aguilera and Rivera, 1992). In addition, salt solutions containing high ionic strength may favour protein denaturation and therefore contribute to a reduction in bean cooking time (Garcia-Vela *et al.*, 1991; and del Valle *et al.*, 1992b). It has also been reported that limitation of water in bean cotyledons may impair the effectiveness of tempering/soaking solutions in reducing the cooking time of pulses (Scanlon *et al.*, 1998).

The present investigation was divided into two experiments in order to facilitate analysis. The first experiment used distilled water to optimize the tempering conditions, specifically tempering level and the tempering time of micronized navy and black beans. The hardness, final moisture content, colour, degree of starch gelatinization and soluble protein were used to monitor this optimization. The second experiment used the optimum tempering conditions found in the first experiment, to evaluate the use of three tempering pre-treatments with respect to the hardness, colour, and percent of gelatinized starch and soluble protein content of micronized navy and black beans. Tempering pre-treatments included a control treatment of distilled water, a solution of 150ppm disodium EDTA, a mix of 1% citric acid and 2% ascorbic acid, and a salt solution containing a mix of 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% sodium phosphate dihydrate.

3.2. MATERIALS AND METHODS

3.2.1. Materials

The beans (both navy and black) used in this study were purchased (2001) from Roy Legumex Ltd (St. Jean-Baptiste, MB, Canada) and were grown in Manitoba during the 2000 crop year. Upon arrival, beans were stored at 5 °C in closed plastic containers

(Trimeld Rubbermaid®). Appendix I indicates the chemical and physical characteristics of both navy and black beans.

3.2.2. Tempering process

Beans (7.5 kg) were divided into three equal parts and mixed thoroughly in 4.5 L Rubbermaid® containers with the predetermined amount of distilled water or tempering solution required to reach the tempering level stated in the experimental design. Uniformity of water absorption was assured by tumbling the samples for every 10 min for the first hour, and then once every hour for at least four hours or until tempering water was visually absorbed by the seeds. Appendix V lists the chemicals used in tempering and chemical analyses of navy and black beans.

3.2.2.1. Experiment with water

The navy and black beans were tempered with distilled water using several tempering conditions (Table 7).

3.2.2.2. Experiment with solutions

The navy and black beans were tempered to 28% for 11.5h and 25.8% for 32h, respectively, with various solutions (Table 8).

3.2.3. Micronization process

A pilot-scale MR2 micronizer unit (Micronizing Company Ltd., UK) fitted with a gas-fired infrared manifold and equipped with a vibrating stainless-steel conveyer trough and four ceramic IR burners was used to process the tempered beans. Using natural gas as a fuel, the MR2 micronizer is able to generate between 8.8 and 29.3 kW of power as specified by its manufacturer.

Table 7. Experimental points used to model response variables

Experimental points	Tempering time (h)	Tempering level (%)	Run order	
			Navy	Black
Star points	6	20	9	6
	6	30	1	5
	18	20	7	10
	18	30	10	12
Centre points	12	25	2	2
	12	25	6	3
	12	25	12	8
	12	25	13	11
	12	25	11	9
Axial points	3.5	25	4	4
	20.5	25	3	1
	12	17.9	8	13
	12	32.1	5	7

Table 8. Tempering solutions and pH values that were used to temper navy and black beans for 11.5h to 28% and for 32h to 25.8% moisture, respectively, before micronization

Tempering Solution	pH
Distilled water	6.1
150 ppm disodium EDTA	5.3
1% citric acid + 2% ascorbic acid	2.2
0.2% sodium bicarbonate + 0.1% sodium carbonate + 0.1% sodium phosphate dihydrate	9.8

3.2.3.1. Experiment with water

Micronization operational conditions were kept constant during processing of samples, and included a vibrating conveyer bed slope setting of 5 (equivalent to a slope of 0.0236 radians) and an air-gas mixture operational setting of 9 (Figure 2). The feed rate was

kept constant at 0.67±0.03 kg/min using a setting of 60 in the vibratory feeder (Figure 2). The micronizer was warmed up for 15 min using a warm-up batch of beans prior to each experimental run of the experimental design. The protocol to micronize both navy and black beans was the same as the one described in section 2.2.3.

Immediately after micronization processing, samples were spread out on a single layer on a table (stainless steel) and allowed to cool down to room temperature. A portion of these samples were bagged in 500 g Zip-lock® plastic-bags and used the same day for colour and moisture determinations. The remaining samples were further dried to 16±1% moisture using a convection oven set at 40±5°C (to avoid protein and starch damage), and samples were then stored at 5 °C. Moisture removal was used so that the effects of tempering pretreatments on hardness, degree of gelatinized starch and percent of soluble protein could be evaluated independently of the moisture level.

3.2.3.2. Experiment with tempering solutions

Micronization operational conditions were kept constant during processing of samples, and included a vibrating conveyer bed slope setting of 2.75 (equivalent to a slope of 0.0130 radians) and an air-gas mixture operational setting of 9 (Figure 2). Results from ongoing experiments indicated that a vibrating conveyer bed slope setting of 2.75 would improve the exposure of beans to more uniform IR radiation. The feed rate was kept constant at 0.67±0.03 kg/min using a setting of 60 in the vibratory feeder (Figure 2). The micronizer was warmed up for 15 min using a warm-up batch of beans prior to each experimental run of the experimental design. Unless otherwise stated in this section, the protocol used to micronize samples tempered with several tempering solutions (Table 8) was the same as the one described in Section 3.2.3.1. In this study, however, the final

moisture content of micronized samples was not reported since all samples were air-dried in a convection oven to 16±1% moisture.

3.2.4. Moisture

The moisture content of processed beans was measured by an air-oven method according to the AACC (1995) approved method 44-15A.

3.2.5. Colour evaluation

The Hunterlab Colour Difference Meter (Hunter Associated Laboratory Inc., McLean, Virginia) was used to measure the colour of micronized beans in both the experiment with water and the experiment with tempering solutions. The instrument was calibrated to the white tile (L=92.37, a=-1.2, b= 0.5). One hundred grams of beans were placed in a clear plastic cell, and placed under the port L, a, and b values recorded. The sample (100 g) filled the measuring cell so that no light could penetrate through it. The sample was placed back in the original container, gently mixed and another reading was measured. The colour of beans was expressed as average L, a, b values, where L is brightness, $+a$ redness, $-a$ greenness, $+b$ yellowness and $-b$ blueness.

3.2.6. Grinding

A Stein Laboratory Mill (Fried Stein laboratories, Inc. Atchison, Kansas) was used to grind approximately 50 g of whole beans so that the meal passed through a 500-μm sieve (35 mesh US Standard Sieve Series). The ground samples were used for all chemical analyses. They were stored for no more than two weeks at 5 °C in opaque containers with lids and were warmed to room temperature prior to use.

3.2.7. Degree of starch gelatinization

The degree of starch gelatinization was measured using the method of Arntfield *et al.* (1997). Glucose was used as a standard.

3.2.8. Soluble protein

Soluble protein was determined according to the method of Arntfield *et al.* (1997), using bovine serum albumin as the standard.

3.2.9. Texture evaluation

3.2.9.1. Evaluation of water tempered beans

Sixty-five grams of micronized beans were placed in 975 mL of boiling deionized water (ratio 1:15 beans to water) and boiled for 1 h. This long cooking time (60 min) was chosen due to constraints with the texture analyzer (load limit = 1000 N) in measuring hardness of extreme data points in the experimental design. Cooked beans were then drained thoroughly for about 3 min, and placed in 250 mL covered plastic containers to cool down for 1 h. Texture measurements were taken within 1 h of cooling. Hardness was defined as the maximum force (N) necessary to compress 30 g of cooked-beans through a 10-cm^2- extrusion-bar. Firmness was defined as the work (Nmm) necessary to compress 30 g of cooked-beans through a 10-cm^2- extrusion-bar. The Lloyd Materials Testing Machine Model L1000R (Lloyd Instruments Ltd., UK) was used to analyze texture, as described by Arntfield *et al.* (1997).

3.2.9.2. Evaluation of different tempering solutions

The protocol to evaluate texture of micronized beans tempered in different salt solutions was similar to the one described in the previous section. However, the cooking time was changed to 42.5 min.

3.3. Experimental design

3.3.1. Evaluation of water tempered beans

This experiment was conducted to evaluate the effects of tempering conditions, using water as tempering medium, on the physicochemical properties of micronized navy and black beans. A central composite response surface methodology (RSM) with two factors and five levels was generated and evaluated using Design-Expert® software (Version 5.0.5, Stat-Ease Inc, MN). Tempering time and tempering level were the two independent variables. The 13 treatments included four axial points, five repetitions of the centre design point, and four star-points (Table 7). Design-Expert® software was used to optimize the tempering pre-treatments with respect to moisture, hardness, colour, protein solubility, and percent of gelatinized starch. Three-dimensional graphs were generated from the fitted model using Design-Expert® software. Correlation analysis of the data was performed using Pearson correlation coefficient analysis of SAS (version 8.02, SAS Institute Inc, 2001, Cary, NC).

3.3.2. Evaluation of different tempering solutions

The optimum tempering conditions for navy and black beans as determined from the evaluation of tempering conditions using water only, were used to evaluate the effects of various tempering solutions (completely randomized design) on moisture, hardness, colour, protein solubility, and degree of starch gelatinization of micronized navy and black beans. Tempering solutions (Table 8) included a control treatment of distilled water or 'Water' (pH=6.1), a solution of 150-ppm disodium EDTA or 'EDTA' (pH=5.3), a mix of 1% citric acid and 2% ascorbic acid or 'Acids' (pH= 2.2), and a salt solution containing 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate or 'Salts' (pH=9.8). Data were analysed by SAS (version

8.02, SAS Institute Inc, 2001, Cary, NC). The General Linear Model (GLM Model) was used for analysis of variance of all dependent variables (treatments=4, repetitions=2; t x r = 8 observations per dependent variable). Treatments means (soaking solutions) were compared using the conservative Scheffe's test (controls for the experiment-wise Type I error rate) to determine significant differences. The significance level 'α' for these comparisons was set to 0.05. Correlation analysis of the data was performed using Pearson correlation coefficient analysis of SAS (version 8.02, SAS Institute Inc, 2001, Cary, NC).

3.4. RESULTS AND DISCUSSION

3.4.1. Evaluation of water tempered beans

3.4.1.1. Moisture

The final moisture content of micronized navy and black beans exhibited a highly significant [R^2= 0.85 ($P \leq 0.0001$), lack-of-fit= 0.10; and R^2= 0.79 ($P \leq 0.0004$), lack-of-fit=0.12, respectively] RSM linear regression model. This indicates that there was a significant relationship between the final moisture content of micronized navy and black beans and the extent to which tempering conditions were varied in this investigation. The linear model for the final moisture content of navy and black beans (Figures 6 and 7) indicated that micronized bean moisture content increased as the extent to which tempering level and tempering time increased.

Following micronization of navy and black beans under identical tempering conditions, the final moisture content was always lower for black beans than for navy beans (Figures 6 and 7). For example, the linear model for the final moisture content of navy beans suggested tempering conditions of 20% moisture for 12 h would yield a final moisture level of 17.3% in micronized navy beans, while using the same tempering

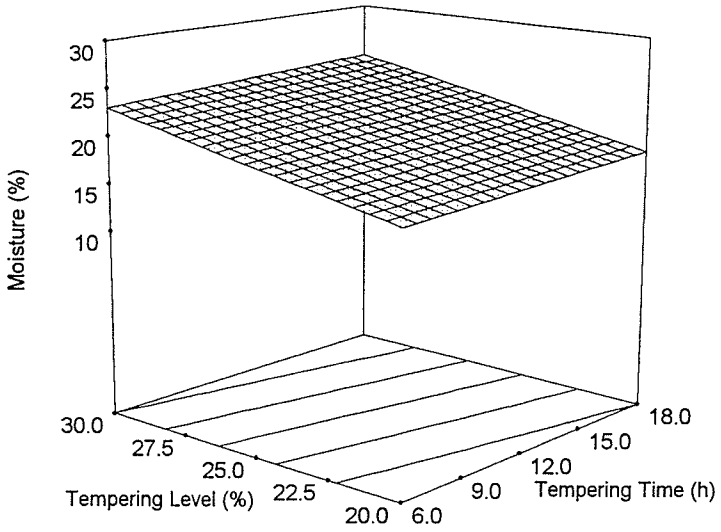


Figure 6. First-order response surface showing effects of tempering time (h) and tempering level (%) on the final moisture content of micronized navy beans. Model was drawn based on the following equation: Moisture (%) = +3.09 + (0.11 x tempering time) + (0.64 x tempering level). R^2=0.85 ($P \leq 0.001$), lack-of-fit=0.10.

conditions, the linear model for the moisture content of black beans yielded a final moisture level of 15.6%, in micronized black beans.

Differences in evaporative water losses for navy and black beans may indicate that, during micronization, black beans absorbed a greater amount of infrared radiation energy than navy beans. Differences in radiation energy absorption characteristics of navy and black beans could be attributed to their optical characteristics (Ginzburg, 1969). Based on the principles underlying absorption of radiation energy (Ginzburg, 1969), dark-coloured black beans were expected to absorb greater amount of radiation energy than navy beans since they are a better approximation of a black body. Black bodies have an absorptivity coefficient of 100%, which in theory indicates that the absorption of IR rays is 100%. Lighter coloured materials like navy beans tend to absorb

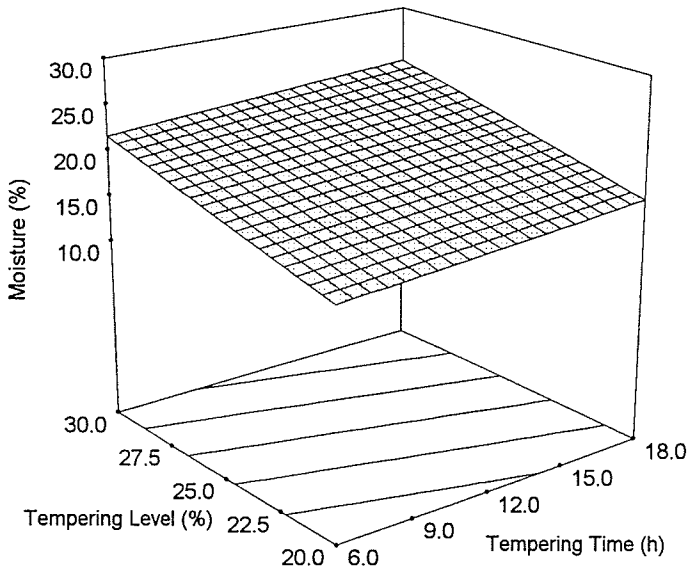


Figure 7. First-order response surface describing effects of tempering time (h) and tempering level (%) on final moisture content of micronized black beans. Model was drawn based on the following equation: Moisture (%) = -0.54 + (0.18 x tempering time) + (0.70 x tempering level). R^2=0.79 ($P \leq 0.0004$), lack-of-fit= 0.12.

less IR radiation energy due to their higher reflectivity coefficient. The denser cell arrangement in the seed coats of black beans than navy beans could also have affected the effectiveness of the overall micronization process.

The analysis of variance (ANOVA) indicated that tempering level had a highly significant effect on the final moisture content of navy and black beans ($P \leq 0.0001$ and $P \leq 0.0002$, respectively). The linear model for the moisture content of navy and black beans further includes the effects of both tempering time and tempering level (Figure 6 and 7). Based on this model, tempering time had a greater effect on the final moisture content of black beans than on navy beans. It is possible that the difference in the influence of tempering time is the result of differences in their water absorption rates.

The rate of water absorption in pulses has previously been related to the ability of water to permeate the seed coat during water imbibition experiments (Powrie *et al.*, 1960; Sefa-Dedeh and Stanley, 1979; Deshpande and Cheryan, 1986; del Valle *et al.*, 1992a). Deshpande and Cheryan (1986) found that the rate of water absorption was slow in seeds with a thick and smooth seed coat, whereas the rate of water uptake was greater in samples with thin and irregular seed coats. The tempering time to reach equilibrium moisture content is therefore expected to be longer for black beans than for navy beans since black beans have thicker seed coats than navy beans (Agbo *et al.*, 1987). As well, dark-coloured seed coats were reported to imbibe less water than light-coloured seed coats, probably due to denser arrangement of seed coat cells in dark-coloured seeds (Powell, 1989) or as a result of oxidative reactions of phenolic substrates resulting in hydrophobic substances in dark-coloured seed coats (del Valle *et al.*, 1992a).

3.4.1.2. Hardness

The RSM analysis of the hardness responses of micronized navy and black beans could not generate a significant regression model using the process variables. It is possible that the long cooking times employed prior to texture analysis softened the texture of both navy and black beans to such an extent that the effects of tempering pretreatments were masked. The hardness values have previously been used for optimization of micronization processing conditions (Scanlon *et al.*, 1998; Zhao, 2000; Toews, 2001). The tempering experiments in the present study, however, did not allow prediction of optimum tempering conditions based on hardness since the RSM analysis of hardness did not yield a significant ($P \leq 0.05$) regression model. The range of hardness values for micronized navy and black was 299-475 N and 428-536 N, respectively (See Appendix VI, Tables 1 and 2).

3.4.1.3. Soluble protein

As with hardness, there was no significant RSM regression model to describe the effect of processing variables (tempering time and tempering level) on the percent of soluble protein for micronized navy and black beans. The percent of soluble protein of cereals and other pulses has previously been found to change as the intensity of IR radiation increased (Arntfield *et al.*, 1997; Zheng *et al.*, 1998; Arntfield *et al.*, 2001). In the present study, the percent of soluble protein ranged from 3.51-22.81% and 2.26-19.69 % for navy and black beans, respectively. However, as it was for hardness, the protein solubility response was unable to be described in terms of tempering conditions by a significant regression RSM model.

3.4.1.4. Starch gelatinization

The degree of starch gelatinization of micronized navy and black beans was described by statistically significant RSM quadratic regression models (R^2=0.97, $P \leq$ 0.0001 and lack-of-fit=0.16, and R^2= 0.97, $P \leq 0.0001$ and lack-of-fit=0.15 for navy and black beans, respectively) based on process variables (Figure 8 and 9). The quadratic regression models for both navy and black beans indicated that both the tempering level and the tempering time, as well as the interaction between the two had significant effects on the degree of gelatinized starch. The quadratic regression model predicts that tempering navy beans to 28% moisture for 11.5 h would give the highest (53.3 %) gelatinized starch level (Figure 8). Similarly, tempering to 25.8% for 32 h would give the highest starch gelatinization level (39.6%) for micronized black beans (Figure 9). For both navy and black beans, the lowest gelatinized starch levels (10.4 and

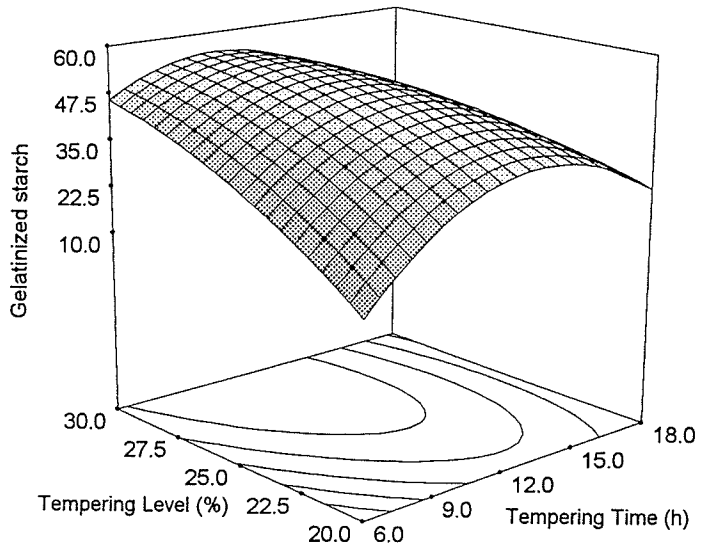


Figure 8. Second-order response surface depicting effects of tempering time and tempering level on the per cent of gelatinized starch in micronized navy beans. Model drawn was based on the equation: Gelatinized starch (%) = - 282.81 + (19.77 x tempering time) + (15.60 x tempering level) – [0.52 x (tempering time x tempering time)] - [0.20 x (tempering level x tempering level)] – [0.31 x (tempering time x tempering level)]. R^2= 0.97 ($P \leq 0.0001$), lack-of-fit=0.16.

14.1%, respectively) are predicted to occur at the lowest tempering level (20%) and tempering time (6 h). Negligible amounts of gelatinized starch (< 2 %) were also found in raw navy and black beans, which could be explained as the result of starch breakdown from alkali hydrolysis during the analysis (Toews, 2001).

The effectiveness of IR radiation in heating food materials (e.g., beans) depends upon several factors including: the distribution of the emitted energy (infrared radiation emissivity), assembly of the material's microstructural components, the optical (colour) and physical (size and shape) characteristics of the sample, and the density and uniformity of emitted power (Ginzburg, 1969). Since both micronization processing conditions and the size and shape

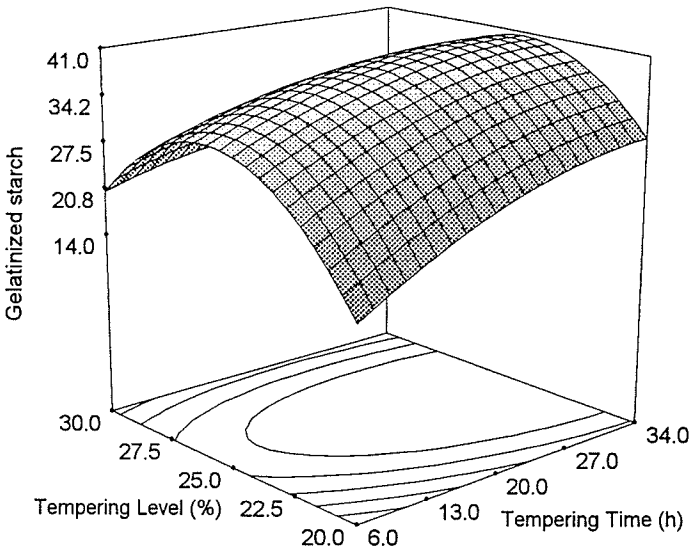


Figure 9. Second-order response surface depicting effects of tempering time and tempering level on the per cent of gelatinized starch in micronized black beans. Model drawn was based on the equation: Gelatinized starch (%) = -349.61 + (2.08 x tempering time) + (29.18 x tempering level) – [0.016 x (tempering time x tempering time)] - [0.57 x (tempering level x tempering level)] – [0.046 x (tempering time x tempering level)]. R^2= 0.97 ($P \leq 0.0001$), lack-of-fit=0.15.

of navy and black beans were essentially the same in the present investigation, it is surmised that differences in the degree of starch gelatinization of navy and black beans were brought about by differences in their chemical composition (i.e. starch source, water availability) and/or optical characteristics (i.e. colour).

The amount of absorbed energy and water available in the seed controlled the degree of starch gelatinization in micronized beans. Starch gelatinization takes place to a greater or lesser extent whenever starch is heated to a temperature between 50-80°C in the presence of water (Aguilera and Stanley, 1999). In this experiment, the high temperature, moist conditions during micronization triggered gelatinization of starch

granules in navy and black beans. The degree of starch gelatinization has been shown to increase with processing temperatures (Lawrence, 1973; Croka and Wagner, 1975; Rusnak *et al.*, 1980, Arntfield *et al.*, 1997) providing water availability is the same in the seed. In this study, the amount of absorbed IR energy of black and navy beans in theory should not be the same since the optical characteristics of their seed coat suggest that they possess different IR radiation absorptive coefficients. As such, dark-coloured black beans should absorb higher amounts of IR radiation energy than light-coloured navy beans (Ginzburg, 1969). During processing the final temperature of black beans (115-125°C) was about 5-10°C higher than that of navy beans (100-115°C). The final surface temperature differences between black and navy beans (5-10°C) may not play an important role in the degree of starch gelatinization. Arntfield *et al.* (2001), for example, found that the percent of soluble protein and the degree of gelatinized starch in lentils was not significantly different between samples micronized to 138 and 170°C. Moreover, operational temperatures during micronization (95-120°C) and the moisture contents (above 25%) were sufficient to initiate gelatinization of starch granules. It is conceivable that the differences in the degree of starch gelatinization between navy and black beans were brought about by differences in the amount of water available in their seeds.

Several studies have reported that the amount of water available for the seed components dictates the extent of the biochemical and physicochemical changes taking place during micronization processing. For example, high tempering levels favoured enzymatic breakdown of antinutritional factors such as oligosaccharides and phytate (Shiau and Yang, 1982; Sarantinos and Black, 1996; Toews, 2001), or promoted physical changes in the seed such as hydration of starch granules, and loosening of the

parenchyma cell walls (Hahn *et al.*, 1977) or a bulk volumetric expansion of the seed (del Valle *et al.*, 1992 a; Scanlon *et al.*, 1999). Free water must be available in the cotyledon of pulses if micronization is to successfully reduce the cooking time of pulses (Scanlon *et al.*, 1997). The optimum time-temperature-moisture conditions necessary for starch gelatinization is expected to correlate well with the ability to shorten cooking times (Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998; Zhao, 2000). High tempering levels (20-40%) have been shown to increase the degree of starch gelatinization of pulses (Arntfield *et al.*, 1997; Zhao, 2000; Toews, 2001). In the present study, the tempering levels of both navy and black beans ranged from 20 to 30% moisture since higher moisture levels can have detrimental effects on the shelf-life of micronized beans. Nevertheless, the amount of water available in the seeds of navy and black beans did not only depend on the tempering pretreatment but also on the water permeation characteristics of their seed constituents, principally the seed coat.

The kinetics of water migration through the seed coats of pulses is a function of the structural characteristics of the seed. The seed coat (Powrie *et al.*, 1960, Sefa-Dedeh and Stanley, 1979), hilum (Hyde, 1954), and micropyle (Kyle and Randall, 1964) have individually been related to water permeability in legume seeds. Although both hilum and micropyle were found to control the initial water uptake (30 to 60 minutes), overall the seed coat was pointed out as the most important barrier to water imbibition, principally due to its larger surface area (Deshpande and Cheryan, 1986). As it was mentioned before, seed coat of black beans are less permeable to water than the seed coats of navy beans (Powell, 1989; Agbo *et al.*, 1987). It is then reasonable to expect that water migration rates for black beans were slower than for navy beans. Garcia-Vela and Stanley (1989) indicated that as water becomes a limiting factor, gelatinization

temperature increases slightly and gelatinization of starch may not even take place. It could be possible that at short tempering times, added water was not fully absorbed throughout the seeds of black beans. This may explain why black beans needed higher tempering times (32h) than navy beans (11h) to achieve the highest level of gelatinized starch. This hypothesis was also confirmed by visible examination of the seeds during tempering experiments. For example, black beans tempered to 25% moisture, had visible water on the surface after 12h of tempering, whereas the tempering water was fully absorbed (in terms of surface appearance) by navy beans after 4h of tempering.

From the results of the present experiment, the highest degree of starch gelatinization was used to predict optimum micronization/tempering conditions. The highest degree of starch gelatinization was achieved in navy and black beans after tempering to 28% moisture for 11h and 25.8% x 32h, respectively. An increase in the degree of starch gelatinization of micronized pulses has been associated with shorter cooking times (Arntfield *et al.*, 1997, Cenkowski and Sosulski, 1998; Zhao, 2000).

3.4.1.5. Colour

Colour is one of the most important quality attributes that is used to grade dry beans (Canadian Grain Commission, 2002). In this study, the RSM analysis of colour of navy beans indicated that the Hunterlab *a* value exhibited a significant ($P \leq 0.05$) linear regression model (R^2= 0.57, $P \leq 0.0154$, lack-of-fit=0.38) with respect to tempering conditions (Figure 10). This regression model showed that tempering time had a greater impact than tempering level on the final colour of micronized navy beans. Figure 10 further indicated that long tempering times are detrimental to the colour quality of navy beans. However, optimum starch gelatinization levels and therefore optimum micronization conditions occurred at relatively low tempering times in navy beans

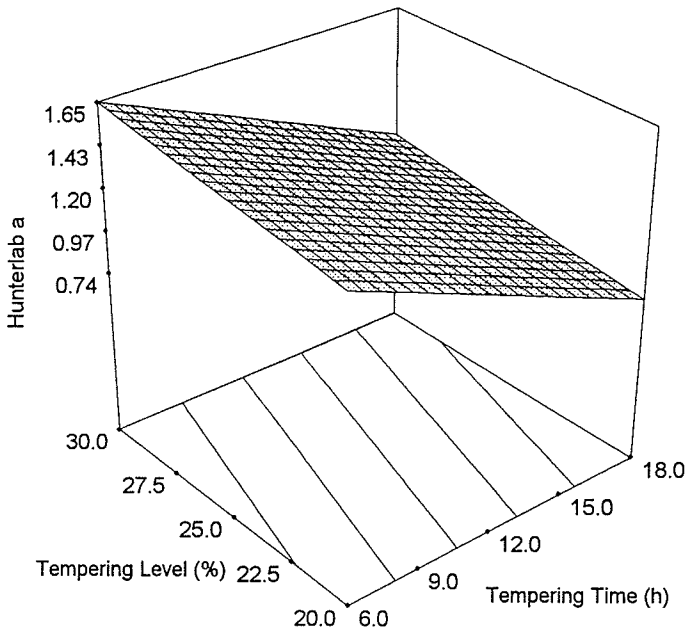


Figure 10. First-order response surface describing the effects of tempering time (h) and tempering level (%) on colour *a* value of micronized navy beans. Model was drawn based on the following equation: Colour *a* = +1.44 - (0.060 x tempering time) + (0.019 x tempering level). R^2=0.57 ($P \leq 0.02$), lack-of-fit= 0.38.

(11.5h). Black bean colour was not significantly affected by process variables (tempering time and tempering level).

For navy beans, the *L* and *a* values were negatively correlated ($P \leq 0.01$) with each other and the *b* value was positively correlated with the percent of gelatinized starch ($P \leq 0.1$) (Table 9). For black beans, the *L* and *a* values were again negatively correlated ($P \leq 0.01$), and the *L* values were also negatively correlated with moisture content and positively correlated with hardness ($P \leq 0.01$) (Table 10). As well, the *a* value of black beans was positively correlated with their final moisture content ($P \leq 0.05$) (Table 10).

Table 9. Correlations between characteristics of micronized navy beans (n=13)

	% moisture content	% starch gelatinized	Hunterlab *L* value	Hunterlab *a* value	Hunterlab *b* value
% moisture content	X	0.39	-0.42	0.15	0.43
% starch gelatinized		X	-0.01	0.01	0.53[a]
Hunterlab *L* value			X	-0.68[b]	-0.29
Hunterlab *a* value				X	0.29
Hunterlab *b* value					X

[a] significant at $p \leq 0.10$.
[b] significant at $p \leq 0.01$.

Table 10. Correlations between characteristics of micronized black beans (n=13)

	% moisture content	Hardness (N)	% soluble protein	Hunterlab *L* value	Hunterlab *a* value
% moisture content	X	0.29	0.29	-0.50[a]	0.49[b]
Hardness (N)		X	0.29	0.48[a]	-0.45
% soluble protein			X	0.07	-0.13
Hunterlab *L* value				X	-0.85[c]
Hunterlab *a* value					X

[a] significant at $p \leq 0.10$.
[b] significant at $p \leq 0.05$.
[c] significant at $p \leq 0.01$

Overall, the colour analysis indicated that optimizing tempering conditions for micronized navy and black beans based on starch gelatinization will result in only minor

colour changes. Colour changes are detrimental to the quality of pulses, so they should be minimized (Arntfield *et al.*, 1997). Nevertheless, in comparison to raw beans, the Hunterlab *L*, *a*, and *b* values of navy beans have changed from 60.2±0.19, 1.6±0.14, and 10.17±0.05 respectively, to an average value of 61.9±0.99, 1.2±0.29, and 11.4±0.76 respectively, due to the micronization process. These changes were more accentuated in black beans but not significant. For example, the average Hunterlab *L*, *a*, and *b* scores for raw black beans were 18.4±0.91, -0.08±0.04, and -1.14±0.68 respectively, whereas after micronization they changed to 22.9±0.24, -1.5±0.16 and –0.43±0.01, respectively. Colour changes have also been reported in micronized lentils as a result of tempering conditions (Arntfield *et al.*, 1997) or micronization temperatures (Arntfield *et al.*, 2001).

3.4.2. Evaluation of tempering solutions

Using the optimum tempering conditions for navy and black beans (28% for 11.5h and 25.8% for 32h, respectively) in water, various solutions (Table 8) were employed to assess their suitability in reducing cooking time, and their influence on the degree of gelatinized starch, per cent of soluble protein and colour. The average final temperature of micronized navy and black beans was 112±5 °C and 117±5 °C, respectively.

Results from the experiment with distilled water (Section 3.4.1) indicated that cooking micronized beans for 60 min tended to overcook samples since the statistical analysis was unable to identify significant differences for the texture responses of experimental treatments. In identifying a more suitable cooking time to screen the cooked texture of micronized beans, it was necessary to evaluate the cooking curves of micronized navy and black beans tempered with distilled water to 28% for 11.5h and 25.8% for 32h, respectively. Both navy and black beans (receiving no soaking

pretreatment) were cooked for 27.5, 35, 42.5, 50, 57.5 and 65 minutes. Based on these cooking curves, it was observed that cooking times of 42.5 min for both navy and black beans would facilitate identification of the effects of tempering pretreatments. After this time, the cooking curve of micronized beans started leveling off, thus increasing the likelihood of reaching a region in which samples are overcooked.

In a separate experiment, an expert sensory panel of five evaluated the cooked texture of unprocessed beans that were soaked overnight (18h) in distilled water and cooked for 27.5, 35, 42.5, 50, 57.5 and 65 minutes. The expert sensory panel agreed that the optimum texture of unprocessed navy and black beans was achieved in both cases after a 42.5-minute cooking time. Using the texture analyzer, this cooking time was associated with hardness (maximum compression force) values of 498 and 456 N for navy and black beans, respectively. It should be kept in mind, however, that micronized beans were not soaked prior to cooking since micronization is intended to reduce preparation times. It was then necessary to compare the hardness of micronized beans to those of unprocessed beans after cooking both samples received no soaking pretreatment.

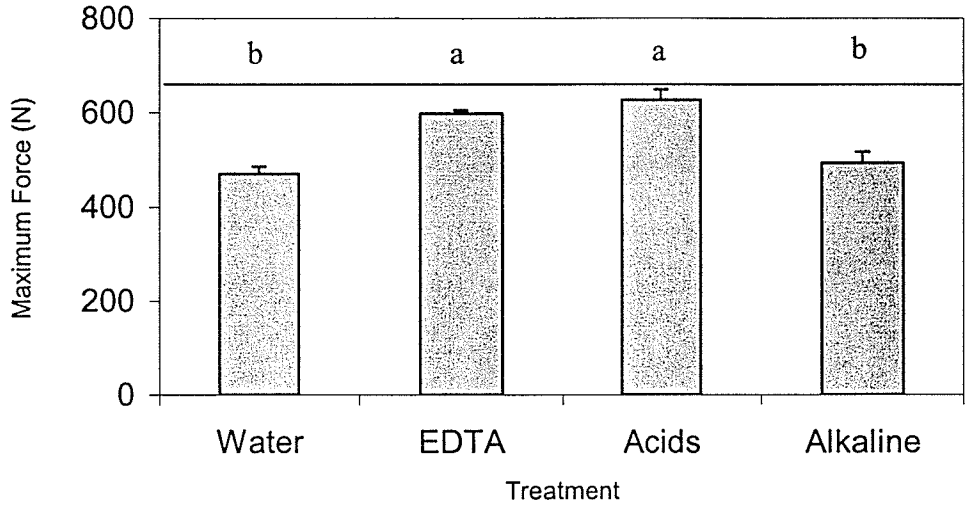
The standard cooking time to examine the texture of unprocessed and micronized beans (no soaking provided in both cases) was set to 42.5 min since longer cooking times tended to overcook micronized beans. Providing that no soaking pre-step was employed, the texture of micronized beans tempered with various solutions (Table 8) was then compared to the one of unprocessed navy (Hardness = 662 N; Firmness = 12730 Nmm) and black (Hardness = 604 N; Firmness = 10480 Nmm) beans cooked for a standard time (42.5 min). The hardness and firmness cooking curves of non-soaked

unprocessed both navy and black beans can be found in Appendix VII (Figures 1 and 2 for navy and black beans, respectively).

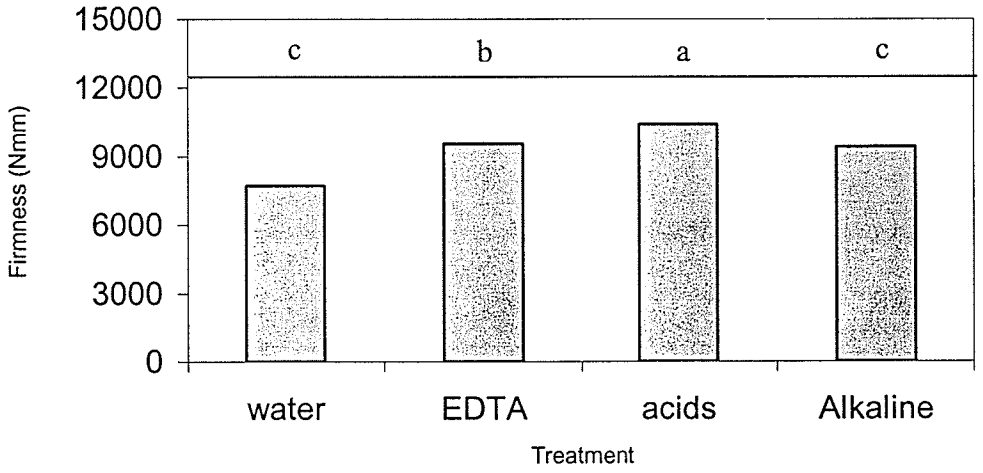
The selection of the tempering solutions was based on their ability to reduce the cooking time of pulses as reported in the literature. The tempering solutions (Table 8), spanned a full range of pHs, and included a control solution of distilled water (pH = 6.08). Scanlon *et al.* (1998) proposed the composition and concentration of the acid and EDTA pretreatments, which were found to be the most effective tempering solutions for reducing hardness of micronized lentils. The alkaline treatment was shown to be the best tempering pretreatment to reduce cooking times in field peas (Toews, 2001).

3.4.2.1. Hardness

The effects of tempering solutions on the hardness of micronized beans are shown in Figures 11 (navy beans) and 12 (black beans). Both unprocessed and micronized beans (not soaked) were cooked for the same time (42.5 min) using a standardized cooking protocol. As shown in Figures 11 and 12, tempering solutions had a significant ($P \leq 0.05$) effect on the hardness of micronized samples. Micronized navy beans tempered in water and with a solution containing salts had significantly ($P \leq 0.05$) lower hardness values (472 and 494 N, respectively) than unprocessed beans (662 N) (Figure 11A). A similar response was found for the firmness of micronized navy beans (Figure 11B). On the other hand, micronized black beans tempered with the salt solution had a significantly ($P \leq 0.05$) lower hardness score (521 N) than unprocessed beans (604 N). The firmness response for black beans tempered with water, however, indicated that the salt treatment was no more effective than water ($P \leq 0.05$) in reducing bean hardness (Figure 12B).

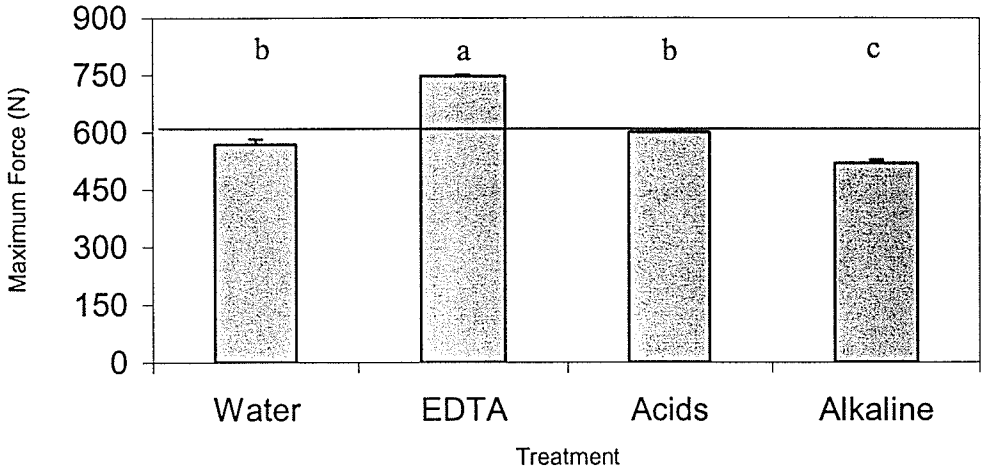


A. Hardness

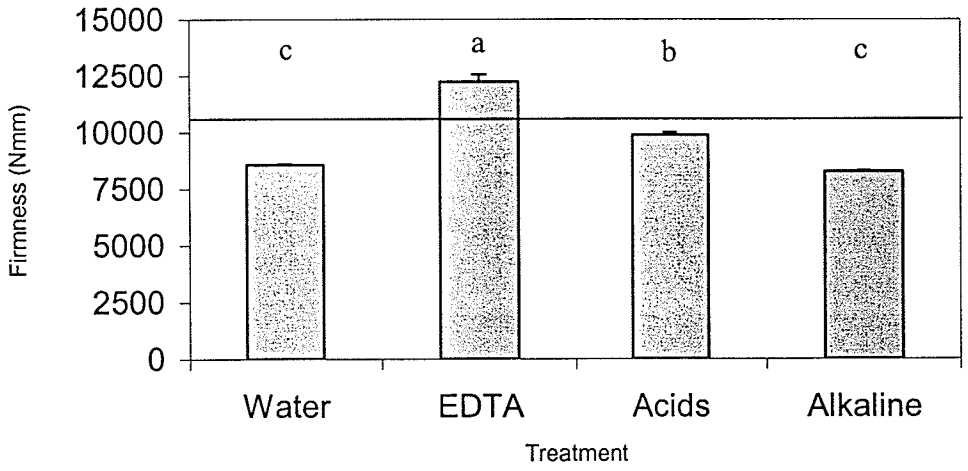


B. Firmness

Figure 11. The hardness (A) and firmness (B) of micronized navy beans tempered for 11.5h to 28% with various solutions. Texture analyses were done after cooking beans (no soaking) for 42.5 min in boiling water. Minimum significance difference was 78.7 N and 1084 Nmm for hardness and firmness, respectively. The horizontal line indicates the hardness of non-soaked unprocessed navy beans after a 42.5-minute cooking time. Different letters above treatment columns indicate significant ($P \leq 0.05$) differences. The vertical error bars indicate standard deviation.



A. Hardness



B. Firmness

Figure 12. The hardness (A) and firmness (B) of micronized black beans tempered for 32h to 25.8% moisture with various solutions. Texture analysis was done after cooking beans (no soaking) for 42.5 min in boiling water. Minimum significance difference was 38.6 N and 774 Nmm for hardness and firmness, respectively. The horizontal line indicates the hardness of non-soaked unprocessed navy beans after a 42.5-minute cooking time. Different letters above treatment columns indicate significant ($P \leq 0.05$) differences. The vertical error bars indicate standard deviation.

Generally, the acid treatment gave the highest hardness score values for navy beans (Hardness = 628 N; Firmness = 10765 Nmm), and the second highest for black beans (Hardness = 601 N; Firmness = 9875 Nmm). These findings concurred with those of McCurdy (1983) indicating that the texture of micronized field peas increased when citric acid (0.25 and 0.50 %) was added to the soaking solution. Toews (2001), however, reported that field peas tempered in a solution containing 1% citric acid and 2% ascorbic acid cooked faster than peas tempered in distilled water. The tempering levels used in the present investigation (20-30%) may be responsible for high maximum force values of beans tempered with this solution. Availability of water in the seed has been postulated as responsible for the poor cooking quality of pulses tempered in various solutions (Scanlon *et al.*, 1998). Scanlon *et al.* (1998) indicated that tempering solutions were effective in reducing the cooking time of lentils when tempering levels were at least 36%.

As shown previously for micronized peas (Toews, 2000), the alkaline treatment (Figure 12A) was an effective tempering solution for improving the cooking quality of black beans beyond what is achieved with water alone. This treatment, however, was no more effective than water in reducing the hardness of navy beans. Garcia-Vela *et al.* (1991) and del Valle *et al.* (1992b) found that softening of black beans could be improved by the addition of salt solutions with high anionic strengths such as carbonates (CO_3^{-2}). They hypothesized that a mechanism involving the solubilization of storage proteins by anionic salts would render proteins more thermally labile and thereby reducing bean hardness. Sodium tripolyphosphate and sodium dihydrate has been reported to reduce cooking time in pulses (Rockland and Metzler, 1967; Toews, 2001), and their combination with sodium carbonate was found to further reduce the texture of

pulses (Toews, 2001). Perhaps sodium tripolyphosphate and sodium dihydrate softened bean texture through the same solubilization mechanism as carbonates since their elevated pH and chelating properties (Al-Nouri and Siddiqi, 1982) are all factors that enhance dissociation of protein subunits and extraction of bean proteins (Garcia-Vela *et al.*, 1991).

Treatment with 150 ppm of sodium EDTA was not as effective as water alone in lowering the maximum force value of either navy or black beans (Figure 11 and 12). These results were not in agreement with previous findings reported in the literature; more specifically those related to the biochemical mechanisms leading to the hardening of beans (hard-to-cook phenomenon). The use of EDTA as a soaking pretreatment has been shown to reduce the cooking time of beans since it is a strong chelating and ionic exchange agent that may facilitate cotyledonary bean cell wall separation (Aguilera and Rivera, 1992). EDTA may further reduce bean-cooking time by favoring thermal denaturation of storage proteins due to its chelating properties (Garcia-Vela *et al.*, 1991). Del Valle *et al.* (1992 a) further corroborated these findings.

The poor textural response of micronized beans tempered in a solution containing EDTA may have arisen from the low EDTA concentrations used in this study. The concentration of sodium EDTA used by del Valle and Stanley (1995) was much greater (5%, pH=7.0) than the concentration used in the present study (0.015%, pH=5.34). Five per cent sodium EDTA, however, is well above its acceptable level (165 ppm) as a food additive (Scanlon *et al.*, 1998). Aguilera and Rivera (1992) reported that the hardness of black beans was reduced by 54% when the concentration of sodium EDTA increased from 20 to 100 mM (pH=4.8-5.0).

The longer cooking times observed for micronized beans tempered in sodium EDTA than those tempered in distilled water (Figure 11 and 12) may suggest that during cooking water permeates easily through the cell interior of micronized beans. Arntfield *et al.* (1997) reported that pectin solubilization was not correlated with the hardness of micronized lentils, though significant ($P \leq 0.05$) increases in the soluble fraction of pectin were reported in micronized lentils (Arntfield *et al.*, 2001). The high degree of penetrability of IR rays (Ginzburg, 1969; Cenkowski and Sosulski, 1998) and the availability of water in tempered seeds (20-30%) may result in *in situ* structural changes within the cells (starch, proteins, pectic substances) (Cenkowski and Sosulski, 1998; Arntfield *et al.*, 2001). Micronization may therefore facilitate cell separation, possibly due to mechanical stresses as a result of *in situ* starch gelatinization, protein denaturation reactions (Rockland and Jones, 1974). Similarly, it is possible that the micronization process promoted solubilization of pectic substances (i.e. middle lamella break down) and this may explain the reduced effectiveness of EDTA as a tempering pretreatment to enhance middle lamella solubilization. Breakdown of the middle lamella of cotyledonary bean cell walls has been reported to take place during cooking (Aguilera and Stanley, 1985). Considering that micronization essentially precooks beans, it would not be surprising that the middle lamella was solubilized, at least partially during the micronization process. It has been reported that treatments that facilitate middle lamella solubilization result in beans with shorter cooking times (Hincks and Stanley, 1986; Aguilera and Rivera, 1992; del Valle and Stanley, 1995). Another possible reason for the reduced effectiveness of sodium EDTA could have been the low concentrations employed in this experiment or the lack of sufficient free water in the cotyledons. These

views will be further discussed in the starch gelatinization and protein solubility sections.

3.4.2.2. Starch gelatinization

Micronized beans tempered in various tempering solutions (Table 6) had a higher degree of starch gelatinization than raw beans (1.5 %). Starch gelatinization of beans was expected to increase after micronization since similar results were found in barley (Lawrence 1973a), maize (Lawrence 1973a, Kouzeh-Kanani *et al.*, 1984), wheat (Lawrence, 1975), sorghum (Croka and Wagner, 1975; Savage and Clark, 1988), lentils (Arntfield *et al.*, 1997; Zhao, 2000, Arntfield *et al.*, 2001), and peas (Cenkowski and Sosulski, 1998; Toews, 2001). The addition of reagents to the tempering solutions (Table 8) did not further increase the degree of gelatinized starch in either navy or black beans beyond that level attainable with water alone (Table 11).

The degrees of starch gelatinization of navy beans tempered in water (24%) and alkaline treatments (19.2%) were not statistically ($P \leq 0.05$) different (Table 11). This is consistent with the texture analysis, which demonstrated that the hardness values of navy beans tempered with these treatments were not significantly different ($P \leq 0.05$). In comparison, the gelatinized starch levels of black beans tempered with water and alkaline treatments were significantly ($P \leq 0.05$) different, with higher levels of gelatinized starch with water. While significant differences had also been noted for the hardness of these two samples (Figure 12), the alkaline product was softer despite having a lower level of gelatinized starch. The degrees of gelatinized starch in navy beans tempered with EDTA (15%) and acid treatments (10.4%) was not statistically ($P \leq 0.05$) different from one another but were significantly lower than for water alone (Table 11). The texture values of navy beans tempered in these solutions (Figure 11) and

Table 11. Percent of gelatinized starch [1] in micronized navy and black beans tempered for 11.5h to 28% and for 32h to 25.8% moisture, respectively, with various solutions

Treatment	% Gelatinized starch (navy beans) [2]	% Gelatinized starch (black beans) [2]
Water	24.0 ± 2.4 [a]	32.5 ± 0.4 [a]
EDTA	15.0 ± 0.2 [bc]	8.7 ± 1.5 [c]
Acids	10.4 ± 0.2 [c]	22.9 ± 1.2 [b]
Alkaline	19.2 ± 0.9 [ab]	22.0 ± 1.1 [b]

[1] mean ± standard deviation.
[2] different letters in the same column indicate significant differences ($P \leq 0.05$).

the starch gelatinization data (Table 11) suggested that lower levels of gelatinized starch produced harder beans. For black beans, these two treatments had significantly ($P \leq 0.05$) lower levels of starch gelatinization than water, and this reduction was significantly greater for the EDTA treatment. Again, lower levels of gelatinized starch resulted in harder beans (Figure 12).

The tempering solution containing sodium EDTA gave the lowest gelatinized starch levels and the highest hardness values in navy (599.3 N) and black (748.9 N) beans. This study indicates that the use of EDTA as a tempering solution restricts starch gelatinization reactions in both navy and black beans. Garcia-Vela and Stanley (1989) indicated that as water becomes a limiting factor, gelatinization temperature increases. However, EDTA has been found to facilitate cell separation (Aguilera and Rivera, 1992). Further Scanlon *et al.* (1998) found that EDTA was effective in reducing the cooking time of lentils even when tempering water was somewhat limited (20%). The

rationale for the adverse effect of this treatment on the hardness of micronized beans still remains unclear and needs further exploration.

The correlation analysis (Tables 12 and 13) indicated that higher levels of starch gelatinization were highly and negatively correlated with the hardness values for both navy and black beans (R^2 = -0.92 and R^2 = -0.78, respectively). Several investigations reported that the degree of starch gelatinization of pulses increased after micronization (Arntfield *et al.*, 1997; Cenkowski and Sosulski, 1998; Zhao, 2000).

The alkaline treatment of sodium phosphates and carbonates was the best tempering solution (other than water) to increase the degree of starch gelatinization in navy and black beans. The high starch gelatinization levels achieved after micronization of navy and black beans occurred concomitant with a reduction in their cooking times (Figure 11 and 12) except for black beans tempered in the alkaline solutions. Black beans tempered in this tempering solution had softer textures despite having lower levels of gelatinized starch than water alone. These findings further confirm that reduction in the cooking time of beans is not only controlled by the level of gelatinized starch but may also be related to other changes in the bean constituents such as pectin solubilization or protein denaturation. Yet overall, the use of tempering solutions did not promote starch gelatinization in micronized beans beyond what is attainable with water alone.

3.4.2.3. Soluble protein

All micronized navy and black beans tempered in various solutions gave lower percentages of soluble protein (Table 14) than that for raw navy (24.42%) and black(21.35%) beans. The reduction in protein solubility of navy beans ranged from

Table 12. Correlations [1] among physicochemical properties of micronized navy beans tempered for 11.5h to 28% moisture with various solutions [2] (n=14)

	Hardness	Soluble Protein	Gelatinized Starch	Colour *L*	Colour *a*	Colour *b*
Firmness	0.93	0.72	-0.92	-0.78	0.72	
Soluble Protein	0.80					
Gelatinized Starch	-0.92			0.77		
Colour *L*			0.77		-0.89	-0.83
Colour *a*				-0.89		
Colour *b*				-0.83		

[1] all values listed are significant at $P \leq 0.05$.
[2] see Table 10 for list of tempering solutions.

Table 13. Correlations [1] among physicochemical properties of micronized black beans tempered for 32h to 25.8% moisture with various solutions [2] (n=14)

	Hardness	Soluble Protein	Gelatinized Starch
Firmness	0.98	0.71	-0.84
Soluble Protein	0.80		-0.90
Gelatinized Starch	-0.78	-0.90	

[1] all values listed are significant at $P \leq 0.05$.
[2] see Table 10 for list of tempering solutions.

10.6% (alkaline treatment) to 21.6% (EDTA treatment), and from 8.3% (water treatment) to 16.4% (EDTA treatment) in black beans. Many investigations found that protein solubility decreased as micronization processing temperatures increased in several products including lentils (Arntfield *et al.,* 1997; 2001), peas (McCurdy, 1992;

Table 14. Percent of soluble protein [1] in micronized navy and black beans tempered for 11.5h to 28% and for 32h to 25.8% moisture, respectively, with various solutions

Treatment	% Soluble Protein (navy beans) [2]	% Soluble Protein (black beans) [2]
Water	15.3 ± 0.3 [bc]	8.3 ± 0.9 [b]
EDTA	21.6 ± 0.2 [a]	16.4 ± 0.5 [a]
Acids	20.3 ± 2.3 [ab]	13.7 ± 1.1 [a]
Alkaline	10.6 ± 1.5 [c]	13.5 ± 0.9 [a]

[1] mean ± standard deviation.
[2] different letters in the same column indicate significant differences ($P \leq 0.05$).

Cenkowski and Sosulski, 1998), soybeans (Kouzeh-Kanani *et al.*, 1981), and maize germ (Kouzeh-Kanani *et al.*, 1984).

Arntfield et al. (1997) suggested that the loss of solubility in micronized lentils was the result of protein denaturation. Zheng *et al.* (1998) further indicated that denaturation of legume proteins after micronization was mainly due to hydrophobic aggregation of polypeptide chains. Protein solubility is affected by several factors including pH, temperature, processing conditions, ionic strength, presence/absence of other components (which are capable of binding with proteins), and solvents (Salunkhe *et al.*, 1989). Micronization had little effect on protein solubility of legumes at pH = 4.0 since the isoelectric point of legume proteins falls in the pH range of 3.5 to 4.5 (Zheng *et al.*, 1998). In the current study, protein solubility was based on extraction at room temperature using 0.5 M NaCl with pH = 7. Protein denaturation can be desirable since it is responsible for a reduction in the level of antinutritional factors in cereals and pulses, such as myrosinase in canola seeds and canola screenings (McCurdy, 1992),

lypoxygenase in soybeans (Kouzeh-Kanani *et al.*, 1982), and trypsin inhibitors (Kouzeh-Kanani *et al.*, 1981) in soybeans. Detachment of protein bodies from the cell matrix may further enhance water penetration into the seed due to a more open structure (Toews, 2001).

Although the percent of soluble protein in micronized beans was significantly correlated to their texture scores ($R^2 = 0.80$, in both cases) (Table 12 and 13), none of the tempering solutions were as effective as water alone in promoting protein denaturation. In navy beans, for example, the percentage of soluble protein was the lowest when water (15.3%) and the salt treatment (10.6%) were employed, although they were not significantly ($P \leq 0.05$) different from one another (Table 14). For black beans, water alone resulted in significantly ($P \leq 0.05$) lower percentages of soluble protein than that brought about by any of the tempering treatments (Table 14). In black beans, however, significantly ($P \leq 0.05$) lower hardness values were observed in samples tempered with the salts. High concentration of anionic salts have been found to facilitate protein denaturation in beans due to their effects on promoting thermal lability of storage proteins (Garcia-Vela *et al.*, 1991; del Valle *et al.*, 1992 b). The results on the percentage of soluble protein of navy beans may suggest that free water was not available in sufficient amounts in the bean cotyledons to allow reagents in the tempering water to reduce hardness further than the reduction caused by water alone (Scanlon *et al.*, 1998).

The lowest decrease in the percentage of soluble protein for navy beans occurred when the sodium EDTA and the acids were used as tempering solutions. These results further corroborate the starch gelatinization and hardness results (Table 11 and Figure

11) indicating that sodium EDTA is detrimental to the cooking quality of micronized navy beans.

The loss of solubility in micronized pulse proteins has been reported to be partly responsible for a reduction in bean cooking times (Arntfield *et al.*, 1997; 2001; Zhao, 2000). A more open structure may result from protein solubility reactions since proteins bodies are tightly packed to other bean cell organelles such as starch (Arntfield *et al.*, 1997). As a result, the water migration rates during normal cooking can be increased thereby reducing cooking times. Starch gelatinization and protein denaturation are indispensable reactions to fully cook beans (Stanley and Aguilera, 1985). Promoting either of those reactions should lead to a reduction in the cooking time of beans (Arntfield *et al.*, 1997). That appears to be the case in the present study, where a decrease in protein solubility or an increase in starch gelatinization occurred concomitantly with a reduction in the texture of both navy and black beans. Micronized black beans tempered with the alkaline pretreatment had significantly lower hardness values than water-tempered beans. Despite of this, water-treated black beans, however, had both higher percentages of starch gelatinization and lower percentages of soluble protein than alkaline-treated beans. These findings supported a mechanistic approach whereby other *in situ* changes (i.e. pectin solubilization, and/or density and porosity changes) favoured the softening of micronized beans.

3.4.2.4. Colour

Although colour changes should be minimized during processing, it is inevitable that heating foods such as beans should result in colour changes. The Hunterlab colorimeter was used to measure the colour (whole seeds) of raw and micronized navy

Table 15. Hunterlab colour *L* value [1] in micronized navy and black beans tempered for 11.5h to 28% and for 32h to 25.8% moisture, respectively, with various solutions

Treatment No.	Colour *L* (navy beans) [2]	Colour *L* (black beans) [2]
Water	60.3 ± 0.7 [a]	18.3 ± 0.3 [a]
EDTA	60.3 ± 0.9 [a]	18.2 ± 0.1 [a]
Acids	54.5 ± 0.8 [b]	18.5 ± 0.1 [a]
Salts	60.3 ± 0.9 [a]	18.5 ± 0.0 [a]

[1] mean ± standard deviation.
[2] different letters in the same column indicate significant differences ($P \leq 0.05$).

Table 16. Hunterlab colour *a* value [1] in micronized navy and black beans tempered for 11.5h to 28% and for 32h to 25.8% moisture, respectively, with various solutions

Treatment No.	Colour *a* (navy beans) [2]	Colour *a* (black beans) [2]
Water	1.04 ± 0.37 [a]	0.35 ± 0.21 [a]
EDTA	1.07 ± 0.37 [a]	0.57 ± 0.19 [a]
Acids	4.63 ± 0.69 [b]	0.97 ± 0.23 [a]
Salts	0.95 ± 0.45 [a]	0.20 ± 0.14 [a]

[1] mean ± standard deviation.
[2] different letters in the same column indicate significant differences ($P \leq 0.05$).

Table 17. Hunterlab colour *b* value [1] in micronized navy and black beans tempered for 11.5h to 28% and for 32h to 25.8% moisture, respectively, with various solutions

Treatment No.	Colour *b* (navy beans) [2]	Colour *b* (black beans) [2]
Water	12.10 ± 0.18 [a]	-1.39 ± 0.40 [a]
EDTA	12.07 ± 0.37 [a]	-1.25 ± 0.07 [a]
Acids	12.75 ± 0.12 [a]	-0.78 ± 0.07 [a]
Salts	12.25 ± 0.31 [a]	-0.99 ± 0.26 [a]

[1] mean ± standard deviation.
[2] different letters in the same column indicate significant differences ($P \leq 0.05$).

and black beans (L = lightness/brightness; $+a$ = redness; $-a$ = greenness; $+b$ = yellowness; $-b$ = blueness).

Generally, micronized black beans, regardless of tempering solutions, were all darker (L = 18.2 to18.5), less green (a = 0.20 to 0.97) and more blue (b = -0.78 to -1.39) than unprocessed beans (L = 22.90; a = -1.50; b = -0.43). Statistical analysis (Table 15,16 and 17) showed that there were no significant ($P \leq 0.05$) differences between the colour responses of micronized black beans tempered with various solutions. Figure 13 shows the physical appearance of micronized black beans tempered in different solutions. However, it was visually observed that during tempering of black beans, soluble colour compounds were leached into the tempering medium. Although the long tempering times assured post absorption of the colour compounds before processing, they were more susceptible to thermal degradation during micronization processing.

The acid treatment was detrimental to colour of navy beans in that they were significantly darker (L = 54.5) and more red (a = 4.63) than the other micronized navy

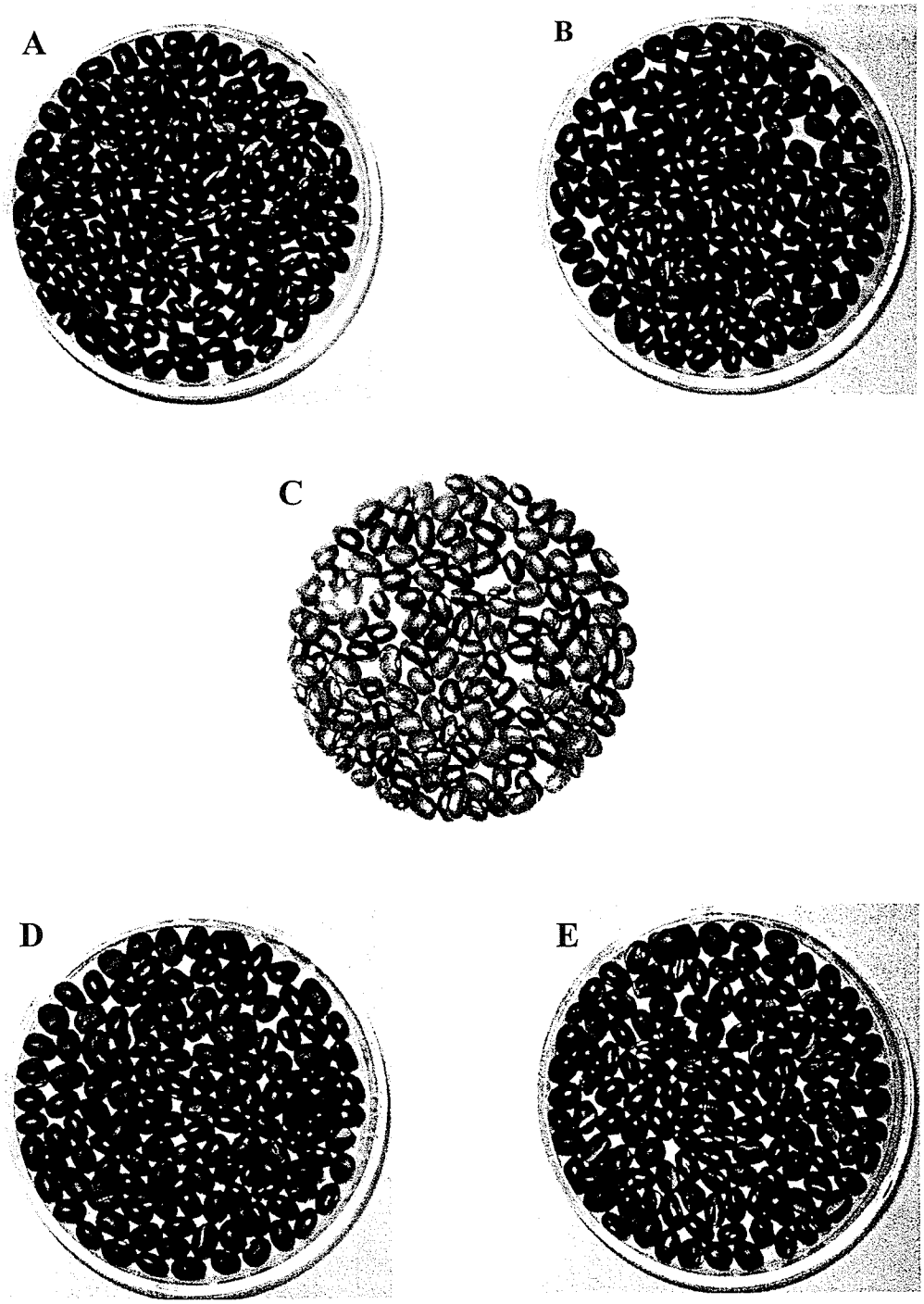


Figure 13. Physical appearance of micronized black beans tempered for 32h to 25.8% with various tempering solutions. (A) Distilled water, (B) 150 ppm of sodium EDTA, (C) unprocessed or control, (D) a mix of 1% citric acid and 2% ascorbic acid, and (E) a mix of 0.2% sodium tripolyphosphate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate.

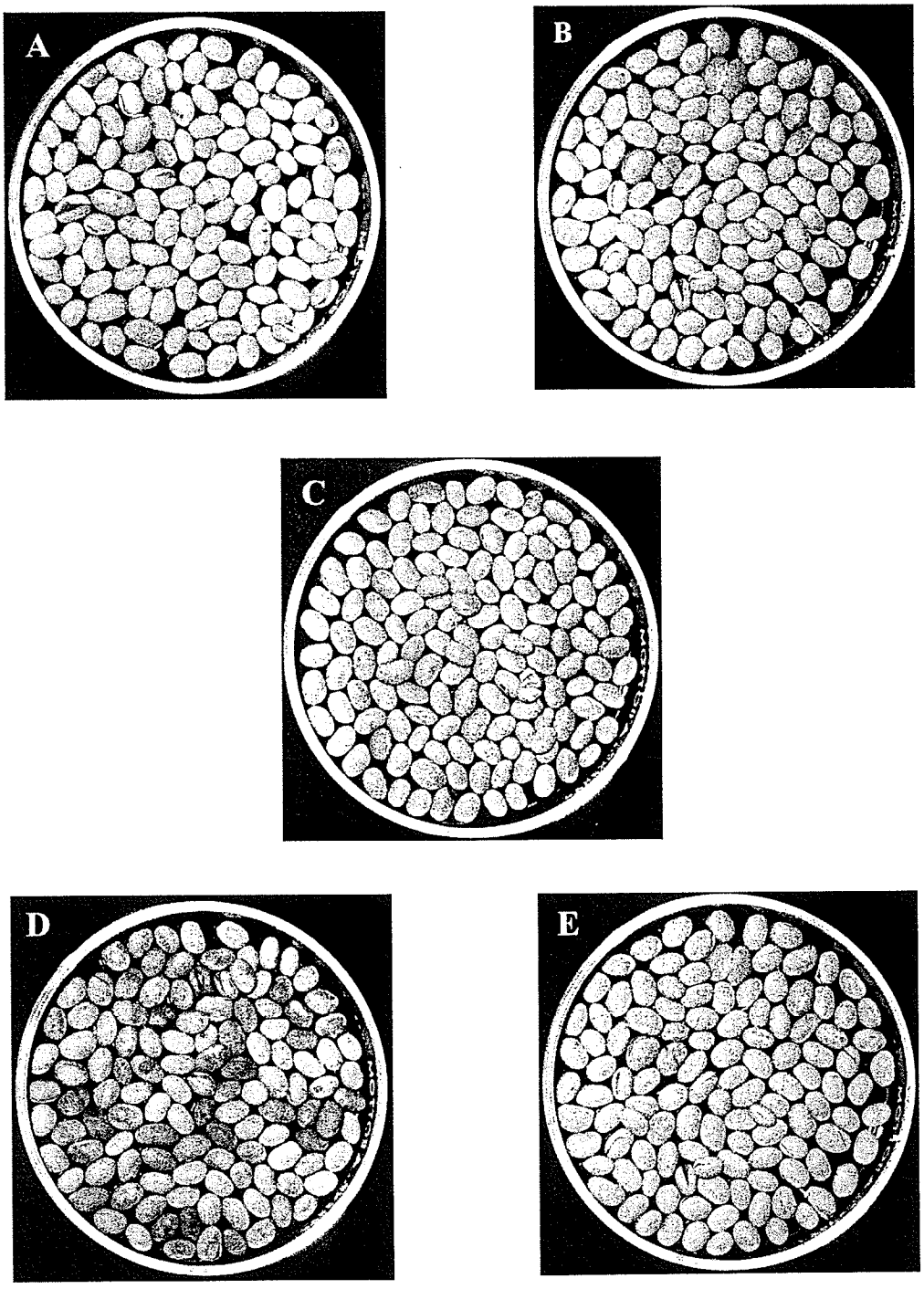


Figure 14. Physical appearance of micronized navy beans tempered for 11.5h to 28% with various tempering solutions. (A) Distilled water, (B) 150 ppm of sodium EDTA, (C) unprocessed or control, (D) a mix of 1% citric acid and 2% ascorbic acid, and (E) a mix of 0.2% sodium tripolyphosphate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate.

beans. Figure 14 illustrates the detrimental effects of this treatment on the colour of navy beans. Toews (2001) reported the development of a brown ring on the surface of micronized green peas after tempering in an acid solution identical to the one used in this study. McCurdy *et al.* (1983) also reported colour changes (less green and more yellow) between peas soaked in a solution containing citric acid and distilled water. Scanlon *et al.* (1998) did not report any changes in the physical appearance of lentils tempered to 20 and 40% with the same citric/ascorbic acid solution. Perhaps, the white colour of navy beans accentuated the detrimental effects of this treatment. The fact that black beans did not exhibit any significant ($P \leq 0.05$) colour changes when tempered with the acid treatment may support the view that initial colour is a factor. This may explain why the results of micronized lentils tempered with a citric/ascorbic acid solution in the study of Scanlon *et al.* (1998) did not exhibit a colour problem. Nevertheless, the present study does not discard the possibility that colour responses of micronized pulses tempered in acid solutions may also be controlled by chemical composition. No other significant ($P \leq 0.05$) changes were observed when other solutions were used to temper micronized navy beans.

As discussed in the present work, water experiment results indicated that optimum tempering conditions for navy and black beans, based on the degree of gelatinized starch, occurred at 28% moisture for 11.5h and 25.8% moisture for 32h, respectively. Using the optimum processing conditions, it was found that a mix of salts (0.2% sodium tripolyphosphate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate) was more effective than a mix of acids (1% citric acid and 2% ascorbic acid) or of 150 ppm of sodium EDTA, in reducing the hardness of micronized beans. As well, the acid solution was detrimental to the physical appearance of micronized navy beans.

Results also indicated that the micronization process significantly ($P \leq 0.05$) reduced the hardness value of navy beans by 28.7 % when tempered with distilled water, and also reduced the hardness of black beans by 13.7 % when tempered with an alkaline mixture of salts. Generally, a decrease in the percentage of soluble protein and an increase in the degree of gelatinized starch were significantly ($P \leq 0.05$) correlated with lower hardness scores in micronized beans.

Chapter 4: Effect of Micronization on Water Imbibition Rates of *Phaseolus Vulgaris* (Var. Navy and Black)

ABSTRACT

This investigation was conducted to study the effect of the micronization process on the water imbibition rates of both navy and black beans (*Phaseolus vulgaris*). The tempering solutions used as part of the micronization process included distilled water (pH=6.08) and an aqueous solution containing 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate termed 'alkaline treated' (pH=9.43). Results indicated that during the first 30 min of water-soaking (at 21 °C), micronized navy beans tempered with distilled water absorbed significantly ($P \leq 0.05$) more water than both unprocessed and alkaline-treated beans. Micronized black beans tempered with distilled water and the alkaline solution significantly ($P \leq 0.05$) imbibed more water than unprocessed beans for the first 60 min of experimentation. SEM microphotographs and porosity measurements indicated that micronization processing has the potential for generating air-bubbles in beans. The higher transportation rates of water through the air phase were thought to be responsible, at least in part, for the improved water imbibition characteristics of both navy and black bean after micronization. In addition, the apparent density of navy and black beans was negatively ($P \leq 0.05$) correlated to helium porosity (R^2 = -0.96 and R^2 = -0.99, respectively), and nitrogen porosity (R^2 = -0.94 and R^2 = -0.94, respectively) values, as well as with their degree of gelatinized starch (R^2 = -0.86 and R^2 = -0.88, respectively).

4.1. INTRODUCTION

There is little doubt that pulses subjected to the micronization treatment can become faster to cook. The cooking time of lentils, for example, was decreased by one-third, and for field peas by a half after micronization processing (Arntfield *et al.*, 1997; Toews, 2001). It is also clear that the tempering conditions prior to micronization, specifically tempering times and tempering levels, do play a major role in achieving desirable textural changes in the product (Abdul-Kadir *et al.*, 1990; Arntfield *et al.*, 1997; Scanlon *et al.*, 1998; Zhao, 2000; Toews, 2001). Moreover, the use of tempering solutions, especially those containing salts (e.g. a mix of carbonates and phosphate salts) has been found to positively affect the cooking quality of micronized lentils and peas (Scanlon *et al.*, 1998; Zhao, 2000; Toews, 2001). It still remains unclear as to what structural and compositional changes have an impact on the textural characteristics of cooked micronized pulses.

Several physicochemical changes have been reported to occur during micronization of pulses and legumes. The following physicochemical events have been found to shorten the cooking time of pulses: high levels of gelatinized starch, low percentages of soluble protein, and high percentages of soluble pectin (Arntfield *et al.*, 1997; Zhao, 2000; Toews, 2001; Arntfield *et al.*, 2001). Proteins, starches, and pectic substances have also been found to influence the cooking quality of unprocessed beans (Garcia-Vela and Stanley, 1989; Aguilera and Stanley, 1985; Jones and Boulter, 1983; Stanley and Aguilera, 1985; Hentges *et al.*, 1991, Aguilera and Rivera, 1992). Generally, it is well accepted that availability of water in the seeds is a prerequisite to quickly cook the interior of beans.

The ability of the seeds of pulses to imbibe water during normal cooking has been reported to influence their cooking times (Sefa-Dedeh and Stanley, 1979; Jackson and Varriano-Marston, 1981; Jones and Boulter, 1983). It has been reported that micronization influences product water imbibition. For example, it has been reported that micronization increased the water absorption index of maize germ (Kouzeh-Kanani *et al.*, 1984) and significantly increased the water hydration rates of peas (Cenkowski and Sosulski, 1998). Scanlon *et al.* (1999) found that micronization of durum wheat resulted in a product with improved maximum water absorption capacity. They also found that micronization caused an increase in porosity of up to 11% and a decrease in stiffness of up to 35%. They concluded that an increase in kernel porosity (i.e. incorporation of air-bubbles) was a prerequisite to produce quick-cooking micronized products since water diffusion rates are greater in the air phase (bubbles) than in the solid phase.

As a continuation of previous studies whereby quick-cooking beans were produced, the primary objective of this study was to evaluate the effects of micronization treatment and tempering solutions (distilled water or a mix of salts) on the water imbibition rates of navy and black beans. As well, the physical and physicochemical characteristics of micronized navy and black beans were evaluated with respect to their water imbibition characteristics.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Commercial grade navy and black beans were obtained from Roy Legumex Ltd (St. Jean-Baptiste, MB, Canada) and were grown in Manitoba during the 2000 crop year. Upon arrival, beans were stored at 5 °C in closed plastic containers (Trimeld

Rubbermaid®). Before experimentation, all extraneous materials were removed by hand after bringing beans to room temperature. For comparing the physical properties of micronized beans to those of unprocessed beans, this study did not use, when possible, processed beans that were either split or had cotyledons partially separated.

4.2.2. Tempering

For each run, navy and black beans (7.5 kg each) were divided into three equal parts and placed in 4.5 L Rubbermaid® containers. Beans were then tempered to 28% for 11.5h (navy beans) and 25.8% for 32h (black beans) using a predetermined amount of tempering solution (room temperature). Previous studies (section 3.4.1.) have shown that these tempering conditions favoured the beneficial effects of micronization in reducing the hardness of both navy and black beans. Tempering solutions included distilled water (pH=6.08) termed 'water-treated', and an aqueous solution containing 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate termed 'alkaline-treated' (pH=9.43) (Toews, 2001). Uniformity of water absorption during tempering was assured by tumbling the samples every 10 min for the first hour, and then once every hour for at least four hours (room temperature).

4.2.3. Micronization process

A pilot-scale MR2 micronizer unit (Micronizing Company Ltd., UK) fitted with a gas-fired infrared manifold and equipped with a vibrating stainless-steel conveyer trough and four ceramic IR burners was used to process the tempered beans. Using natural gas as a fuel, the MR2 micronizer is able to generate between 8.8 and 29.3 kW of power as specified by its manufacturer.

Micronization operational conditions were kept constant during processing of samples, and included a vibrating conveyer bed slope setting of 2.75 (equivalent to a

slope of 0.0130 radians), an air-gas mixture operational setting of 9, and distance between the conveyer trough and radiation source of 11.7 cm (Figure 2). The feed rate was kept constant at 0.67±0.03 kg/min using a setting of 60 in the vibratory feeder (Figure 2). The micronizer was warmed up for 15 min using a warm-up batch prior to an experimental run.

Soon after micronization processing, samples were spread out on a single layer on a stainless-steel table, and cooled to room temperature. Samples were then dried to 14.3 ± 1 % moisture using a convection oven set at 40 ± 5°C (to avoid protein and starch damage). Moisture removal was used so that the effects of the micronization process on bean density and porosity as compared to unprocessed beans (about 13.8 % m.c.) could be evaluated independently of the final moisture level. It is noteworthy that the present experiment intended to examine the effect of the micronization process (including the tempering pretreatment) and not the infrared heat treatment alone.

4.2.4. Density measurements

All density measurements were performed at room temperature. For each treatment, mean values of results on the subsamples (n=3 for the volume measurements using gases, n=4 for the bulk density, and n=10 for the apparent volume) were calculated.

4.2.4.1. Apparent density

The apparent density (ρ_{ap}) of beans was determined by dividing the sample's weight by the average apparent volume of beans (10 seeds) in that sample. A triaxial ellipsoid was used to calculate the apparent volume of a sample (n=10) by measuring the major, minor, and intermediate axes of individual seeds with a digital Mituyo calliper (Mituyo Corp., Japan) having accuracy to 0.02 mm. To estimate the error associated with the measurement of the major, minor, and intermediate axes, the combined percentage error

(CPE) was calculated as follows: CPE = ($\Delta V/V$) * 100%. The volume 'V' for a triaxial ellipsoid is: V = ($4\pi/3$) (abc); where 'a', 'b', and 'c' are the major, intermediate, and minor semi-axes, respectively. Further, 'ΔV' is the combined error in calculating volume or $\Delta V = [(\partial V/\partial a * \Delta a)^2 + (\partial V/\partial b * \Delta b)^2 + (\partial V/\partial c * \Delta c)^2]^{-1/2}$, where, for example, '$\partial V/\partial a * \Delta a$' is the error in the volume calculation due to the error in the measurement of the major semi-axis. $\Delta a = \Delta b = \Delta c = 0.02$ mm.

4.2.4.2. Bulk density

For calculating bulk density (ρ_B), seeds were placed in a metal funnel (minor diameter = 3.81 cm; major diameter = 22 cm; height = 16.5 cm), which had at its bottom a metal gate. The funnel was then placed at 3.81 cm on top of a 0.5 L weighing bucket (chrondrometer). After the funnel's gate was opened, the seeds freely fell into the weighing bucket. The surface of the weighing bucket was then levelled with a wooden rod in such a way as not to influence the packing of seeds. The weight of beans in the half-a-litre weighing bucket was used for determining bulk density (kg/m^3). This weight, also known as bushel weight or test weight in the grain industry, was calculated from the weight difference in the weighing bucket before and after sample loading. The bulk density of each treatment repetition was the mean of the subsample repetitions (in this case, n=4).

4.2.4.3. Particle density

In measuring the particle density (ρ_p) of beans, the volume of approximately 15 g of beans was estimated with a gas compression pycnometer (multi-volume pycnometer 1305, Micromeritics Instrument Corp., GA). Either helium or nitrogen was used as the displacement medium to measure the particle density of beans at a gas pressure of 140-170 kPa.

4.2.5. Porosity

The open pore porosity (ϵ_{op}) of micronized beans was expressed as a percentage and it was calculated from the following equation: ϵ_{op} (%) = [1 – (ρ_{ap} / ρ_p)]*100; where 'ρ_{ap}' is the apparent density of beans – density of beans including all pores in the material – and 'ρ_p' is the particle density – density of beans including the volume of all enclosed pores but not the externally connected pores. Hereafter, the open pore porosity will simply be referred to as porosity.

4.2.6. Water imbibition

Micronized bean samples (10 seeds) were placed in customized 5 x 5 x 5 cm metallic baskets (2 mm mesh). Each metallic basket was then placed into 1-litre beakers containing each 500 mL of distilled water at 21 ± 1°C. For determination of the water imbibition rates of beans, samples were retrieved from the soaking water, blotted dry in paper towel, and their weight (±0.001) recorded as 'drained weight'. The water imbibition capacity of soaked beans was measured every 10 min for the first hour and then every hour until samples reached constant weight. After each water imbibition measurement, samples were returned to the soaking water at which point, time was resumed. Without changing the soaking water, the weight of leached matter in 2 mL of the soaking water was found after evaporating to dryness at 100 °C for 24 h. The total leached solids after 2, 4, and 8 h of soaking was calculated by multiplying the dry matter weight in 2 mL of collected water by the total remaining water volume. Water imbibition was calculated as follows:

$$\text{Water imbibition (\%)} = \frac{[(\text{drained weight}) - (\text{initial weight} - \text{solids lost})]}{(\text{Initial dry weight} - \text{solids lost})} \times 100$$

4.2.7. Scanning electron microscopy

For scanning electron microscopy (SEM) studies, both raw and micronized navy and black beans were freeze-dried at -40 °C for 48 h. After drying, samples were fractured with a razor blade and mounted on aluminium stubs with carbon conductive paint. Then, samples were coated with gold/palladium and viewed in a Cambridge Stereoscan 120 Scanning Electron Microscope (Cambridge Instruments, Montreal, QC, Canada) at an accelerating voltage of 20 kV. The SEM images were recorded as digital images using an IBAS II Image Analyzer (Kontron Elektronic, Germany). For measuring the size of the intercellular spaces among cotyledonary cell walls, the SEM images were fully enlarged (13.6 x 13.6 cm) and the average diameter was calculated from the arithmetic mean of the maximum diameter of at least four intercellular spaces. The dimensions were measured by comparison with the image scale bar using a plastic ruler (±1 mm).

4.2.8. Experimental design

A complete randomized design was used to evaluate the effects of the micronization process on the physical and physicochemical properties of navy and black beans. Treatments included a treatment control (unprocessed beans), and two micronization processing treatments in which beans were tempered either with distilled water (pH=6.08) or with a solution containing 0.2% sodium bicarbonate, 0.1% sodium carbonate and 0.1% of sodium phosphate dihydrate or 'alkaline treatment' (pH=9.43). Navy and black beans were tempered to 28% moisture for 11.5 h and to 25.8% moisture for 32 h, respectively. Data were analysed by SAS (version 8.02, SAS Institute Inc, 2001, Cary, NC). The General Linear Model (GLM Model) was used for analysis of variance of all dependent variables (treatments = t = 3, repetitions = r = 2; t x r = 6 observations per dependent variable). Significant differences among treatment means

were compared using the conservative Scheffe's test (controls for the experiment-wise Type I error rate). Correlation analysis of the data was performed using Pearson correlation coefficient analysis of SAS.

4.3. RESULTS AND DISCUSSION

4.3.1. Density measurements

4.3.1.1. Apparent density

Tables 18 and 19 indicate that regardless of the tempering pretreatment, the mean minor-axes of micronized navy and black beans were significantly ($P \leq 0.05$) greater than the axes of the unprocessed beans. The minor axis of beans is also known as thickness since it is the height of beans when the seed's hilum is placed horizontally. The major and intermediate axes of micronized black beans receiving the alkaline treatment were significantly ($P \leq 0.05$) smaller than those of unprocessed beans. Significant differences in the physical dimensions of navy and black beans after micronization were an indication of volumetric changes of their seeds. The physical dimensions (major, minor, and intermediate axes) of beans provide valuable information in process optimization since the shape and size of beans are important attributes for establishing quality. A volumetric expansion of the seeds of kidney beans, black beans, lentils, and pinto beans has previously been reported to occur because of micronization (Fasina *et al.*, 1997). The consistently higher apparent volumes for micronized beans, compared to unprocessed beans (Table 18 and 19) indicated that micronization processing of beans resulted in a product with increased volume. In addition, it should be noted that the bean volumes, as measured by the geometrical method of approximation to a triaxial ellipsoid, exhibited little variation (CV up to 5%) among treatment repetitions (n=2), but showed greater

Table 18. Average physical dimensions[1] of both unprocessed[2] and micronized navy beans tempered for 11.5h to 28% moisture with either water or an alkaline solution[3] (n=2)

Tempering solution	Major axis[4] (mm)	Intermediate axis[4] (mm)	Minor axis[4] (mm)	Volume (mm^3)	CPE (%) [5]
Unprocessed	9.04 ± 0.51 [a]	6.18 ± 0.30 [a]	5.16 ± 0.44 [a]	151.4 ± 2.67	0.55
Distilled water	8.72 ± 0.50 [a]	6.29 ± 0.29 [a]	5.70 ± 0.22 [b]	163.9 ± 4.53	0.53
Salts	8.76 ± 0.44 [a]	6.31 ± 0.30 [a]	5.84 ± 0.32 [b]	169.6 ± 0.92	0.52

[1] Dimensions in a triaxial ellipsoid: mean ± standard deviation.
[2] raw navy beans.
[3] a mix of 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate.
[4] different letters in the same column indicate significant differences ($P \leq 0.05$).
[5] Combined percentage errors (CPE) due to the measure of the major, minor, and intermediate semi-axes of individual seeds with a digital calliper (± 0.02 mm).

Table 19. Average physical dimensions [1] of both unprocessed [2] and micronized black beans tempered for 32h to 25.8% moisture with either water or an alkaline solution[3] (n=2)

Tempering solution	Major axis[4] (mm)	Intermediate axis[4] (mm)	Minor axis[4] (mm)	Volume (mm^3)	CPE (%) [5]
Unprocessed	9.38 ± 0.33 [b]	6.40 ± 0.34 [b]	5.14 ± 0.39 [a]	159.1 ± 1.00	0.54
Distilled water	9.14 ± 0.46 [ab]	6.36 ± 0.21 [ab]	5.64 ± 0.29 [b]	171.8 ± 5.23	0.52
Salts	8.95 ± 0.46 [a]	6.15 ± 0.33 [a]	5.57 ± 0.28 [b]	163.0 ± 1.48	0.53

[1] Dimensions in a triaxial ellipsoid: mean ± standard deviation.
[2] raw black beans.
[3] a mix of 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate.
[4] different letters in the same column indicate significant differences ($P \leq 0.05$).
[5] Combined percentage errors (CPE) due to the measure of the major, minor, and intermediate semi-axes of individual seeds with a digital calliper (± 0.02 mm).

variability within subsamples (CV up to 12%). Using the same technique, Fasina *et al.* (1997) reported a coefficient of variability of 15% for both unprocessed and micronized black beans. These results suggested that beans are quite variable plant materials, and natural variability in beans should not be overlooked when interpreting results.

The apparent densities of both unprocessed and micronized navy beans were not different ($P \leq 0.05$) one from another (Table 20). Conversely, micronized black beans preconditioned with distilled water showed a lower ($P \leq 0.05$) apparent density than both unprocessed and micronized black beans tempered with the alkaline solution (Table 21). Furthermore, the combined percentage errors (CPE) due to the measurement of the beans semi-axes were very low, below 0.6% (Table 18 and 19). The method used to measure bean apparent density may therefore be adequate to monitor apparent volumetric changes that are thought to take place because of micronization.

Table 20. Density[1] of both unprocessed and micronized navy beans tempered for 11.5h to 28% moisture with water and a solution containing salts[2]

Tempering solution	[3]Apparent density (kg/m^3)	[3] Bulk density (kg/m^3)	[3] Helium density (kg/m^3)	[3] Nitrogen density (kg/m^3)
Unprocessed	1427 ± 24^{a}	804 ± 0.4^{a}	1427 ± 1.4^{a}	1425 ± 0.5^{a}
Distilled water	1354 ± 16^{a}	716 ± 0.8^{b}	1423 ± 2.1^{a}	1417 ± 7^{a}
Salts	1363 ± 30^{a}	694 ± 1.1^{c}	1413 ± 2.1^{b}	1410 ± 2.5^{b}

[1] mean ± SD.
[2] a mix of 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate.
[3] different letters in the same column indicate significant differences ($P \leq 0.05$).

Table 21. Density[1] of both unprocessed and micronized black beans tempered for 32h to 25.8% moisture with water and a solution containing salts[2]

Tempering solution	[3] Apparent density (kg/m^3)	[3] Bulk density (kg/m^3)	[3] Helium density (kg/m^3)	[3] Nitrogen density (kg/m^3)
Unprocessed	1415 ± 14 [a]	796 ± 2 [a]	1443 ± 0.7 [a]	1438 ± 1 [a]
Distilled water	1249 ± 11 [b]	689 ± 9 [b]	1432 ± 0.5 [b]	1430 ± 1 [b]
Salts	1373 ± 15 [a]	694 ± 2 [b]	1439 ± 0.5 [a]	1426 ± 2 [b]

[1] mean ± SD.
[2] a mix of 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate.
[3] different letters in the same column indicate significant differences ($P \leq 0.05$).

The correlation analyses in Tables 22 and 23 showed that the apparent density of both navy and black beans was highly correlated ($P \leq 0.01$) with bean porosity (helium and nitrogen). This negative correlation indicated that treatments that resulted in more porous beans also resulted in beans with low apparent densities. This, in turn, could also be translated into beans with higher apparent volumes (assuming constant final moisture contents for micronized beans). This is not surprising since the apparent volume of beans and porosity of durum wheat kernels have previously been reported to increase after micronization (Fasina *et al.*, 1997; Scanlon *et al.*, 1999). Further, apparent densitywas significantly correlated ($P \leq 0.05$) with the degree of gelatinized starch of both navy and black beans. This correlation indicated that processed beans that had high degrees of gelatinized starch had lower apparent densities. Perhaps the combined effect of water conditioning and the high radiation energy absorbed by the product during micronization is not only responsible for promoting physicochemical changes but also for physically opening both the macrostructure and microstructure of beans.

Table 22. The correlation coefficients [1] between characteristics of micronized navy beans (n=8)

	Apparent density (kg/m3)	Particle density-helium (kg/m3)	Particle density-nitrogen (kg/m3)	Porosity-helium (kg/m3)	Porosity-nitrogen (kg/m3)	Gelatinized Starch (%)
Apparent density	1	n.s.	n.s.	-0.96***	-0.94***	-0.86**
Particle density-helium		1	0.95***	n.s.	n.s.	n.s.
Particle density-nitrogen			1	n.s.	n.s.	n.s.
Porosity-helium				1	0.99***	0.78*
Porosity-nitrogen					1	0.73*
Gelatinized starch (%)						1

[1] n.s. indicates not significance.
* indicates significant at p ≤0.10.
** indicates significant at p ≤0.05.
*** indicates significant at p ≤0.01.

Table 23. The correlation coefficients [1] between characteristics of micronized black beans (n=8)

	Apparent density (kg/m^3)	Particle density-helium (kg/m^3)	Particle density-nitrogen (kg/m^3)	Porosity-helium (kg/m^3)	Porosity-nitrogen (kg/m^3)	Gelatinized starch (%)
Apparent density	1	0.94***	n.s.	-0.99***	-0.94**	-0.88**
Particle density-helium		1	n.s.	-0.94***	-0.94***	-0.85**
Particle density-nitrogen			1	n.s.	n.s.	-0.75*
Porosity-helium				1	0.99***	0.88**
Porosity-nitrogen					1	0.85**
Gelatinized starch (%)						1

[1] n.s. indicates not significance, * indicates significant at $p \leq 0.1$, ** indicates significant at $p \leq 0.05$, and *** indicates significant at $p \leq 0.01$.

4.3.1.2. Bulk density

The micronization process significantly ($P \leq 0.05$) reduced the bulk density values of both navy and black beans compared to that of unprocessed beans (Tables 20 and 21). Bulk density has been used as a quality index for grading grains since healthy and fully mature grains have a characteristic bulk density. Factors that commonly reduce bulk density are insect infestation, excessive foreign matter and high percentage moisture content. Based on this approach, lower bulk density values for micronized beans compared to that of raw beans would suggest that micronization processing was

detrimental to the quality of beans. In the present studies, however, bean samples were almost free of extraneous materials, and the moisture content of micronized beans was essentially the same as that of unprocessed materials. Thus, the bulk density values of processed beans are consistent with the apparent density results in that they reflected a change in the seed size and seed shape as measured by the lower degree of compactness when beans were packed as a bulk (Table 20). The bulk density of micronized navy beans preconditioned with the alkaline solution had a significantly ($P \leq 0.05$) lower bulk density than both unprocessed beans and water-tempered micronized beans. This suggested that the addition of a mix of carbonates and phosphates to the tempering solution may further open the seed structure of beans, perhaps by enhancing denaturation of cotyledonary proteins (Garcia-Vela *et al.*, 1989; del Valle *et al.*, 1992 b) and thus loosening protein bodies tightly arranged in the cells of unprocessed beans.

Overall, the lower degree of compactness of both micronized navy and black beans indicated that, generally, the bean size was increased and the shape altered after micronization processing. Changes in the size and shape may reflect the nature of the micronization process in which beans are first hydrated during tempering and then dehydrated by means of an intense-heat drying process. Unlike conventional drying techniques though, micronization could make the microstructure of beans more porous (formation of air-bubbles) due to rapid vaporization of superheated steam at weak points of the intercellular bean structure (Arntfield *et al.*, 1997; Scanlon *et al.*, 1999; Arntfield *et al.*, 2001). These views are further discussed in the following sections.

4.3.1.3. Particle density

For navy beans tempered with the alkaline solution, micronization significantly ($P \leq 0.05$) reduced particle density using both the helium and nitrogen as displacement

fluids in a gas pycnometer (Table 20). These results are consistent in demonstrating that particle density is not only influenced by the micronization process itself, but also by the tempering reagents during conditioning of samples.

Conversely, for black beans tempered with both distilled water and the alkaline solution, micronization significantly ($P \leq 0.05$) reduced density (Table 21) based on nitrogen density but only water-tempered samples had reduced density when using helium. Helium and nitrogen greatly differ in their molecular size. Since nitrogen gas has a molecule approximately fourteen times greater than that of helium, it was expected that, at similar measuring conditions, helium could penetrate to small spaces that, at least in theory, were inaccessible to nitrogen. Although this could explain why the helium density of micronized black beans was greater than their nitrogen density, differences in density of this magnitude could also be ascribed to variability within this cultivar.

The higher particle density values for unprocessed black (1438 kg/m^3) than for navy (1425 kg/m^3) beans may reflect the thicker and microstructurally denser seed coats of black beans (Agbo *et al.*, 1987). Density of the cotyledons of black and navy beans may also have accounted for part of the particle density variation. Differences in seed coat water permeability have been shown to affect processing conditions. A previous experiment (Chapter III) found that the thicker seed coats of black beans were responsible, at least in part, for the long tempering times necessary to fully absorb the tempering water.

4.3.2. Porosity

Porosity increased after micronization processing of black beans tempered with distilled water only (Table 24). Micronized black beans tempered with distilled water exhibited the greatest porosity values (helium = 13.0 %; nitrogen = 12.6 %). In this

Table 24. The porosity of both unprocessed and micronized[1,2] navy and black beans tempered with either distilled water alone or a salt solution (a mix of 0.2% sodium bicarbonate, 0.1% sodium carbonate, and 0.1% of sodium phosphate dihydrate).

Tempering solution	Variety	Bean porosity (%) [4]	
		Helium [5]	Nitrogen [6]
Unprocessed [3]	Navy	0.63 [a]	0.10 [A]
	Black	1.91 [w]	1.60 [m]
Distilled water	Navy	4.81 [a]	4.54 [A]
	Black	13.0 [y]	12.6 [n]
Alkaline	Navy	3.51 [a]	3.29 [A]
	Black	4.56 [w]	3.72 [m]

[1] Micronized navy beans were tempered for 11.5 h to 28% moisture.
[2] Micronized black beans were tempered for 32 h to 25.8%.
[3] Raw beans.
[4] different letters in the same column indicate significant difference ($P \leq 0.05$).
[5,6] Type of gas used in the gas pycnometer to calculate the particle density of beans that was used for determining bean porosity.

study, porosity or open pore porosity is the ratio of the volume of pores connected to the outside to the total bean volume. The pressurized (140-170 kPa) helium and nitrogen gases and the equilibration times (about 2 min) used during particle density measurement, assured gas penetration to practically every possible open pore. Thus, an increase in porosity suggested that micronization could indeed generate air-bubbles in processed beans.

Tables 22 and 23 indicate that helium and nitrogen measured the same type of porosity, as evidenced by their high correlation coefficient (R^2=0.99). The significant correlations between the degree of starch gelatinization and the porosity of micronized navy ($P \leq 0.10$) and black ($P \leq 0.05$) beans suggested that open pore porosity could also be used as a good indicator of product texture. Tables 22 and 23, however, indicated that

the helium density of black beans only was significantly ($P \leq 0.05$) correlated with porosity. Open pore porosity can be strongly affected by the ability of the helium and nitrogen molecules to penetrate externally connected bean capillaries (void spaces). The porosity values of micronized both navy and black beans were marginally greater when helium was used as the displacement fluid, compared to nitrogen. As mentioned before (Section 4.3.1.), this difference could have arisen from the differences between helium and nitrogen molecule sizes. In addition, there was uncertainty on whether all the possible closed pores formed during processing of beans were equally inaccessible to the compressed gases, which could also explain why particle density was not a good indicator for measuring the overall impact of the micronization process on the physical structure of beans. The water imbibition characteristics of beans as well as their microstructure may provide a more comprehensive idea of what changes beans might undergo during the micronization process.

4.3.4. Water imbibition

Figures 15 and 16 show the combined effect of micronization and pretreatments on the water imbibition rates of navy and black beans, respectively. Figure 15 indicated that the water imbibition rates for micronized salt-treated navy beans were relatively similar to that for the unprocessed beans. This was the case for soaking times of up to 125 min. After this time, micronized salt-treated navy beans imbibed water at a slower rate, until they reached a water absorption equilibrium value of 1.02 g H_2O/g dry matter. Conversely, unprocessed samples kept imbibing water to a maximum level of 1.23 g water/g dry matter, after 520 min of soaking. By comparison, during the first 30 min, the beans tempered with distilled water imbibed significantly ($P \leq 0.05$) more water than both unprocessed and salt-treated micronized beans. For example, micronized navy

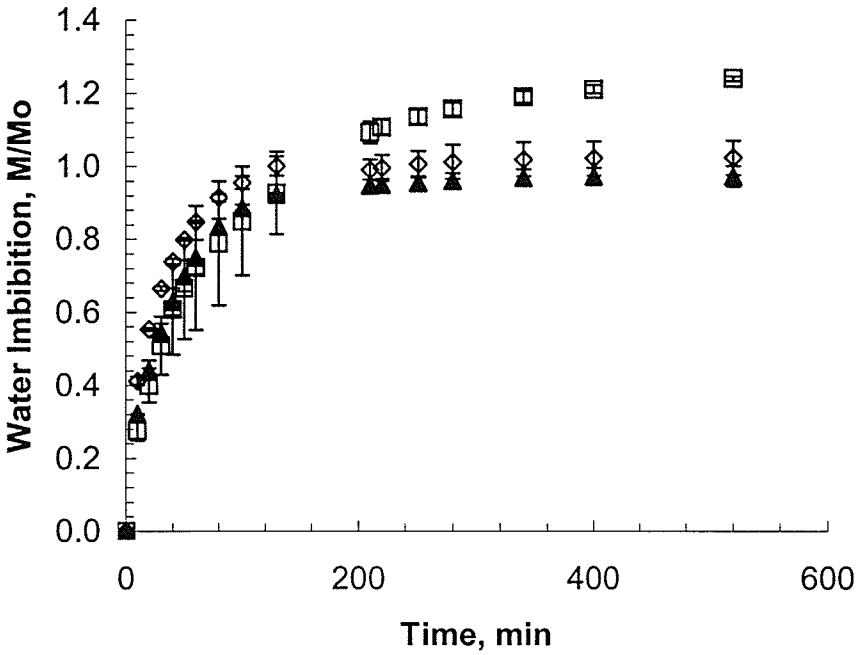


Figure 15. Water imbibed as a function of initial dry bean weight (Mo) for raw (□) and micronized navy beans tempered with distilled water (◊) and an alkaline solution (▲). Micronized navy beans were tempered for 11.5 h to 28 % moisture. Error bars indicate standard deviation of duplicates.

beans tempered with distilled water imbibed 46, 38 and 31% more water than unprocessed beans after soaking times of 10, 20 and 30 min. With black beans, on the other hand, micronization improved the water imbibition rates when tempered with both distilled water and with the alkaline solution. Unlike navy beans, this was the case for the first 480 min of soaking. After this time, micronized black beans tempered with distilled water reached their maximum water absorption capacity (1.16 g water/g dry matter). Micronized beans tempered with the salt solution and unprocessed black beans continued imbibing water to a maximum level of 1.28 and 1.36 g water/g dry matter, respectively. These findings indicated that micronized beans had higher water imbibition

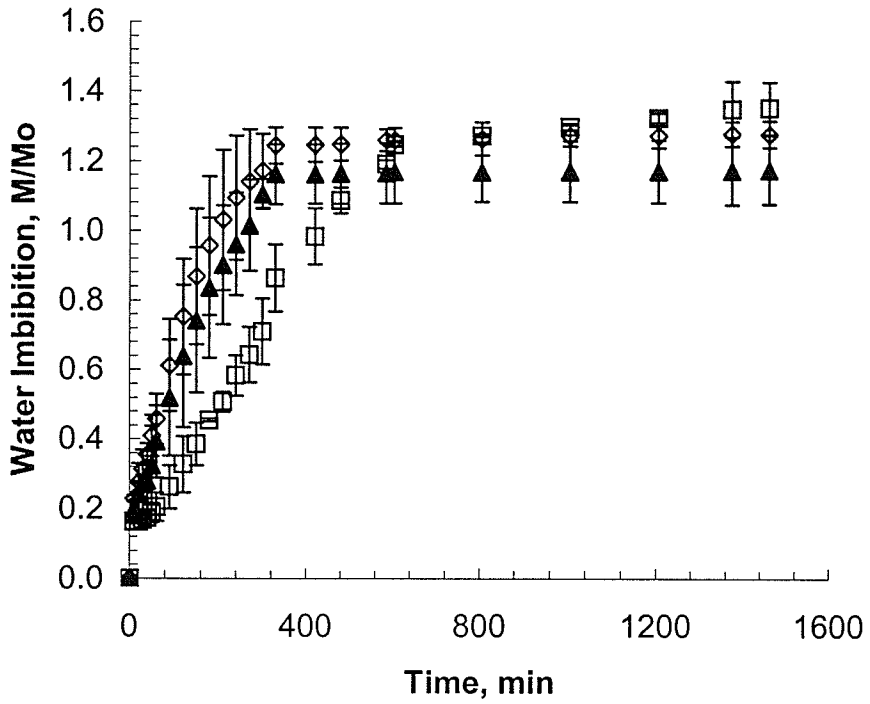


Figure 16. Water imbibed as a function of initial dry bean weight (Mo) for raw (□) and micronized black beans tempered with distilled water (▲) and an alkaline solution (◊). Micronized black beans were tempered for 32 h to 25.8 % moisture. Error bars indicate standard deviation of duplicates.

rates than unprocessed beans, in the linear portion of the water imbibition curves. For example, micronized black beans tempered with distilled water absorbed 44, 88, 100, 100, 116 and 124% more water per gram of dry matter than unprocessed beans after 10, 20, 30, 40, 50 and 60 min of soaking, respectively. Similarly, micronized black beans tempered with the alkaline solution absorbed 44, 75, 83, 112, 116 and 119% more water per gram of dry matter than unprocessed beans after 10, 20, 30, 40, 50 and 60 min of soaking, respectively. The first thirty minutes and first sixty minutes of soaking of navy and black beans, respectively, will also be referred from now as 'initial water imbibition' of navy and black beans, respectively. As well, during this first hour of soaking,

micronized black beans tempered with water and with the alkaline solution imbibed significantly ($P \leq 0.05$) higher amounts of water than raw beans. The higher water transportation rates found at the initial stages of water absorption is an indication that the water diffusion through the seed was facilitated, possibly due to both physicochemical and physical changes of the seeds of micronized beans (Scanlon *et al.*, 1999).

Figures 15 and 16 indicated that cultivar influenced initial water imbibition rates since navy beans imbibed water more rapidly than black beans. Several investigations (Deshpande and Cheryan, 1986; Agbo *et al.*, 1987; del Valle *et al.*, 1992 a) have indicated that white-coloured bean varieties imbibed water more rapidly than dark-coloured seeds. This phenomenon could be due to the denser arrangement of seed coat cells in dark-coloured seeds (Powell, 1989) or as result of oxidative reactions of phenolic substrates resulting in hydrophobic substances (Hincks and Stanley, 1986; Hohlberg and Stanley, 1987; del Valle *et al.*, 1992 a; Stanley, 1992). As well, cultivar influenced both the water holding capacity and the response to the alkaline tempering solution. For example, the use of the alkaline tempering solution increased the water absorption at equilibrium to black beans, while had the opposite effect on navy beans. More specifically, the water holding capacity in unprocessed, water-treated and salt-treated micronized beans was smaller for navy (1.23, 1.03, and 0.93 g water/g dry matter, respectively) than for black (1.36, 1.16, and 1.28 g water/g dry matter, respectively) beans.

Since seed composition and microstructure have previously been found to influence water imbibition rates (Agbo *et al.*, 1987), the degree of gelatinized starch and the percentage of soluble protein data previously studied in this investigation (Chapter III) were incorporated in this section to broaden the scope of the present discussion. The

degree of gelatinized starch in micronized navy beans tempered with water (24 %) and tempered with the alkaline (19.2 %) solutions were not significantly ($P \leq 0.05$) different (Table 11). These two samples, however, had greater levels of gelatinized starch than unprocessed navy beans (below 2 %). The water imbibition curves in Figure 15, however, indicated that at the initial stages of soaking, unprocessed and micronized navy beans tempered with the alkaline solution had relatively the same water imbibition rates, whereas micronized navy beans tempered with distilled water had significantly higher imbibition rates. These results suggested that the water imbibition rates of navy beans were influenced by factors other than the degree of gelatinized starch. For black beans, however, the significantly ($P \leq 0.05$) higher levels of gelatinized starch for beans tempered in water or the alkaline solution appeared to influence the water imbibition responses (at least for the first 2 h) since they also exhibited a significantly ($P \leq 0.05$) higher water imbibition rates than unprocessed beans. From these results, it is possible that for some beans starch gelatinization affects water imbibition or factors affecting starch gelatinization also affect water imbibition.

Micronized navy beans tempered with water and the alkaline solution did not have significantly ($P \leq 0.05$) different levels of soluble protein (Table 14). These two samples, however, had lower levels of soluble protein than unprocessed navy beans (Appendix I). The effect that the experimental treatments had on navy beans in terms of the percentage of soluble protein (water $\approx$ salts < unprocessed) was similar to the response on water holding capacity (water $\approx$ salts < unprocessed). By comparison, the percentage of soluble protein in black beans (water < salts < unprocessed) that received the experimental treatments showed a similar response in their water holding capacity

(water < salts < unprocessed). Sefa-Dedeh and Stanley (1979) reported that protein content controlled water imbibition kinetics at the later stages of soaking, with high protein beans having higher water absorption capacities. Abdel-Kader (1995) found that there was no significant correlation between the total protein content and the total absorbed water of unprocessed faba beans. For micronized beans, however, the conformational changes that storage proteins have undergone during micronization may render them more hydrophobic. Zheng *et al.* (1998) suggested that the loss of solubility of micronized legume proteins could be the result of hydrophobic aggregation of polypeptide chains. Therefore, it does appear reasonable to think that a reduction in the water holding capacity of beans following micronization is associated with the reduced percentages of soluble protein. Nevertheless, compositional changes give only partial information on the complex structural changes that beans undergo during micronization processing. The water holding capacity of beans, for example, is influenced not only by the protein and state of the starch matrix of the cotyledons but also by anatomical organelles and their physical dimensions i.e. seed coat thickness or size of raphe, hilum and micropyle (Powrie *et al.*, 1960; Sefa-Dedeh and Stanley, 1979).

The physical structure of beans is known to play an important role during water imbibition (Deshpande and Cheryan, 1986; Agbo *et al.*, 1987), especially at the initial stages of water uptake (Sefa-Dedeh *et al.*, 1979). Scanlon *et al.* (1999) suggested that because of the high processing temperatures and high tempering regimes, it is likely that air-bubbles are developed within the structure of micronized pulses and grains. It was hypothesised that air-bubbles were created due to internal venting of superheated steam at weak points of the cell structure, principally in the intercellular spaces. Soaking controls hydration of beans by the diffusion process. Such a mechanism could explain

the higher water imbibition rates for both navy and micronized beans, especially at the initial stages of water imbibition.

SEM microphotographs of all samples were taken to afford a better understanding of the microstructural changes that seeds undergo during processing. Figure 17 gave evidence that micronization has the potential for inducing air-bubble formation in the structure of beans. SEM microphotographs showed almost negligible air spaces at the interstitial joints of the cotyledonary cell walls of unprocessed navy (Figure 17A) and black (Figure 17B) beans. Contrarily, micronized navy (Figures 17C and 17E) and black beans (Figures 17D and 17F) exhibited distinguishable pores, or bubbles, specifically in the intercellular spaces. Micronized beans tempered with distilled water had pore sizes of 7.8-10 μm (navy beans) and 7.7-8.8 μm (black beans). Interestingly, addition of a mixture of salts to the tempering solution resulted in micronized beans with pore sizes of 4.3-9.8 μm (navy beans) and 8.2-14.3 μm (black beans). Generally, these results supported the lower particle density of both micronized navy and black beans, compared to unprocessed beans (Tables 20 and 21). Results shown in Table 24 indicated that the micronization process significantly ($P \leq 0.05$) increased the porosity of black beans tempered with water only. These results, however, did not reflect the findings from SEM analysis indicating that black beans tempered with the salt solution had the highest pore sizes (8.2-14.3 μm). For micronized navy beans, the use of a mixture of salts as a tempering pretreatment did not improve porosity (Table 24), compared to water-treated beans. The pore sizes of micronized navy beans tempered with either water or the salt solutions was relatively the same (salts = 4.3-9.8 μm; water = 7.8-10 μm), but greater than that of unprocessed beans (almost negligible). Nonetheless, average pore size measurements gave an indication that air-bubbles could,

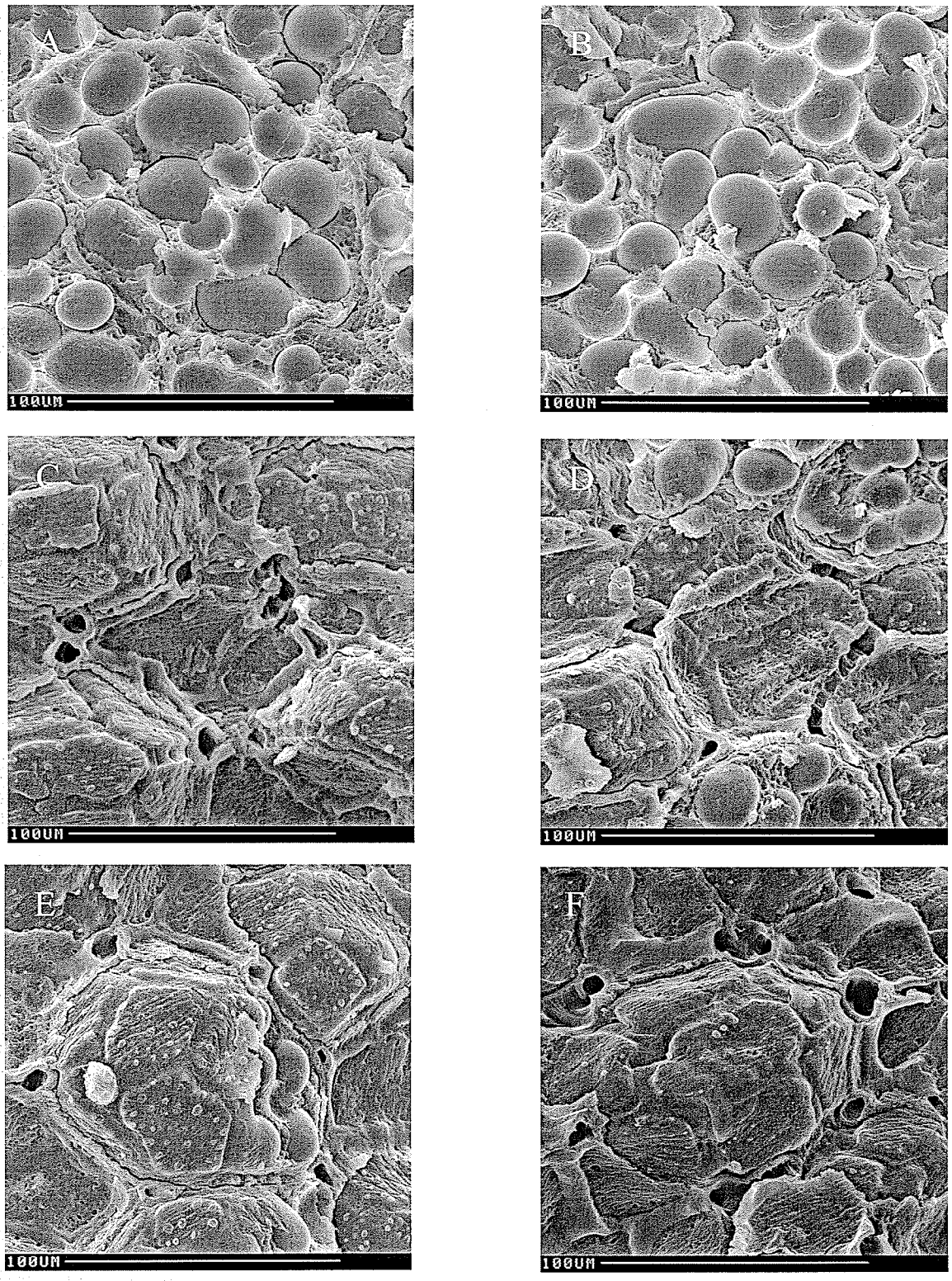


Figure 17. SEM microphotographs of navy beans: unprocessed (A), micronized tempered with distilled water (C), and micronized tempered with salts (E); and black beans: unprocessed (B), micronized tempered with distilled water (D), micronized tempered with salts (F).

indeed, be produced in micronized beans. The porosity of both unprocessed and micronized beans were calculated in the present study to establish how physical changes influenced their water imbibition characteristics. This is in accord with the view that not all intercellular spaces were accessible to the displacement fluids used in this study (i.e. helium and nitrogen) despite their low molecular weights and the relatively long times they had to penetrate the testing material (beans). Further, if the water present in the intercellular spaces prior to micronization (after tempering) were superheated as theorized by Scanlon *et al.* (1999), steam (superheated) would create void paths (capillary pores) before leaving the solid structure of beans. These potential open pores, however, were totally or partially closed due to the viscoelastic nature of plant materials. These could partly explain the poor correlations coefficients between the particle density and porosity of unprocessed and processed beans, except for the helium density of micronized black beans tempered with salts.

On the other hand, the hydration of beans is controlled during soaking through the diffusion process (Aguilera and Stanley, 1995). Since water diffusion rates are greater in the air phase (bubbles) than in the solid phase, it is expected that higher water absorption rates, arising from the incorporation of void spaces in micronized beans, would result in a product that is easier to cook. Although, this experiment was carried out at 21 °C, water transportation phenomena theory would indicate that boiling temperatures should increase the water transportation rates during cooking. Theoretical work based on Fick's second law of diffusion could be used to illustrate water diffusion through seeds during water imbibition experiments. Fick's equation for steady state diffusion can be written as follows,

$$\partial X/\partial t = D * (\partial^2 X/\partial r^2)$$

where X is the moisture content (kg water/kg dry solids), r is the diffusion path (m), t is time (s), and D is the overall diffusivity coefficient (m^2/s) (Crank, 1975). From the equation, it is evident that the higher the D is, the faster water would migrate to the seed interior. In addition, the Einstein relationship could be used to estimate the effect of soaking medium temperature on D. The Einstein relationship can be expressed as follows: $D_T = D_{294} * (T/294) * (\eta_{294}/ \eta_T)$; where T is temperature (K), η_T is dynamic viscosity at temperature T (Pa.s), and D_T is the overall diffusion coefficient at temperature T (m^2/s). The viscosity of water at 21 °C is about 1 mPa.s, while at 100 °C (boiling temperature at atmospheric pressure) is 0.2838 mPa.s (Bourne, 1982). Based on the aforementioned equation, the effect of increasing the temperature of the soaking medium from room to boiling temperature on the overall water diffusivity coefficient thorough beans can be obtained. It was found that D_{373} should be about four times greater than D_{294} or better $D_{373} = 4.47 * D_{294}$. Thus, suggesting that as temperature increases (i.e. 21 to 100 °C) so does the overall diffusion coefficient D. Consequently, higher temperatures during soaking (i.e. cooking) should result in a higher D or, in other words, higher water transportation rates (to the beans' interior) during cooking. The rate of water absorption in faba beans has also been reported to increase with temperature (Abdel-Kader, 1995).

In addition, SEM microphotographs and porosity results suggested that micronization processing has the potential for generating air-bubbles in beans. Structure dictates to a large extent the water imbibition characteristics of beans, particularly during the initial stages of water imbibition process. Since the transportation rates of water are higher through the air phase than through the solid phase, it is safe to assume that the initial imbibition rates for processed beans were influenced by the number and size of

bubbles engendered in the seeds during micronization processing. Other seed components (e.g. hilum, raphe, seed coat) are also known to influence water imbibition characteristics of beans (Agbo *et al.*, 1987). However, the individual contribution of each of these components to the overall water imbibition characteristics of micronized beans exceeds the scope of this project. For navy beans, the effects ($P \leq 0.05$) of micronization and pretreatments on hardness (water-treated $\approx$ salt-treated $<$ unprocessed), degree of gelatinized starch (water-treated $\approx$salt-treated $<$ unprocessed), and percentage of soluble protein (water-treated $\approx$salt-treated $<$ unprocessed) (Figure 11, and Tables 11 and 14) differed from their effect on physical characteristics. For example, the initial water imbibition rates (water-treated $>$ salt-treated $\approx$unprocessed) and porosity values (water-treated $\approx$salt-treated $\approx$unprocessed) (Figure 15 and Table 24) suggested that water-treated micronized navy beans should have better cooking quality than salt-treated beans. Perhaps, the influence of physical characteristics on the textural quality of micronized navy beans was not as important as that arisen from changes in navy bean chemical composition. For black beans, on the other hand, the effects of micronization and pretreatments on hardness (unprocessed $>$ water-treated $>$ salt-treated), degree of starch gelatinization (unprocessed $<$ salt-treated $<$ water-treated), and percentage of soluble protein (unprocessed $>$ salt-treated $>$ water-treated) were quite different than for navy beans in that higher degrees of gelatinized starch and lower percentages of soluble proteins did not necessarily translate into lower hardness scores (Figure 12, Tables 11 and 14). The effects ($P \leq 0.05$) of micronization and pretreatments on black bean initial water imbibition rates (unprocessed $<$ water-treated $\approx$salt-treated) and porosity values (unprocessed $\approx$ salt-treated $<$ water-treated) indicated that low hardness values were associated with high porosity values and high initial water

imbibition rates. Therefore, the physical characteristics of micronized black beans partially influenced the textural quality of black beans since chemical characteristics partially explained the textural behaviour of black beans. Those chemical changes, however, were not totally monitored in this investigation. For example, changes in the solubility of pectic substances could explain textural behaviour of beans since it has previously been pointed out as a factor influencing the texture of micronized lentils (Arntfield *et al.*, 1997; Arntfield *et al.*, 2001) and peas (Toews, 2001).

Results from the present study indicated that during the first 30 min of water-soaking (at 21 °C), micronized navy beans tempered with distilled water absorbed significantly ($P \leq 0.05$) more water than both unprocessed and alkaline-treated beans. Micronized black beans tempered with distilled water and the alkaline solution significantly ($P \leq 0.05$) imbibed more water than unprocessed beans for the first 60 min of experimentation. SEM microphotographs and porosity measurements indicated that micronization processing could result in beans with a more porous structure due to an increase in the size of their intercellular spaces. Micronized beans could benefit from porosities by improving their water hydration rates during cooking, compared to unprocessed beans. In addition, the apparent density of navy and black beans was negatively ($P \leq 0.05$) correlated to helium porosity (R^2 = -0.96 and R^2 = -0.99, respectively), and nitrogen porosity (R^2 = -0.94 and R^2 = -0.94, respectively) values, as well as with their degree of gelatinized starch (R^2 = -0.86 and R^2 = -0.88, respectively).

GENERAL DISCUSSION AND CONCLUSIONS

The first experiment of this investigation (Chapter 2) was undertaken to optimize the effect of processing conditions of a pilot-scale MR2 micronizer unit on micronized product attributes of navy beans. These attributes were product temperature at micronizer outlet, hardness, final moisture content, percent of soluble protein, hydration capacity, and density. Processing conditions included the slope of the conveyer bed (SL), instrumental setting of a gas-air mixture valve (GL), and the distance between the conveyer bed and the infrared radiation source (DL). As the extent to which SL and GL increased, so did the final temperature of the micronized navy beans. Bean moisture content was reduced as the extent to which SL and GL increased. The percent of soluble protein and hydration capacity of the micronized navy beans were reduced as SL increased. Overall, results indicated that SL and GL, but not DL, made significant ($P \leq 0.05$) contributions to the optimization of micronization processing conditions by controlling the rate and extent of the micronization process. Optimum micronization operational conditions occurred at GL=9 and SL=5. It is noteworthy that the principle of ceteris paribus (all other factors were kept constant) was applied for other micronization operational parameters throughout the course of this investigation. For example, the feed rate controller was set to 60 (approx. 0.67 ± 0.03 kg/min) and the distance of the feed gate gap was set in such a way that allowed a single layer of water-conditioned beans to be fed into the vibratory conveyer trough (approx. 5 mm).

The objective of the second experiment (Chapter 3) was twofold. Firstly, the optimum operational conditions given by the previous experiment (Chapter 2) were used to evaluate the effect of tempering conditions (tempering time and tempering level),

using distilled water, on the cookability of both navy and black beans. Secondly, using the optimum tempering conditions from the previous experiment, a completely randomized design was used to study the effect of tempering solutions on the cookability of both navy and black beans. Changes in the percentage of soluble protein, degree of gelatinized starch and colour of whole beans were also monitored. Tempering solutions included a mix of salts (0.2% sodium bicarbonate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate), a mix of acids (1% citric acid and 2% ascorbic acid), and 150 ppm of sodium EDTA. It is necessary to add that the micronization operational setting SL was changed to 2.75, while all other operational parameters were kept constant. This decision was based on the results of ongoing experiments indicating that a slope of 2.75 would improve the exposure of bean samples to more uniform infrared radiation, which would in turn result in a product of more consistent quality.

As discussed in Chapter 3, water experiment results indicated that optimum tempering conditions for navy and black beans, based on the degree of gelatinized starch, occurred at 28% moisture for 11.5h and 25.8% moisture for 32h, respectively. Using the optimum processing conditions, it was found that a mix of salts (0.2% sodium bicarbonate, 0.1% sodium carbonate and 0.1% sodium phosphate dihydrate) was more effective than a mix of acids (1% citric acid and 2% ascorbic acid) or of 150 ppm of sodium EDTA, in reducing the hardness of micronized beans. As well, the acid solution was detrimental to the physical appearance of micronized navy beans. Furthermore, based on the hardness results, the use of sodium EDTA and the acid solution was discontinued since their use did not contribute to reduce bean cooking hardness beyond what was attainable with water alone. The lack of sufficient free water in the bean cotyledons arising from the low water conditioning regimes (navy =28%; black =

black = 25.8%) and/or an increase in the soluble fraction of pectin after processing of beans were thought to account for the poor textural response of micronized beans tempered with a solution of sodium EDTA. Results also indicated that the micronization process significantly ($P \leq 0.05$) reduced the hardness value of navy beans by 28.7 % when tempered with distilled water, and also reduced the hardness of black beans by 13.7 % when tempered with an alkaline mixture of salts. Generally, a decrease in the percentage of soluble protein and an increase in the degree of gelatinized starch were significantly ($P \leq 0.05$) correlated with lower hardness scores in micronized beans. Nevertheless, it was still unclear as to what structural and compositional changes had a major impact on the textural characteristics of micronized cooked-beans.

Generally, it is well accepted that availability of water in the seeds is a prerequisite to quickly cook beans. The ability of the seeds of pulses to imbibe water during normal cooking has been reported to influence their cooking times (Sefa-Dedeh and Stanley, 1979; Jackson and Varriano-Marston, 1981; Jones and Boulter, 1983). It could be expected that studying the water imbibition characteristics of beans at room temperature would afford us a better understanding of the effects of micronization on the physical structure of beans. Several investigations have reported the strong relationship between the physical structure of beans (e.g. microstructure) and their water imbibition characteristics during soaking (Powrie *et al.*, 1960; Sefa-Dedeh and Stanley, 1979; Aguilera and Stanley, 1999). The objective of the final experiment (Chapter 4) was therefore to study the effects of the micronization process on the water imbibition characteristics of both navy and black beans tempered with distilled water or a mix of salts, as related with their physical (apparent density, bulk density, particle density and porosity) and physicochemical (the degree of gelatinized starch and the percentage of

soluble protein) characteristics. Results indicated that the micronization process significantly ($P \leq 0.05$) reduced the bulk density values of both navy and black beans compared to unprocessed beans. Furthermore, the bulk density of processed beans supported the apparent density results in that they reflected a change in the size and shape of the seeds as measured by the lower degree of compactness when beans were packed as a bulk. The addition of salts to the tempering solution resulted in micronized beans with significantly ($P \leq 0.05$) higher bulk densities than both unprocessed and water-treated processed beans. These results intimated that both the micronization treatment and the addition of salts to the tempering solution have a major impact on the physical structure of micronized beans. Additionally, changes in the size and shape of beans could have reflected the nature of the micronization process in which beans are first hydrated during tempering and then dehydrated by means of an intense-heat drying process. Unlike conventional drying techniques, micronization could make the microstructure of beans more porous (formation of air-bubbles) due to rapid vaporization of superheated steam at weak points of the intercellular bean structure (Arntfield *et al.*, 1997; Scanlon *et al.*, 1999; Arntfield *et al.*, 2001).

The water imbibition studies suggested that compared to unprocessed beans, micronization resulted in beans that imbibed more water during the initial stages of soaking (first 30 min for navy beans, and first 60 min for black beans). Several works have indicated that the physical structure of beans plays an indispensable role in determining the extent and rate of the water imbibition process (Deshpande and Cheryan, 1986; Agbo *et al.*, 1987), especially at the initial stages of water uptake (Sefa-Dedeh *et al.*, 1979). Scanlon *et al.* (1999) suggested that because of the high processing temperatures and high tempering regimes, it is likely that air-bubbles are developed

within the structure of micronized cereal grains and pulses. It was hypothesised that air-bubbles were created due to internal venting of superheated steam at weak points of the cell structure, principally in the intercellular spaces. Since soaking controls hydration of beans by the diffusion process, such a mechanism could be valid to explain the higher water imbibition rates for both navy and micronized beans, especially at the initial stages of water imbibition.

Scanning electron microscopy (SEM) was used to link microstructural properties of beans with their physical properties. SEM photomicrographs also revealed the impact of the micronization process on the microstructure of beans. SEM photomicrographs showed almost negligible air spaces at the interstitial joints of the cotyledonary cell walls of unprocessed navy and black beans. In micronized navy and black beans, SEM microphotographs clearly showed open-holes at junction points of the cotyledonary bean cell walls. Micronized beans tempered with distilled water had pore sizes of 7.8-10 μm (navy beans) and 7.7-8.8 μm (black beans). Addition of salts to the tempering solution resulted in micronized beans with pore sizes of 4.3-9.8 μm (navy beans) and 8.2-14.3 μm (black beans). The numbers and sizes of intercellular pores supported the lower particle density values and the higher porosity values of both micronized navy and black beans, compared to unprocessed beans. The formation of air-bubbles in the interior of micronized beans was therefore a plausible mechanism in which a more porous structure was developed due to rapid removal of water at the intercellular spaces of cotyledonary bean cells. Since water diffusion rates are greater in the air phase (bubbles) than in the solid phase, it could be expected that the higher water absorption rates of micronized beans would result in a product that is easier cook.

Chapter 4 results also indicated that cultivar influenced initial water imbibition rates since navy beans imbibed water more rapidly than black beans. Several investigations (Deshpande and Cheryan, 1986; Agbo *et al.*, 1987; del Valle *et al.*, 1992 a) have indicated that white-coloured bean varieties imbibed water more rapidly than dark-coloured seeds. This phenomenon could be due to the denser arrangement of seed coat cells in dark-coloured seeds (Powell, 1989) or as result of oxidative reactions of phenolic substrates resulting in hydrophobic substances (Hincks and Stanley, 1986; Hohlberg and Stanley, 1987; del Valle *et al.*, 1992 a; Stanley, 1992). The slower water imbibition rates for navy beans than for black beans was in line with the findings in Chapter 2 (first experiment) in which navy beans needed shorter times than black beans (11.5 vs. 32 h) to maximize the degree of starch gelatinization in their seeds after standard processing conditions. As well, cultivar influenced both the water holding capacity and the response to the alkaline tempering solution. For example, the use of the alkaline tempering solution increased the water absorption at equilibrium to black beans, while it had the opposite effect on navy beans. More specifically, the water holding capacity in unprocessed, water-treated and salt-treated micronized beans was smaller for navy (1.23, 1.03, and 0.93 g water/g dry matter, respectively) than for black (1.36, 1.16, and 1.28 g water/g dry matter, respectively) beans. The effect of cultivar was also noticeable during density analysis. For example, the higher particle density values for black beans than for navy beans (navy=1425 kg/m^3, and black =1438 kg/m^3) was attributed to the thicker and microstructurally denser seed coats of black beans (Agbo *et al.*, 1987). The bulk density of micronized navy beans preconditioned with the alkaline solution had a significantly ($P \leq 0.05$) lower bulk density than both unprocessed beans and water-tempered micronized beans. Overall, results suggested that the addition of a

mix of carbonates an phosphates to the tempering solution may further open the seed structure of beans, perhaps by enhancing denaturation of cotyledonary proteins (Garcia-Vela *et al.*, 1989; del Valle *et al.*, 1992 b) and thus loosening protein bodies tightly arranged in the cells of unprocessed beans.

On the other hand, the experiment in Chapter 4 indicated that the total imbibition capacity of beans decreased after micronization processing. The water imbibition capacity of beans (hydration capacity) is defined as the degree to which the cotyledon and cell contents (starch, protein and cell wall components) become fully saturated with water. Earlier studies (Chapter 1) suggested that the water imbibition capacity of beans after micronization was negatively correlated to both outlet temperature and bean density. Generally, it was suggested that beans subjected to the micronization process had a lower water imbibition capacity than unprocessed beans. Results from Chapter 4 were also consistent in demonstrating that micronization of beans resulted in a product that imbibed less water at the later stages of soaking. Surprisingly, these findings were not in accordance with the results of previous studies carried out at the University of Manitoba in which the water imbibition capacity of durum wheat kernels increased after micronization processing (Scanlon *et al.*, 1999). However, the differences in the structural components of pulses and cereal grains could account, at least partly, for their different responses in total water imbibition capacity. Sefa-Dedeh and Stanley (1979) reported that protein content controlled water imbibition kinetics at the later stages of soaking, with high protein beans having higher water absorption capacities. The lower water imbibition capacity of beans after micronization was concomitant with a reduction in their percentage of soluble protein. Similar results were reported in lentils (Arntfield *et al.*, 1997; Zhao, 2000) and peas (Toews, 2001). Further, Zheng *et al.* (1998) suggested

the loss of solubility of micronized legume proteins could be the result of hydrophobic aggregation of polypeptide chains. It was then suggested that denaturation and/or aggregation of cotyledonary cell proteins after micronization could reduce the amount of active water-binding sites, and thus reduce the total water imbibition capacity of micronized beans.

In addition, the Pearson correlation analysis indicated that the apparent density of navy and black beans was significantly ($P \leq 0.05$) correlated with their helium porosity (R^2 = -0.96 and R^2 = -0.99, respectively) and nitrogen porosity (R^2 = -0.94 and R^2 = -0.94, respectively) values, as well as with their degree of gelatinized starch (R^2 = -0.86 and R^2 = -0.88, respectively). The correlation analysis suggested that certain physical properties of beans could be as good predictors of the cooking quality of beans as it is the case for certain physicochemical parameters (e.g. starch gelatinization). Despite the relatively same chemical structure, size, and shape of black and navy beans, their responses (physical and chemical characteristics) to the tempering pretreatments and micronization were quite different. In short, this study indicated that the degree of starch gelatinization, percentage of soluble proteins, open pore porosity, and apparent density are good indicators of texture in micronized beans. Both physical and physicochemical events occur simultaneously during micronization processing and should not be disregarded or overlooked.

Recommendations for Future Studies

- The potential of micronization for generating air-bubbles in foods is one of the most important benefits of this innovative technique. Calculation of the water diffusivity coefficients of micronized beans could be helpful in gaining a better understanding of the role of air-bubbles in soaking and cooking.
- A high moisture content of the final product would certainly compromise the shelf stability of the micronized beans. Although a larger conveyer trough would ideally solve this problem, a second passage in the micronizer could have the same effect. Based on preliminary trials, however, it was observed that re-micronization could result in a burnt product. Perhaps, keeping beans in isothermal containers immediately after micronization could further reduce operational costs.
- A study of the fundamental mechanical properties (e.g. modulus of elasticity, strength, toughness, bioyield point) of beans could be very useful for gaining a more comprehensive understanding of the consequences of the structural changes that beans undergo during processing. Since mechanical properties are related to texture, they could also be used as good indicators of product cookability.
- It could be of interest to study the effect of micronization treatment per se (independently of the effect of tempering pretreatments) on the physicochemical properties of beans. This could be accomplished by running a control sample that should be dried at low temperatures (to avoid starch and protein damage) using conventional drying techniques, instead of being infrared heat-treated.

- One of the most devastating post harvest losses in beans is that related to the hard-to-cook phenomenon. It has been thought that, among other detrimental effects, the hard-to-cook phenomenon reduces the ability of cotyledonary bean cell walls to separate during normal cooking (i.e. boiling water). For the present investigation, based on the unusual response of beans to pretreatments containing sodium EDTA, it appeared that micronization could alter/facilitate the separation of cotyledonary bean cell walls during normal cooking due to solubilization of pectic substances (e.g. middle lamella) during processing. A study of the effect of micronization on the structure of cotyledonary bean cell walls (i.e., pectic substances, calcium-magnesium-phytic acid-cell wall interactions) may be worthwhile. Furthermore, micronization may be used to prevent hardening of beans by producing a biochemically less active product.

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APPENDIX I

Table 1. The chemical composition[1], texture and colour characteristics[1] of raw navy and black beans

	Navy beans	Black beans
% Gelatinized starch	1.55 ± 0.09	1.85 ± 0.14
% Soluble Protein	24.42 ± 1.34	21.35 ± 0.11
Maximum Force Value[2] (N)	325 ± 17[3]	455 ± 2.1[3]
	498 ± 39[4]	456 ± 6.8[4]
Firmness[2] (Nmm)	7466 ± 239[4]	6793 ± 178[4]
Hunterlab colour L	60.17 ± 0.19	22.90 ± 0.24
Hunterlab colour a	1.6 0 ± 0.06	-1.50 ± 0.06
Hunterlab colour b	10.17 ± 0.05	-0.43 ± 0.07

[1] Mean ± standard deviation.
[2] Beans soaked overnight in distilled water and cooked for 42.5 min.
[3] Texture analyses of beans from the 1999 crop year.
[4] Texture analyses of beans from the 2000 crop year.

APPENDIX II

Summary of the sensory evaluation test for determining the optimum cooking time of unprocessed navy beans.

Objective:

To determine the optimum cook time of unprocessed navy beans.

Panellists:

The expert sensory panel was composed of five members.

Methods:

Initially the group discussed the various uses for navy beans and the textural characteristics associated with each use. Among the potential uses of navy beans discussed in this sensory test, we could name the following: baked beans with sauce, intact beans or mashed beans with a thicker sauce, canned beans, salads in which beans have relatively good firmness, and soups in which beans are mostly intact, showing some resistance to bite and they are not mushy.

During this session it was agreed that the objective of the sensory test was to determine the bean sample that was optimally cooked for use as intact beans with sauce. Further discussion determined that the optimal textural characteristics for intact beans were that the beans could be cracked and their external seed coats should be tender without leaving a residue of the seed coat in the mouth. Texture of the interior should be soft but not mushy, smooth but not granular, and homogeneous with no particles left on breakdown of the sample.

Samples were evaluated individually and textural descriptors noted. The ballot used in this study can be seen in Appendix III. Discussion followed regarding the sample with the optimum texture for an intact bean product based on these descriptors.

Five samples of navy beans cooked for different lengths of time were presented. Based on the textural characteristics, the sample closest to the ideal optimally cooked sample was selected based on consensus of the group.

Sample Preparation and Presentation:

Beans were rinsed with deionized water, placed in deionized water (ratio 1:3 beans to water) and soaked about 15 hours. For cooking beans were placed in deionized boiling water (ratio 1:10 beans to water) and boiled at a constant rate for 20, 27.5, 35, 42.5 and 50 minutes. Samples were then drained and cooled to room temperature for about one hour before evaluation.

For each sample approximately 30 g of cooked beans were placed in 125 mL Styrofoam cups that were coded with 3 digit random numbers. Filtered water was provided for rinsing between samples. Samples were evaluated in random order.

Results:

Detailed comments from the panellists of cooked unprocessed navy beans cooked for several cooking times.

Cook time (min)	Comments
20	Too hard, coarse particles, granular sensation, needs to be cooked, although appearance is good, they don't have adequate texture, seed coat has more resistance
	Intact, too much resistance in softness for first and second bite, very grainy, tough to swallow, hard seed coat when swallowing
	Quite firm, granular during chewing, every single seed coat has remained in my mouth, not cooked enough (even a bit firm for salad)
	Hard, can feel intact cotyledons, sort of granular, tiny granules
	Hard, grainy, seed coat smooth
27.5	Relatively hard; before swallowing the seed coats were not perceived, cotyledons yielded coarse particles, some residues of the seed coat
	Beans are still too hard; much more grainy, seed coat was sharper (more predominant) and remained in mouth
	Bit firm, bit granular, lots of residual seed coat, good for salad, too firm for intact cooked bean
	There are some hard cores and softer outside, slightly granular, not much difference between seed coat and interior
	Not as hard like 286 (20 minute sample), grainy, not evenly cooked, skin is chewy
35	Hardness is okay; seed coats were almost imperceptible, smooth texture.

	Softness is perfect, first bite is good, slight residual particles/pasty like remaining, seed coat remaining in mouth is prickly, visually is mushed
	Soft, some resistance, reasonably smooth, still mouth full of seed coats, except for seed coats good for use as intact bean
	A little tiny bit mealy, seed coat noticeable, left in mouth, soft but intact interior, not 'diluted', smooth
	Easy to chew, skin is little chewy, not mushy but soft, not grainy
42.5	Too mushy, too smooth, too chewy, overcooked
	Ideal softness, least grainy, no particulates left in mouth, seed coat soft and remained in mouth
	Soft, bit of resistance, quite smooth, not too bad on the residual seed coat, appearance not ideal, reasonable for cooked bean product
	Soft but very intact interior, homogeneous, stiff, smooth
	Soft but not mushy, seed coat little chewier
50	Too mushy, seed coat can be felt slightly, before swallowing, seed coats still remain in the mouth, too chewy.
	Softness, too soft, very little particulates, slight residual pasting, seed coat softer, but still remained in mouth
	Very soft, almost mushy, reasonably smooth, still mouth full of seed coats (looks messy), too soft for straight cooking
	Soft, can feel big difference between seed coat and inside, interior is smooth
	Soft, inside mushy, not grainy, seed coat is chewier

General Comments:

Five out of six panellists agreed that the optimum sample was the one cooked for 42.5 min due to following reasons:

1) It had a tender seed coat.
2) It exhibited a soft intact interior (cotyledons were cooked).
3) Beans in that sample had homogenous texture.

Only one panellist thought that the optimum sample was the one cooked for 35 min because the 42.5-minute sample was too soft whereas the 35-minute sample had optimal smoothness.

The 50-minute sample had a smooth interior but the difference between interior and exterior was extreme. The 27.5-minute sample could be suitable for salads due to firmer texture.

Conclusion:

1. The expert sensory panel indicated that the optimum cooking time of navy beans was 42.5 min.
2. Using a Lloyd Materials Testing Machine Model L1000R (Lloyd Ltd., UK), it was found that after cooking navy beans for 42.5 min their hardness (maximum compression force) value was 325±17 N. It was also found that cooking times of 50 and 60 min did not reduce hardness values beyond to what was attainable with a cooking time of 42.5 min (325±17 N).

APPENDIX III

Ballot given to panellist for evaluation of texture of navy beans

Evaluation of Beans

Instructions:

Place one teaspoon of beans in your mouth, move to the molars and evaluate the force required to bite through the sample.
Chew the sample slowly and evaluate the way the sample is perceived in the mouth throughout the chewing and just prior to swallowing. Repeat this procedure taking another teaspoon of sample.

Note your observations in the space provided.

Evaluate the samples in the order listed.

Code No. _____

Code No. _____

Code No. _____

Code No. _____

Code No. _____

APPENDIX IV

Table 1. Full factorial design [1] including observed experimental data for studying the effect of micronization operational conditions on the physicochemical properties of navy beans

Std order	Run order	Factor GAS	Factor SLOPE	Factor DISTANCE	Response Outlet Temp. (°C)	Response Hydration capacity (mL/g)	Response Particle density (kg/m^3)	Response Hardness (N)	Response Moisture (%)	Response Protein solub. (%)
1	11	5	-5	11	55.0	126.4	1358	263	23.4	13.9
2	5	9	-5	11	67.5	126.3	1365	268	22.9	11.7
3	6	5	5	11	77.5	126.4	1373	225	22.2	5.7
4	4	9	5	11	95.0	121.3	1381	281	19.9	9.0
5	3	5	-5	12.4	53.0	125.0	1392	206	22.6	10.6
6	10	9	-5	12.4	62.5	126.3	1369	267	22.2	13.1
7	1	5	5	12.4	95.0	122.7	1397	216	21.6	9.3
8	9	9	5	12.4	101.5	123.6	1373	252	18.3	8.9
9	7	7	0	11.7	77.5	122.8	1379	247	21.7	11.5
10	8	7	0	11.7	90.0	124.4	1371	280	19.9	8.4
11	2	7	0	11.7	77.5	123.4	1380	252	21.3	11.3

[1] Gas = operational setting of a air-mixture valve, Slope = slope of the vibrating conveyer trough, and Distance = distance (cm) between the vibrating conveyer trough and the radiation source in a pilot-scale gas-fired micronizer unit MR2 (Micronizing Ltd., Framlingham, U.K.)

APPENDIX V

Table 1. Chemicals used in tempering and chemical analyses of navy and black beans

Manufactures	Chemical
Sigma Chemical Co.	Bovine Serum Albumin Glucose *0*-toluidine *Rhizopus* Amyloglucosidase Sodium carbonate
Fisher Scientific International Inc.	Acetic acid glacial Acetone, reagent grade Disodium EDTA, reagent grade Hydrochloric acid Sodium tripolyphosphate, laboratory grade Trichloroacetic acid
BDH Chemical, Toronto	Sodium acetate anhydrous
Canada Colours and Chemicals Ltd.	Ascorbic acid
Mallinckrodt Specialty Chemicals Co., Paris, KT.	Sodium hydroxide Sodium phosphate dihydrate, analytical reagent
Pierce (Illinois)	Coomasie® Protein Assay Reagent Kit
Glass Microfibre Filters (England)	Whatman GF/C 125 mm filter

APPENDIX VI

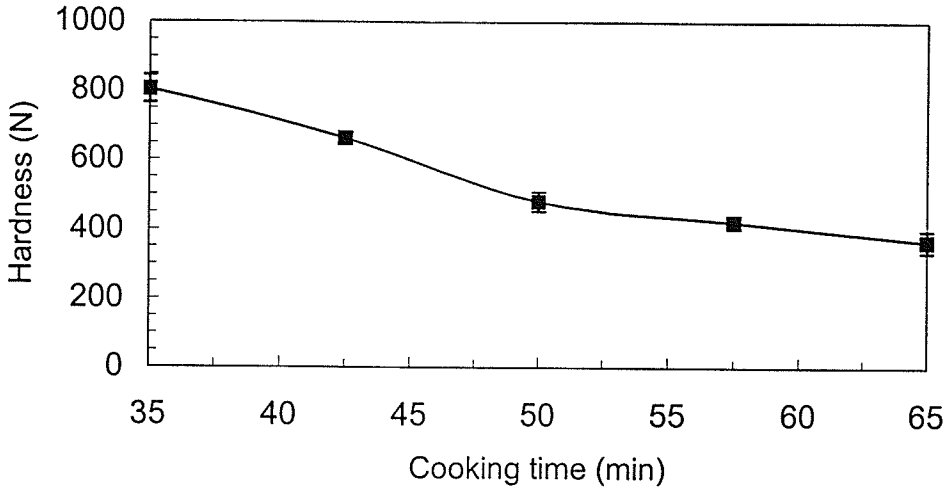
Table 1. RSM experimental design including observed experimental data for determining the effect of processing conditions on the physicochemical properties of navy beans

Std order	Run order	Tempering time (h)	Tempering Level (%)	Moisture (%)	Hardness (N)	Protein Solubility (%)	Gelatinized Starch (%)	Color *L*	Color *a*	Color *b*
3	1	6.0	30.0	22.4	332	3.5	41.0	61.2	1.4	11.8
9	2	12.0	25.0	20.4	436	12.1	51.3	62.1	1.5	12.1
6	3	20.5	25.0	22.0	389	22.8	10.8	61.1	1.1	11.3
5	4	3.5	25.0	20.1	476	17.8	19.9	62.0	1.7	11.4
8	5	12.0	32.1	27.7	439	19.4	55.1	61.0	1.6	12.4
10	6	12.0	25.0	19.4	403	11.4	52.4	61.5	1.3	12.4
2	7	18.0	20.0	18.6	369	18.7	23.5	63.3	0.4	10.9
7	8	12.0	17.9	16.6	408	18.5	29.5	62.6	1.1	12.0
1	9	6.0	20.0	16.2	299	7.2	7.1	61.1	1.8	10.2
4	10	18.0	30.0	22.4	396	4.4	20.3	62.3	0.8	11.0
11	11	12.0	25.0	21.3	423	17.7	48.8	60.7	1.2	11.3
12	12	12.0	25.0	19.6	429	8.6	47.3	63.1	0.8	11.0
13	13	12.0	25.0	20.0	413	7.0	48.2	62.8	0.9	10.4

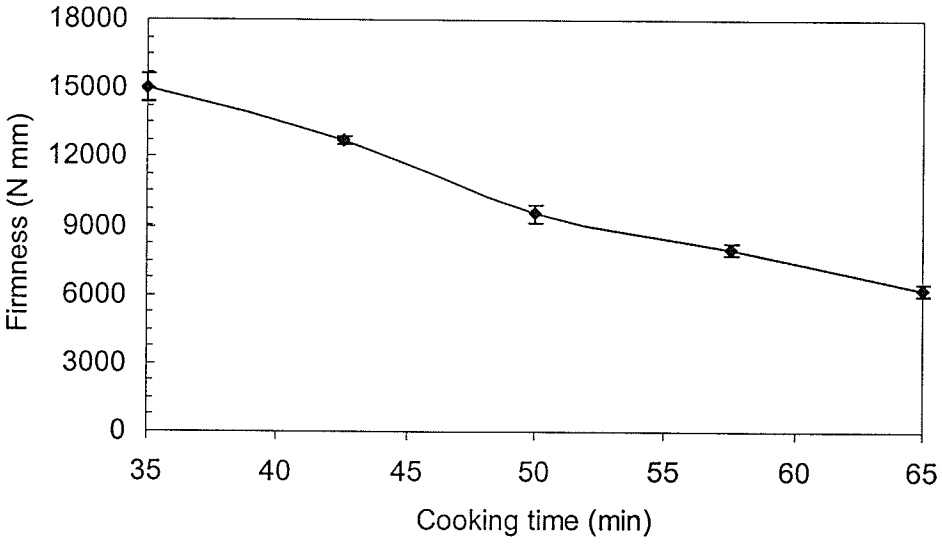
Table 2. RSM experimental design including observed experimental data for determining the effect of processing conditions on the physicochemical properties of black beans

Std order	Run order	Tempering Time (h) h	Tempering Level (%) %	Moisture %	Hardness N	Protein Solub. %	Gelatinized starch %	Color *L*	Color *a*	Color *b*
6	1	20.5	25.0	22.8	478	14.4	39.3	18.1	0.8	-1.1
11	2	12.0	25.0	18.2	477	19.7	38.2	18.5	0.3	-0.8
10	3	12.0	25.0	19.2	504	8.2	35.5	18.8	-1.1	-0.8
5	4	3.5	25.0	16.9	428	2.3	32.3	17.5	0.5	-1.2
3	5	6.0	30.0	19.7	531	10.8	17.6	18.1	0.2	-1.8
1	6	6.0	20.0	16.6	536	8.3	11.6	20.9	-1.8	-1.5
8	7	12.0	32.1	25.9	496	11.2	11.2	17.6	0.6	-1.1
13	8	12.0	25.0	20.7	485	11.8	34.0	17.8	0.6	-1.2
9	9	12.0	25.0	18.8	480	13.3	33.8	18.5	0.1	-1.4
2	10	18.0	20.0	14.6	490	19.5	23.0	19.4	-2.1	-0.2
12	11	12.0	25.0	20.3	496	14.9	34.9	17.6	0.6	-1.2
4	12	18.0	30.0	22.1	495	9.4	23.5	18.2	0.0	-1.5
7	13	12.0	17.9	13.5	441	4.5	6.1	18.5	0.4	-1.0

APPENDIX VII

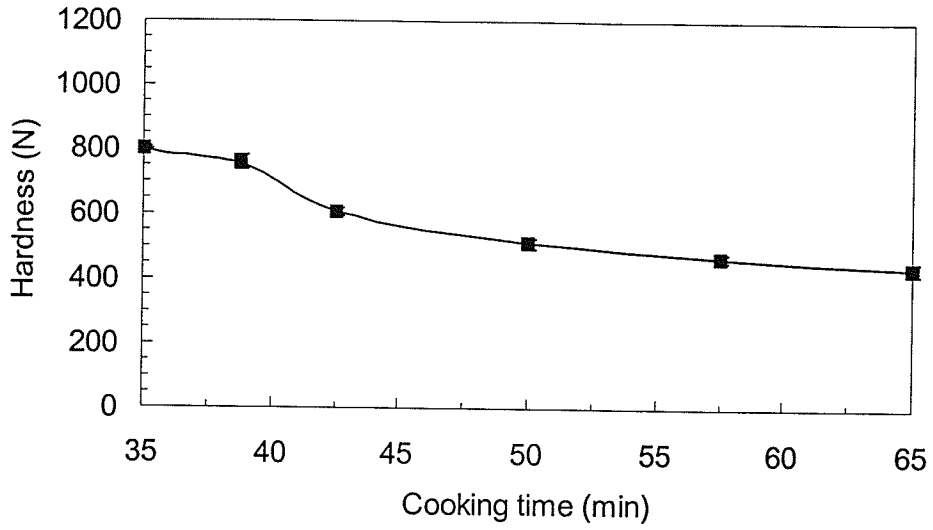


A. Hardness of navy beans

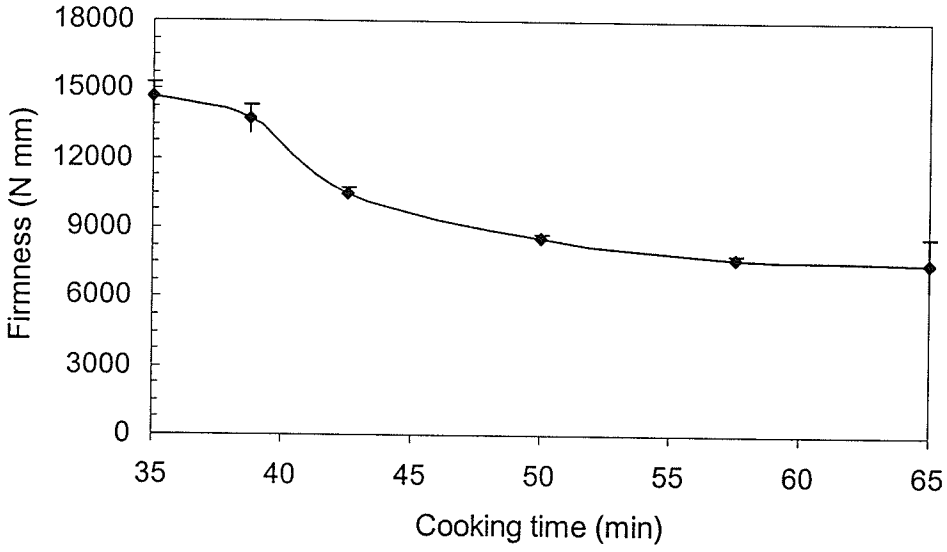


B. Firmness of navy beans

Figure 1. The hardness (A) and firmness (B) cooking curves of non-soaked unprocessed navy beans (2000 crop year). Error bars indicate standard deviation of triplicate measurements.



A. Hardness of black beans



B. Firmness of black beans

Figure 2. The hardness (A) and firmness (B) cooking curves of non-soaked unprocessed black beans (2000 crop year). Error bars indicate standard deviation of triplicate measurements.