The Effects of Pretreatment Conditions and Micronization on the Anti-nutritional Factors, Cookability, and Microorganisms in Navy Beans (*Phaseolus vulgaris* L.)

BY

Jennifer Richardson

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

MASTER OF SCIENCE

Department of Food Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

*****

COPYRIGHT PERMISSION PAGE

THE EFFECTS OF PRETREATMENT CONDITIONS AND MICRONIZATION ON THE ANTI-NUTRITIONAL FACTORS, COOKABILITY, AND MICROORGANISMS IN NAVY BEANS (*PHASEOLUS VULGARIS* L.)

BY

Jennifer Richardson

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

I hereby declare that I am the sole author of this thesis.

I authorize the University of Manitoba to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Jennifer Lynne Richardson

I further authorize the University of Manitoba to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Jennifer Lynne Richardson

The University of Manitoba requires the signatures of all persons using or photocopying this thesis. Please sign below and give address and date.

ACKNOWLEDGEMENTS

Much love and thanks goes out to my family for their love, patience, and endless support. Without the love, continuous support and encouragement of my Mother and Father, this thesis would not be complete today. They made the "bad" or "difficult" days not seem so terrible. They are wonderful, caring people whose loving support helped me through this thesis.

I would like to thank Dr. Susan Arntfield for her guidance and support over the last 3 years. Your encouragement and support has been instrumental. I would also like to thank Dr. Stefan Cenkowski and Dr. Rick Holley for always welcoming questions and providing assistance to the many bumps along the road.

It goes without saying that many individuals at the University of Manitoba have not only supported my thesis academically, but made it an enjoyable place to be. I'd like to specially thank Aniko Bernatsky and Georgina Mejia for not only their technical assistance, but also their friendly smile every morning. Additionally, the assistance of Peter Frohlich in the summer of 2002 was extremely helpful in the completion of this project.

Last, but not least, I'd like to thank Yvonne Halden. In addition to her excellent help and direction with numerous questions, she was always there to listen. Regardless of how her day was, she'd always ask how you were. She makes the Food Science Department an enjoyable and fun place to be. Thank you very much Yvonne.

TABLE OF CONTENTS

8.0 LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Micronization is a new technology that has the potential to increase the utilization of pulse crops in North America. Several anti-nutritional factors and the long cooking time of pulse crops, such as navy beans, have limited their use in North America. Micronization has the potential to reduce several anti-nutritional factors and reduce the cooking time for pulses. In this experiment, several combinations of tempering time and temperatures in conjunction with 20 and 25% moisture levels proved that micronization will eliminate the pre-soaking period for navy beans, but will not reduce their cooking time. Starch was not significantly ($p \leq 0.05$) gelatinized under any conditions, and hence, cooking time was not reduced. No tempering treatment or moisture level combination significantly ($p \leq 0.05$) reduced phytic acid, tannins, or trypsin inhibitors. The hard-to-cook phenomenon has an integral affect on the ability of micronization to produce significant reductions in cooking time and anti-nutritional components. The benefits of micronization are limited by the quality of the raw product.

A microbiological investigation into the micronization process determined that tempering for 18h at room temperature (R.T.) significantly increases bacterial growth while micronization significantly decreases it. By varying the tempering treatments it was determined that tempering for 18h at R.T. and 1.5h at 60°C produced significantly ($p \leq 0.05$) greater growth during tempering compared to 6h at R.T. and 4.5h at 45°C. They also yielded the highest bacterial reduction after micronization. Tempering to 25% moisture for 18h at R.T. had

significantly higher bacterial growth than 20% moisture under the same conditions. Moisture content had no impact on microbial growth at tempering or micronization for all other treatments. Yeast and mould did not grow during tempering and were almost completely eliminated by micronization. *Bacillus* spore-formers are the primary organisms remaining on navy beans tempered for 18h at R.T. followed by micronization. However, when beans were tempered to 25% moisture one of the 14 colonies examined was gram negative. The survival of *Enterobacteriaceae* needs to be looked at in more detail in future studies, for pilot scale micronization to be deemed a safe processing treatment. A minimal slope and greater output temperature allow for commercial micronization to reduce more bacteria than pilot scale micronization.

With no significant differences in anti-nutritional factors or cookability between tempering at 20% and 25% moisture, future micronization work should be done with tempering for 6h at R.T. and 20% moisture to control microbial growth. Tempering for 4.5h at 45°C could also be chosen, but the added energy and capital costs of heating during tempering would not be desired.

INTRODUCTION

Malnutrition is a chronic problem for over 800 million people in developing countries (FAO, 1996). On a global basis, over 65% of food protein and 80% of food energy is supplied by plants (Deshpande and Damodaran, 1990). Pulses, which contain 20 - 40% protein (Ruperez, 1998; Iyer, 1980), are commonly consumed as the main source of protein and calories in tropical and subtropical areas where animal protein is too expensive or not religiously accepted (Deshpande and Damodaran, 1990).

Canadians obtain some of their nutrition through the consumption of meat and meat products, while most developing countries must obtain their nutrients through plants, such as pulses. In 2000, Canadians produced 4,439,000 metric tons of pulses and only utilized 15.6% as food. Unlike Canada, developing countries such as those in South America, have increased their pulse consumption rates from 85.1% to 94.6% from 1990 to 2000 respectively. Their overall production of pulses has also increased by 1.2%. Other developing countries such as Africa have followed the same trends over the past 10 years. (FAO, 2002)

Pulses are a good source of potassium, calcium, thiamin, niacin, and most other B vitamins (Koehler et al., 1987; Kadam and Salunkhe, 1989). They are also good sources of the minerals iron, magnesium, zinc, and copper (Haytowitz et al., 1981; Koehler et al., 1987; Iyer et al., 1980). Pulses contain no cholesterol, ¼ the sodium, and 80% less fat than lean ground beef (Haytowitz et al., 1981). Still, with many nutritional components, several nutritional and

convenience related problems counteract the benefits of pulses and impede their use in the diet.

Nutritionally, pulses are deficient in the sulfur containing amino acids, methionine and cystine (Koehler et al., 1987). They contain several anti-nutritional components such as lectins, trypsin inhibitors, tannins, and phytic acid. Despite high protein contents, these antinutritional factors lower protein digestibility and nutrient bioavailability, thereby limiting the widespread use of pulses (Antunes and Sgarbiere, 1980; Durigan et al., 1987; Salunkhe and Kadam, 1989). Although not anti-nutritional, the presence of raffinose oligosaccharides, which induce flatulence (Reddy et al., 1984; Flemming, 1981; Iyer et al., 1980; Jood et al., 1985; Abdel-Gawad, 1992), are definite factors in limiting the consumption of pulses.

In conjunction with nutritional deficiencies, anti-nutritional factors, and social discomfort, the slow cooking time of pulses inhibit their growth and widespread use in convenience-orientated countries. Prolonged cooking times of 1 - 4h and preparation (soaking) times of 12 - 15h (Silva and Braga, 1982; Deshpande et al., 1984) are the primary impediments in North America where convenience is demanded. These long cooking times can be reduced in legumes by using an infra-red heat treatment called micronization.

Micronization is a treatment that has the potential to pre-cook and substantially reduce the cooking time of pulses (Arntfield et al., 1997; Scanlon et al., 1998; Abdul-Kadir et al., 1990; Sarantinos and Black, 1996). Micronization uses wavelengths from the infra-red range of 1.8 - 3.4 microns. This radiation

penetrates the product, releasing its energy, and ultimately heats the product (Sarantinos and Black, 1996). Micronization impacts many quality parameters of pulses. Micronized lentils exhibit reduced cooking time and phytic acid (Cenkowski and Sosulski, 1997), while micronization reduces several anti-nutritional factors and oligosaccharides in peas (Toews, 2001). To achieve these benefits, micronization requires a pre-treatment step called tempering. Tempering is the addition of a specified amount of water to pulses, to obtain a desired final moisture content. Dry navy beans, which are commonly canned and used in bean salads, stews, and chilies, have not yet been examined to see if micronization can offer similar advantages.

Preliminary research on navy beans suggests that the tempering conditions of 25% for 18h at room temperature (R.T.), provided a suitable environment for microbial growth. Various organisms can be found on navy beans due to their close contact with soil and water during growth. Under the above tempering conditions, the type of micoorganisms present, and the direct effect of micronization on navy bean microflora, needs to be investigated for food safety. Microbial responses to micronization will be addressed in the following study.

One of the primary goals of this research was to determine how tempering and micronization will impact the growth and survival of the predominant organisms on navy beans. Preliminary results indicated that tempering for 18h at R.T. and 25% moisture significantly increased the number of bacterial colonies, while micronizing significantly reduced them. As a result, microbial growth can

be controlled at either of these two stages of the process. Since tempering conditions are suspected to play a major role in reducing anti-nutritional factors and cooking times, they must be considered when applying different pre-treatments to control microbial growth.

Objectives:

1. Investigate the potential for microbial hazards during tempering and their responses to tempering and micronization.
2. Reduce the microbial growth at tempering using four different temperature and time combinations with 20% and 25% moisture levels.
3. Using these treatments, reduce tannin and phytic acid concentrations, and reduce trypsin inhibitor activity, while increasing the amount of gelatinized starch.
4. Reduce navy bean cooking time

LITERATURE REVIEW

1.1. Navy beans

1.1.1. Nutritional composition

Pulses are high in protein, containing almost two to three times more protein than cereal products (Deshpande and Damodaran, 1990). They are a nutritionally sound source of protein containing no cholesterol, ¼ the sodium and 80% less fat than lean ground beef (Haytowitz et al., 1981). Pulses are also a good source of complex carbohydrates (Haytowitz, et al., 1981). They have been shown to improve all aspects of diabetic control (Simpson et al., 1981) and lower cholesterol (Anderson et al., 1990) when incorporated into the diet.

Yet, there are nutritional impediments to bean consumption, particularly their low protein digestibility and quality (Koehler et al., 1986). Soaking pulses, prior to cooking, will increase protein quality, but this time consuming process is not desirable in fast paced convenience oriented countries such as Canada and the United States.

Another nutritional impediment is the unbalanced amino acid profile (Salhunke and Kadam, 1989). In particular, navy beans are deficient in the sulphur containing amino acids, methionine and cystine (Kakade, 1974; Koehler et al., 1987). The composition of navy beans is listed in Table 1.

1.1.2. Structure

Figure 1 and 2 illustrate the structure of a legume seed and the cell wall of a plant cell respectively. In Figure 1, three important water imbibition points have

Table 1: The Composition of Navy Beans

Component	Amount	Reference
Fat (%)	1.83	Koehler et al., 1987
Ash (%)	4.4	Koehler et al., 1987
Nitrogen (%)	3.78	Koehler et al., 1987
Protein (%)	23.6	Ockerman, 1991
Total Fiber (%) [a]	17.7	Kereliuk and Kozub, 1995
Starch (%)	27.0 – 52.7	Reddy, et al., 1984
Lipids (%)[b]	2.24	Drumm, et al., 1990
Minerals (mg/100g)		
Iron	6.59	Koehler et al., 1987
Zinc	2.87	Koehler et al., 1987
Calcium	216	Koehler et al., 1987
Magnesium	177	Koehler et al., 1987
Potassium	1361	Koehler et al., 1987
Phosphorus	463	Koehler et al., 1987
Vitamins (mg/100g)		
Thiamin	0.805	Koehler et al., 1987
Riboflavin	0.195	Koehler et al., 1987

All values are an average of 3 samples unless otherwise noted
[a] Average of 12 samples
[b] Average of 2 samples

been identified: the hilum, micropyle, and raphe (Swanson et al., 1985). The cotyledons, which constitute the majority of the seed, contain parenchyma cells bound by a distinct cell wall and middle lamella (Stanley and Aguilera, 1985). At seed maturity, these parenchyma cells contain highly specialized organelles, protein bodies and starch granules (Liu, 1995; Stanley and Aguilera, 1985), which play significant roles in the cooking process. The three layers of the parenchyma cell wall are shown in Figure 2. The middle lamella is the exterior layer of the cell wall containing pectic substances and acting as the primary adhesive to other plant cells (Lui, 1995; Stanley and Aguilera, 1985). For cooking to occur, these pectic substances must dissolve and allow for cell separation (Stanley and Aguilera, 1985). If the pectic substances do not dissolve during cooking, the middle lamella will not release adjacent cells, inhibit cell separation, and interfere in cooking. The hard-to-cook (HTC) phenomenon will affect beans in this way. HTC will be discussed at a later point in more detail.

1.1.3. Production

Pulses are produced all over the world. Developing countries produce more pulses, and export much less, on a global basis than developed countries. In 2000, developed countries produced 15,430 thousand metric tonnes, while developing countries produced more than double at 39,370 thousand metric tonnes (FAO, 2002). Yet, interest in pulse production is increasing in developed countries such as Canada. The seeding area of pulses in Canada has increased 3500% from 1980 to 2001 (AAFC, 2002a).

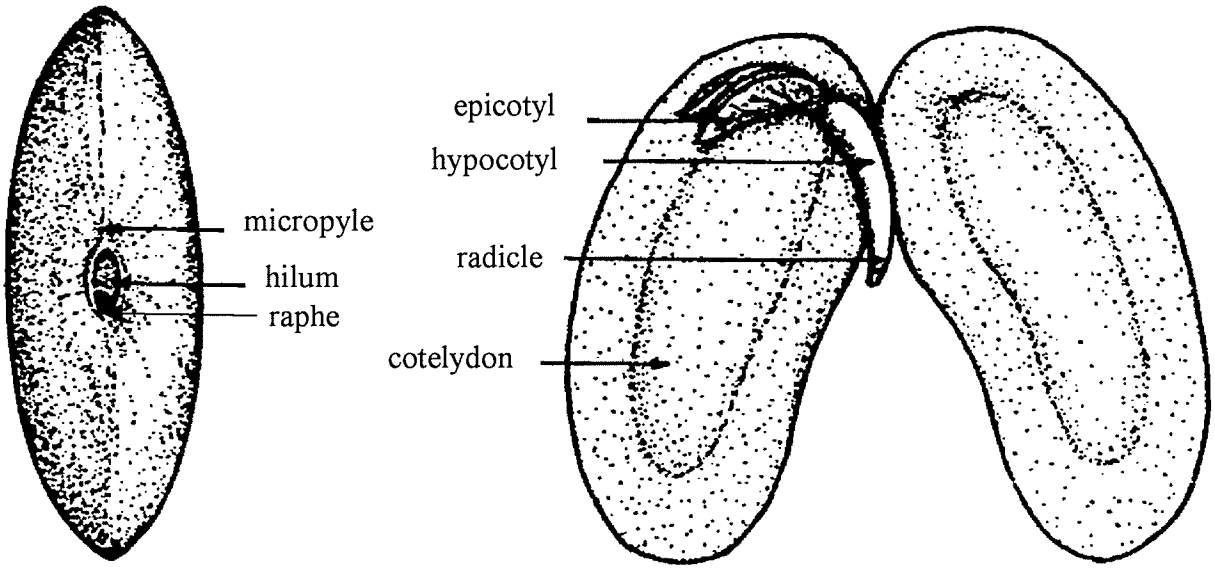

Figure 1. Legume seed. Left-external view; Right-internal view.
Source: Stanley and Aguilera, 1985

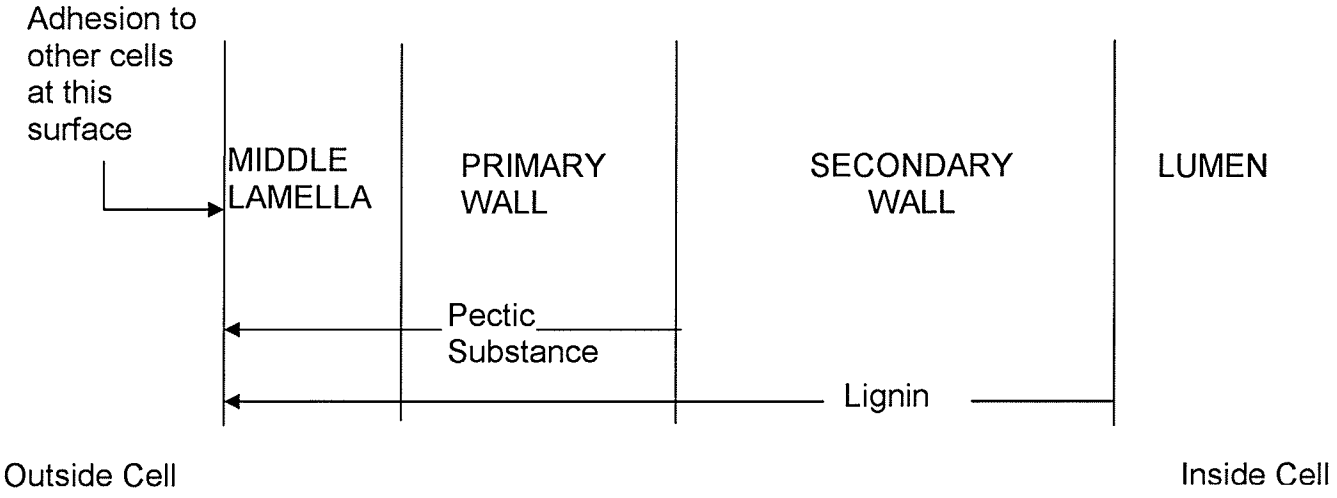

Figure 2. A schematic diagram of a plant parenchyma cell wall.
Source: Stanley and Aguilera, 1985

Dry beans follow the same statistics. Developing countries produced 6.8 times more beans than developed countries and exported 2.25 times less in 2000 (FAO, 2002). Still, dry bean production is increasing in developed countries. In 2000, the United States produced 1.18 times more dry beans than in 1980 (USDA, 2002b). During the 2002 and 2003 season, dry bean production increased 41% in Canada and the total U.S. and Canadian supply increased by 34% (AAFC, 2002b) from the previous crop year.

1.1.4. Utilization

In areas of the world where animal protein is expensive or not religiously accepted, legumes serve as the main source of protein and caloric intake (Deshpande and Damodaran, 1990). Developing countries exported only 6.1% of their pulses, while developed countries, that rely upon animals for protein, exported 46.6% of their pulses in 2000 (FAO, 2002).

Over the last two decades, local utilization of field beans has been increasing in developed countries. Perhaps an increased interest in a vegetarian lifestyle has inspired the use of pulses in the diet as a nutritious source of protein. The United States reduced their field bean exports from 34% to 21.2% from 1980 to 2000 respectively (USDA, 2002b). These reduced exports coincided with increases in field bean utilization as per capita consumption increased from 2.5 to 3.5 kg (USDA, 2002b).

1.1.4.1. Human consumption

Dry beans are commonly prepared using processes such as cooking, soaking, and fermenting in developing countries. For example, Asians typically use fermented pulse flours to produce idli, which is consumed as a staple food in their diet (Batra and Millner, 1976).

In developed countries a common preparation method for dry beans is canning. In 2000, more than 90% of navy beans in the U.S. were canned as baked beans or navy bean soup (USDA, 2002a). The beans are initially hydrated in water, followed by canning in brine and/or tomato sauce (Uebersax and Bedford, 1980). Canned beans are often used in salads, chilies, and as side dishes in North America.

Stove-top cooking is another common method for preparing *Phaseolus vulgaris* beans (which includes navy beans). It is a method common to both developed and developing countries. Due to the resistance of *Phaseolus vulgaris* beans to soften during cooking, they need to be soaked overnight in water to reduce their cooking times. This long preparation time is the major impediment in their utilization as human food in developed countries where convenience is a necessity.

1.1.4.2. Livestock feed

Non-food grade dry beans are often used for livestock feed. The world utilized 9.20% of dry beans produced as feed in 2000 (FAO, 2002). Their high protein concentration makes their incorporation into animal diets beneficial.

When ingested by cattle or sheep, navy beans are quickly digested to yield high metabolizable energy levels (NSW Agriculture, 2002). Additionally, the anti-nutritional factors (lectin and trypsin inhibitors) commonly associated with the consumption of dry beans by humans and monogastrics, have no negative effects when fed at levels less than 10% (sheep and beef cattle) or 20% (dairy cattle) to livestock (NSW Agriculture, 2002).

1.2 Factors contributing to the variability in bean quality

1.2.1. Growing location

Growing location can have a strong impact on bean composition and its functional and textural properties such as hardness. When two *Phaseolus vulgaris* cultivars were grown in two locations with low and high Ca and Mg contents, regardless of storage or soaking time, both cultivars of beans grown in soils with lower Ca and Mg concentrations had shorter cooking times (Paredes-López et al., 1989 a). Proctor and Watts (1987) also reported that differences in the cookability of three navy bean cultivars grown in three different Manitoba locations were affected more by location than genetic variability. This indicates that different soils, and agricultural practices, could be a large source of variability for bean cookability and texture.

1.2.2. Weather

Weather can have a significant impact on seed quality. Field peas exposed to heat stress and those exposed to low temperatures, rain and cloud,

plus severe mildew infection, exhibited poorer cooking quality than those grown in less stressed environments (Gubbels et al., 1985). Quenzer et al. (1978) also showed that when pinto beans were subjected to a late frost, they were unable to imbibe water. The length of growing season, temperatures, and microbial infestation will all strongly impact seed quality.

1.2.3. Seed maturity

Seed maturity is another important factor relating to bean quality. Pulses harvested early, prior to maturity, exhibit poor quality (Gubbels et al., 1985; Chernick and Chernick, 1963). Small seeds within a variety and crop can indicate immaturity and exhibit poorer cooking quality as well (Chernick and Chernick, 1963).

1.2.4. Storage conditions

During storage, if navy beans are exposed to high temperatures and/or high relative humidities, they can develop the hard-to-cook characteristic (El-Tabey Shehata, 1992) which involves the inability to absorb water. The hard-to-cook phenomenon and how it can affect bean cooking quality will be discussed in a subsequent stion.

1.3. Anti-nutritional components in navy beans

1.3.1. Phytic acid

Phytic acid is the principle storage form of phosphate, representing 70% of the total phosphate in dry beans (Chang, 1977; Lolas and Markakis, 1977). One such bean is navy beans which contains 1.29% - 1.58% phytic acid (Lolas and Markakis, 1975). Phytic acid from *Phaseolus vulgaris* is 99.6% water soluble (Lolas and Markakis, 1975) and commonly present as a salt of mono- and di-valent cations such as calcium and magnesium (Deshpande and Cheryan, 1984). Phytic acid is often referred to as myo-inositol hexa-phosphoric acid or its mono-dodeca anion form, phytate (Maga, 1982).

The interaction of phytate with proteins, vitamins, and several minerals is considered to be one of the factors limiting the nutritive value of plant foods (Deshpande and Cheryan, 1984). Nutritionally, phytic acid can have harmful implications. When more than 1% of a diet is comprised of phytic acid, mineral utilization and bioavailability decreases (Iyer et al., 1980). Phytic acid sequesters and interacts with cations, such as Fe, Mg, and Ca, to form complexes that are insoluble or otherwise unavailable for absorption (Cheryan, 1980; Kon et al., 1973; Lolas and Markakis, 1977).

In poor developing countries, where humans lack proper nutrition, this anti-nutritional factor needs to be reduced to improve the nutritional value of their crops. The exposure of soaked beans to temperatures above room temperature (R.T.) destroys heat sensitive cell membranes and activates the endogenous enzyme, phytase (Chang, 1977). Phytase hydrolyzes phytic acid to inositol and

phosphoric acid (Lolas and Markakis, 1977), thus removing the reactive phosphate groups, and reducing the amount of mineral binding (Cheryan, 1980). Becker et al. (1974) discovered that in California small white beans, phytase activity was optimal when soaking at 35 - 45°C, while Chang (1977) found optimal activity to be at 50 - 60°C.

Inactivating all phytic acid may not be desirable if short cooking times are required. When phytic acid is not present, these divalent cations, such as magnesium and calcium, act to form cross-links between pectic substances in the middle lamella (Stanley and Aguilera, 1985). Cross-linking leads to reduced cell separation and longer cooking times. When phytic acid is present, it chelates these divalent cations, thereby limiting cross-linking (Jones and Boulter, 1983; Moscoso et al., 1984). This leads to increased solubility of the middle lamella pectic substances and cell separation, followed by quicker cooking times (Jones and Boulter, 1983; Moscoso et al., 1984). Reducing phytic acid in dry beans by 65% increased the cooking time five times that of the original (Kon and Sanshuck, 1981). Kon (1979) also discovered that California small white beans, soaked under moderate temperatures of 45°C for 4.5h, had reduced phytic acid, but still maintained a relatively short cooking time of 26 min.

1.3.2. Tannins

Tannins are essential sondary metabolites produced by plants to defend against fungal, bacterial, and viral attack (Salunkhe, 1990). These flavanol polyphenols are primarily located in the seed coat with negligible amounts in the

cotyledon (Salunkhe, 1990; Elias et al., 1979). Dry bean tannin concentrations vary with seed coat colour. White colored field beans have tannin concentrations as low as of 1.3 mg/g while black colored beans contain 42.5 mg/g (Elias et al., 1979).

The ability of tannins to inhibit digestive enzymes and interfere with mineral absorption is a nutritional concern in field beans. High molecular weight condensed tannins present in pulses use multiple phenolic hydroxyl groups to effectively cross-link with, and further precipitate proteins and other macromolecules (Salunkhe, 1990), rendering them unabsorbable in the human body. Complexing and inhibiting proteolytic and digestive enzymes, such as trypsin, alpha-amylase, and lipase (Griffiths, 1979; Griffiths and Moseley, 1980; de Lumen and Salamat, 1980) decrease net metabolizable energy, protein utilization, and the efficiency ratio (Moseley and Griffiths, 1979; Salunkhe, 1990). Additionally, tannins naturally chelate divalent metal ions, to form insoluble complexes, rendering them less absorbable (Srikantia, 1976; Narasinga Rao and Prabhavathi, 1982) in the body.

In contrast, other researchers have indicated that tannins are not nutritionally harmful when ingested. Digestive enzymes that appear to be inhibited by tannins *in vitro*, were not significantly inhibited *in vivo* (Salunkhe, 1990). It has been suggested that the major dietary effect of condensed tannins on digestion, is not the inhibition of endogenous enzymes (proteins), but rather the formation of less digestible compounds with dietary proteins (Salunkhe, 1990; Reddy et al., 1985). It is suspected that tannins preferentially bind to prolamins

and glycoproteins (Butler et al., 1984) that do not play an important role in the *in vivo* digestion of bean proteins (Durigan et al., 1987).

Nevertheless, the elimination of tannins in field beans would be desirable to increase field bean nutrition, but their high heat resistance makes their removal and/or inactivation difficult. Studies have shown that approximately 50% of faba bean tannins are heat stable at temperatures of 100°C for 12h and 125°C for 2h (Ziena et al., 1991). White field beans required cooking for 20 min at 121°C to lose 52.6% of their tannin concentration (Elias et al., 1979). Based on the light color of navy beans, we do not expect a large amount of tannins to be present, but their high heat resistance may allow what is present, to remain active after micronization.

1.3.3. Trypsin inhibitors

Trypsin inhibitors are located in the cotyledons and testa (seed coat) of field beans (Wilson et al., 1972). Their direct function is unknown, but it has been suggested that they can control endogenous proteinases, act as storage proteins, and participate in a plant's defense mechanism against microbial or inst diseases (Ryan, 1973). Trypsin inhibitors are one of many potential anti-nutritive components of field beans (Griffiths, 1979). Sayeed and Njaa (1985) discovered a 0.828 negative correlation between *in vitro* protein digestibility and trypsin inhibitor activity from *Phaseolus vulgaris* beans.

Raw navy beans contain 18,703 - 23,070 trypsin inhibitor (TI) activity units per gram (Dhurandhar and Chang, 1990; Occena et al., 1997). Trypsin inhibitors

(TI) represent 2.6% of total navy bean protein and 40% of the protein's cystine (Kakade et al., 1969). The nutritional significance of cystine in the diet makes trypsin inhibitor inactivation, and not removal, very desirable. Kakade et al. (1969) showed that when feeding chicks raw navy bean trypsin inhibitors, they lost 1.8 g/day and only 44.5% of the cystine ingested was absorbed. When fed heat treated navy bean trypsin inhibitors, chicks gained 3.3 g/day and 76.3% of the cystine consumed was absorbed. Micronizing reduces trypsin inhibitors in field beans from 2.48 to 0.21 TIU/mg (McNab and Wilson, 1974). This decrease in trypsin inhibitors is responsible for increases in food conversion efficiency and nutritive value compared to a raw bean diet (McNab and Wilson, 1974).

Trypsin inhibitors can exhibit high heat or low heat resistance depending on the physical state of the substrate (Carvalho and Sgarbieri, 1997; Rayas-Duarte et al., 1992). van der Poel et al. (1990) agree, but also state that the effectiveness of a treatment on trypsin inhibitors is influenced by moisture, temperature, and time. Under moist conditions at 102°C and 300 kPa for 40 min, TI activity was reduced to 9.4% (van der Poel et al., 1990) in dry beans. Increasing the temperature to 119°C under the above conditions reduced TI activity to 1.9% (van der Poel et al., 1990). Higher temperatures resulted in greater reductions of TI activity (van der Poel et al., 1990). A combination of high moisture and high temperature, are the best conditions to inactivate trypsin inhibitors in whole beans. It is expected that when micronizing whole beans at temperatures of 98 - 104°C, and moisture contents of 25%, some trypsin inhibitors will be inactivated.

1.3.4. Oligosaccharides

Navy beans contain approximately 60% carbohydrates, of which, 5.3 - 8.1% are the oligosaccharides sucrose, raffinose, stachyose, and verbascose (Reddy et al., 1984). Navy beans contain 2.2 - 3.5% sucrose, 0.4 - 0.7% raffinose, 2.6 - 3.5% stachyose, and 0.1 - 0.4% verbascose (Reddy et al., 1984). The oligosaccharides from the raffinose family, raffinose, stachyose, and verbascose, are responsible for the pain and discomfort associated with flatulence production in humans and animals (Reddy et al., 1984; Vishalakshi et al., 1980; Flemming, 1981; Iyer et al., 1980; Jood et al., 1985; Abdel-Gawad, 1992). Humans do not posses the necessary alpha-galactosidases to break down these oligosaccharides. These sugars pass through the gut and into the colon where bacteria metabolize these sugars, producing carbon dioxide, hydrogen, and methane (Becker et al., 1974).

The concentration of oligosaccharides in beans can be reduced by diffusion and enzymatic degradation. Oligosaccharides are water soluble, making them extractable, by diffusion, during soaking and cooking (Vidal-Valverde, 1993). The enzyme alpha-galactosidase is present in beans. If it is released and activated, this enzyme will break down these oligosaccharides into smaller sugars such as sucrose. Beans soaked at temperatures of 45-65°C will optimize alpha-galactosidase action on stachyose and raffinose (Becker et al., 1974). Incubating navy beans at 60°C for 1.5 h and 45°C for 4.5 h during tempering may activate the alpha-galactosidases and reduce the amount of oligosaccharides present.

1.4. Navy bean microflora

1.4.1. Bacteria

Plants are often contaminated with numerous types of organisms through their contact with soil and water. Depending on the type of soil and environmental conditions, the types of bacteria present can largely differ (Banwart, 1989). The bacteria *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Alteromonas*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Cytophaga*, *Escherichia*, *Flavobacterium*, *Klebsiella*, *Micrococcus*, *Moraxella*, *Paenibacillus*, *Pseudomonas*, *Psychrobacter*, *Streptococcus*, *Streptomyces*, *Vagococcus*, and *Vibrio* are all common contaminants in water and/or soil (Banwart, 1989; Jay, 1996). However, a plant environment is not suitable to sustain life for many of these organisms. The organisms that tend to adhere and sustain life on plants are lactic acid bacteria, *Corynebacteria*, *Curtobacterium*, *Pseudomonas*, and *Xanthomonas* (Jay, 1996).

Of the soil organisms, there are two important spore-forming bacteria, *Bacillus* and *Clostridium*. They are both gram-positive rods, but differ in their growth requirements (Francis, 2000). *Bacillus* is aerobic and *Clostridium* is anaerobic (Francis, 2000). *Bacillus cereus* and *Clostridium botulinum* are organisms from these genera that have the capability to cause food poisoning and food intoxication respectively (Francis, 2000). These organisms, in addition to non-sporeforming pathogens, *Listeria monocytogenes* and *Escherichia coli*, need to be investigated for their presence on pulses to ensure that micronized navy beans are safe for human consumption.

1.4.2. Yeast and mould

Mould spoils approximately 10 - 20% of the annual cereal crop in the world. Most moulds can grow at a water activity of 0.8, while some xerophilic moulds can grow at water activities as low as 0.65 (Francis, 2000; Ockerman, 1991). Field crops develop mould microflora involving the genera *Cladosporium*, *Alternaria*, *Phoma*, *Drechslera*, *Epicoccum*, and *Fusarium*. Once dried and placed into storage, field microflora are overgrown by storage fungi including the genera *Penicillium*, *Aspergillus*, and *Eurotium*. The only field fungi that persist with storage fungi is *Fusarium*. The most important factor allowing the overgrowth of storage fungi is water activity. Field fungi can't grow on dry surfaces, but storage fungi can. Storage fungi have the ability to produce small, resilient spores that persist on plants and eventually sporulate. To inhibit the spoilage of pulses during storage, the moisture content must remain below 12.5 - 13%. (Robinson et al., 2000)

Yeasts are also present, but do not play an integral role in dry product storage. Most yeast are unable to grow at water activities below 0.87, making their impact on dry bean microflora minimal (Francis, 2000).

1.5. Water activity and its relationship to microbial activity

For millions of years people have reduced water activity to control microbial spoilage and preserve their foods (i.e. Salting, drying, and concentrating) (Jayaprakasha et al., 1997; Francis, 2000). Water activity is a measure of the amount of free water in a food system and is represented by the water vapor

pressure of the substance divided by the vapor pressure of pure water (Jayaprakasha et al., 1997; Giese, 1997). Water activity is an important factor contributing to microbial, chemical, and enzymatic reactions leading to spoilage (Jayaprakasha et al., 1997; Fontana, 2000).

Water activity is a major factor affecting the microbial responses of foods (Francis, 2000). Altering a food's water activity will impact a microorganism's survival, growth, sporulation, germination, morphology, and metabolite production (Jayaprakasha et al., 1997). Each organism has its own critical or minimum water activity for various lifecycle and metabolic stages. Table 2 outlines many organisms and their required water activity for growth. To inhibit all microbial growth and have microbiologically stable beans the water activity should be 0.60 or below during storage and transport (Labuza, 1980; Jayaprakasha et al., 1997; Ockerman, 1991; Francis, 2000).

1.5.1 The effect of storage conditions on water activity

Storage conditions will affect water activity. When foods are stored in an environment of higher or lower relative humidity (R.H.), the beans will equilibrate over time by absorbing or desorbing water (Jayaprakasha et al., 1997). Since temperature affects R.H, both temperature and R.H. are key environmental storage conditions that need to be monitored to prevent microbial growth on navy beans.

Table 2: Water activity's relationship to microbial growth

Minimum Water Activity For Growth	Microorganisms Inhibited
1.0-0.95	Gram-negative rods, bacterial spores, some yeasts
0.95-0.91	Most cocci, lactobacilli, vegetative cells of bacilli, some molds
0.94	*Clostridium botulinum* growth and toxin
0.91-0.87	Most yeasts
0.80	Most molds
0.86	Aerobic growth of *Staphylococcus aureus*,
0.8-0.75	Most halophilic bacteria
0.8	Production of mycotoxins
0.75-0.65	Xerophillic molds
0.68	Practical limit for fungi
0.65-0.6	Osmophillic yeasts

(Francis, 2000)

1.6. Thermal inactivation of microorganisms

All microorganisms can be destroyed by heat. Many factors impact the time and temperature required to inactivate specific organisms (Nickerson and Sinskey, 1972). Individual organism characteristics, as well as the food's water

activity are two important factors that will impact the ability of heat treatments such as micronization to kill navy bean microflora.

1.6.1. Microorganism type and lifecycle stage's effect on thermal inactivation

The type of microorganisms and their lifecycle stages have a large impact on the susceptibility of an organism to heat. Vegetative bacteria are much less heat resistant than bacterial spores (Nickerson and Sinskey, 1972; Prescott et al., 1990; Kamat et al., 1989) and much more heat resistant than vegetative yeast and mold. However, yeast spores have approximately the same heat resistance as vegetative bacteria, while mould spores have higher heat resistance than either (Prescott et al., 1990), but not as high as bacterial spores. When *B. cereus* spores and vegetative cells were heated at 90°C, the spores required up to 39 times longer exposure than the vegetative cells to obtain a 1 log reduction (Kamat et al., 1989).

1.6.2. Water activity and its effect on thermal inactivation

When minimal growth conditions are not met, organisms often enter the dormancy. The dormant phase, often associated with organisms on dry foods, is a phase where cells do not grow, but survive on minimal metabolic activity. These cells have adapted to adverse conditions making them resilient and much harder to kill. Organisms in high moisture or high water activity foods are more heat labile than those in dry foods (Ray, 1996; Nickerson and Sinskey, 1972).

When organisms are in high water activity foods, they begin to reproduce and metabolize (Ray, 1996). When undergoing growth and metabolic reactions, organisms are much more susceptible to damage and death by heat treatments. Although high moistures and water activities during tempering will allow for the growth of many organisms, it will also enhance the destruction of microorganisms during micronization.

1.7. Micronization

1.7.1 The process

Micronization is a food processing method that heats foods through the use of infra-red heat (Sarantinos and Black, 1996). This process involves the application of electromagnetic radiation, with a wavelength of 1.8 - 3.4um, onto a food that has been tempered to a set moisture content (Sarantinos and Black, 1996; McCurdy, 1992). To do this on a large scale, the product is funneled through a vibratory feed onto a vibrating bed, to provide maximum surface exposure and minimum heat damage (Blenford, 1980). While moving down the bed, gas fired ceramic radiators emit infra-red radiation onto the beans causing constituent molecules to vibrate and release energy in the form of heat (McCurdy, 1992). Once exiting the bed, the product is cooled and sent on for further treatments (i.e. flaking etc.) or packaging. Figure 3 is a picture of a commercial micronizer at Infra-Ready Foods Ltd. in Saskatoon, Saskatchewan.

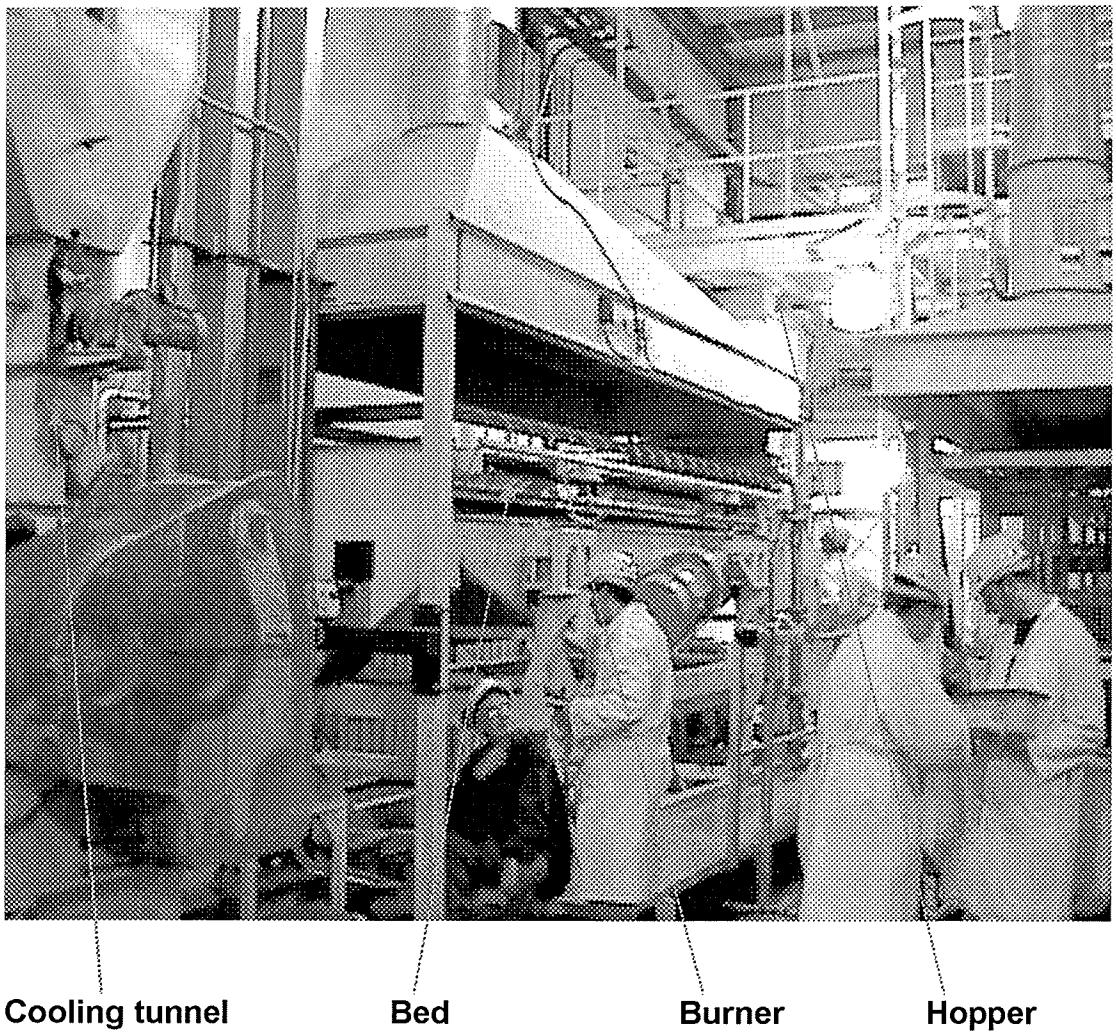

Figure 3. Photograph of the commercial micronizer at Infra-Ready Foods Inc. in Saskatoon, Saskatchewan.

1.7.2. Applications

Micronization has previously been used to remove cocoa bean shells, roast and toast products, and reduce cocoa bean bacterial loads (Murray, 1987). It has also been established as a method to gelatinize starch in cereals and legumes, and to eliminate enzymes, such as lipase, and some anti-nutritional factors in oilseeds (Blenford, 1980).

The ability of micronization to pre-cook grain legumes and substantially reduce their cooking time has been controversial (Sarantinos and Black, 1996). Early work determined that pinto beans soaked to 17% moisture, and micronized at 99°C, exhibited an increase in water absorption rate and capacity, but also a 25% increase in cooking time (Abdul-Kadir et al., 1990). Similarly, chickpeas at 17% moisture (soaked for 1 min) and micronized at 88°C for 300 s, exhibited seed hardening and decreased cookability compared to raw chickpeas (Sarantinos and Black, 1996). More recent work using lentils, has indicated that moisture content and its uniformity across the seed, prior to micronization, are the critical factors governing the ability of micronization to reduce cooking times (Cenkowski and Sosulski 1997; Arntfield et al. 1997). These researchers have indicated that when a critical moisture content is reached through tempering, micronization will gelatinize starch and increase protein solubility, leading to softer lentils and reduced cooking times. Based on this, perhaps micronization can offer similar advantages to navy beans when a critical moisture content and the correct micronization parameters are used. Recent research has also identified infra-red heating as an effective method to reduce microbial contamination in foods (Sakai and Hanzawa, 1994; Sarantinos and Black, 1996). Micronizing fungally infected chickpeas for 300 s at 88°C eliminated all xerophilic fungi (Sarantinos and Black, 1996). The primary mode of destruction has not been determined, but is suspected to be due to thermal effects (Sakai and Hanzawa, 1994). Infra-red heating does not have enough power to destroy microorganisms immediately, but when penetrating the food, energy is absorbed

at the surface, and the product's temperature rises (Sakai and Hanzawa, 1994), implying possible thermal destruction.

1.8. Cooking and the role of starch

1.8.1 The cooking process

When dry beans are first introduced to water, water can penetrate the hilum, micropyle, and raphe (Swanson et al., 1985). The hilum is the primary site for water imbibition. Water penetrates the seed at the hilum and is transported to the periphery of the cotyledon through the spongy parenchyma cells and into the inter-cotyledon space (Varriano-Marston and Jackson, 1981). These three critical points of water entry can be seen in Figure 1. Regardless of the method of entry, water must enter the seed and be fully distributed to fully cook the bean. Aguilera and Stanley (1985) have suggested that legume cooking is a two-phase process. Initially the middle lamella breaks down and cell separation occurs. Once separation has occurred, starch granules within the parenchyma cells will gelatinize, leading to bean softening. This indicates that the ability of beans to imbibe water, which leads to middle lamella breakdown, cell separation, and starch gelatinization, will strongly impact the cookability of legumes.

Soaking solutions can also affect cookability. Cooking cowpeas in hard water, containing large amounts of Mg and Ca, increased bean hardness, and reduced pectin solubility and water absorption (Uzogara et al., 1990). Contrary

to this, cooking in salt solutions (e.g. sodium carbonate) reduced cowpea hardness at the same cooking time (Uzogara et al., 1990; Singh et al., 1988).

1.8.2. Starch gelatinization

Starch is a prominent and important component in foods, in that it contributes to its textural characteristics (Elbert and Witt, 1968). Starch is commonly used industrially and modified in many foods to achieve a desired texture (Lee et al., 1995). During traditional stove-top cooking, starches swell and gelatinize to achieve a softer, more edible texture.

The prominent components of starch, amylose and amylopectin, are both involved in starch gelatinization. When water, in conjunction with heat, is added to starch, weak hydrogen bonds in the amorphous regions of the granule rupture and the granule swells (Lineback and Inglett, 1982). The stronger bonds remain intact and hold the granule together during swelling (Lineback and Inglett, 1982). When a specific temperature, characteristic of that starch, is reached, these swollen granules gelatinize, which leads to softening and a loss of birefringence (Lineback and Inglett, 1982; Lee et al., 1995).

The gelatinization temperature of starch granules will vary depending on granule size and amylose content (Banks and Greenwood, 1975). Larger granules are less resistant to gelatinize and higher amylose contents result in lower gelatinization temperatures (Banks and Greenwood, 1975). Additionally, environmental conditions, such as available water and space, will affect the gelatinization temperature and degree of gelatinization (Fujimura and Kugimiya,

1994). It has been shown that dried beans, with 9% moisture, do not fully gelatinize after soaking for 2 h at 70°C followed by cooking for 60 min. Yet, fresh beans that were not soaked and contained 57% and 63% moisture, completely gelatinized after 60 min of cooking (Elber and Witt, 1968). With higher moisture, prior to cooking, starch is gelatinized much faster.

Navy bean starch is reported to have a gelatinization temperature of 66 - 77°C (Schoch and Maywald, 1968; Biliaderis et al., 1979). With an external output temperature of 98 - 103°C during micronization and an input moisture contents of 20% and 25%, it is expected that micronization will partially gelatinize navy bean starch, leading to quicker cooking times.

1.9. The effects of storage conditions on bean cookability and texture: The hard-to-cook phenomenon

The storage environment of beans is a critical factor affecting cookability. Storage under high relative humidities and temperatures can induce hard shell and the hard-to-cook (HTC) phenomenon (Stanley and Aguilera, 1985). Hard shell affects the rate of absorption, while HTC beans involve cotyledon hardening. Hard shell beans can be identified at the initial hydration stage by a reduced rate or lack of water absorption, but cotyledon hardening in HTC beans can not be detected that early.

HTC beans develop primarily by chemical means rather than physical, as is the case for hard shell (Stanley and Aguilera, 1985). HTC beans exhibit equal

or greater swelling rates compared to fresh beans (Varriano-Marston and Jackson, 1981). Their water absorption rates and capacities can vary from being equal, to slightly greater than, to slightly less than non-HTC beans (Plhak, et al., 1989; Varriano-Marston and Jackson, 1981). One distinct difference is the loss of 10 times more solubles when soaking HTC beans than normal beans, due to cellular damage of the plasma lamella during storage (Shomer et al., 1990). At the microscopic level, starch granules appear structurally damaged in HTC beans (Paredes-López, et al., 1988; Paredes-López et al., 1989a), but the gelatinization temperatures or transition enthalpies remain unchanged (Paredes-Lopez, et al., 1988; Hohlberg and Stanley, 1987).

HTC beans are most often associated with storage under high temperatures and relative humidities for long periods of time (Burr et al., 1968; Aguilera and Stanley, 1985; Jones and Boulter, 1983). These conditions lead to reduced cell separation (Jones and Boulter, 1983) and cotyledon hardening (Varriano-Marston and Jackson, 1981), resulting in hard textured beans. Since cell separation is the first stage of cooking, HTC beans require prolonged cooking times to achieve the desired cooked texture, if at all possible.

The HTC phenomenon is suggested to develop due to limited middle lamella dissolution and limited cotyledon cell separation. The exact mechanism is not known, but several researchers have suggested that reversible and irreversible mechanisms are involved (Aguilera and Rivera, 1992; del Valle and Stanley, 1995). The reversible mechanism involves the formation of insoluble cationic crosslinkages between demethoxylated pectic substances in the middle

lamella due to the inactivation of phytic acid (Jones and Boulter, 1983; del Valle and Stanley, 1995). During bean storage at high R.H. and temperatures, enzymes like phytase break down phytic acid, releasing bound divalent cations. These cations migrate to the middle lamellar pectin where they are enzymatically demethoxylated, and the cations engage in crosslinking reactions forming calcium magnesium pectates (Mafuleka et al., 1993; del Valle and Stanley, 1995; Shomer et al., 1990). Not as much is known about the irreversible effects. The irreversible mechanism is suspected to involve the strengthening of cell walls by lignification of the middle lamella (Stanley and Aguilera, 1985). It is suspected that lignification involves the accumulation of aromatic compounds, such as tannins (Stanley, 1992), at cell wall surfaces where they act as precursors in lignification-like reactions (del Valle and Stanley, 1995). The reversible mechanism is primarily affected by water activity, while the irreversible mechanism is impacted more by temperature (del Valle and Stanley, 1995). Ultimately, the lack of water absorption and cell separation could inhibit many navy bean reactions that can take place during micronization.

Sensory evaluations are commonly used to determine the desired cooking time and textural properties of a food. Garruti and Bourne (1985) investigated the sensory characteristics of red kidney beans stored at 30°C and 40°C and 80% R.H.. These beans exhibited increased hardness, fracturability, lumpiness, chewiness, and skin toughness compared to beans stored at 2°C. This indicates that bean textural characteristics are strongly affected by high temperatures and R.H., which are likely to yield HTC beans.

MATERIALS AND METHODS

2.1. Materials

2.1.2. Navy beans

Raw navy beans were grown in rural Manitoba during the 2000 and 2001 crop years. They were dried and stored over the winter (temperatures ranging from 25°C to -10°C) at Roy Legumex Inc., St. Jean Baptiste, Manitoba. Beans were delivered to the research facility in the spring of 2001 and 2002, where the beans were stored at 4°C.

2.1.3. Chemicals and supplies

Chemicals and supplies used in the analysis of micronized navy beans are listed in Table 3.

Table 3: Chemicals and supplies

Analysis	Material	Company	Catalog number
Gelatinized starch	Amyloglucosidase	Sigma Chemical Co.	A7255
	Grade GF/C Whatman glass microfiber filters	Whatman, Fisher Scientific	09-874-37
	O-toluidine reagent	Sigma Chemical Co.	635-6
	Trichloracetic acid	Fisher Scientific	A322-3
	D-(+)-Glucose	Sigma Chemical Co.	G-7528

	Anhydrous sodium acetate	Fisher Scientific	BP 333-500
Bacterial Enumeration	Standard methods agar	BBL, Fisher Scientific	B11638
	Potato dextrose agar	BBL, Fisher Scientific	B11550
	Tartaric acid	BBL, Fisher Scientific	A314-500
	GasPak plus	BBL, Fisher Scientific	B71040
	Sterile calcium alginate swabs	Fisher Scientific	14-959-81
	Sterilized disposable petri-dishes	Fisher Scientific	08-757-13
	GasPak jar systems	BBL, Fisher Scientific	11-814-21
	Anaerobic jar indicator strips	BBL, Fisher Scientific	11-814-36
	Brilliant green lactose bile broth (BGLB)	BBL, Fisher Scientific	B11080
	Lauryl sulfate tryptone (LST)	Difco, Fisher Scientific	DF 0241-17-0
	Lactobacilli MRS agar	Difco, Fisher Scientific	DF 0882-17-0
	Nutrient Broth	BBL, Fisher Scientific	B11-479
	Tetramethyl-p-Phenylenediamine	Sigma Aldrich Chemicals	23,469-9
	Potassium Hydroxide	Fisher Scientific	P25-1
Moisture Content	Aluminum weighing dishes	Fisher Scientific	08-732 & 08-732-1
	U.S. Standard stainless steel 80 and 100 mesh sieves	Fisher Scientific	04-884-1AV & 04-81X

Phytic acid	AG-1-X8 Resin	Bio-Rad Laboratories	140-1451
	Phytic acid (Inositol-hexaphosphoric acid)	Sigma Chemical Co.	P-8810
	Chromaflex columns size 23	Fisher Scientific	420100-0023
Tannin	Vanillin (4-hydroxy-3-methoxyben-aldehyde)	Sigma Chemical Co.	V2375
	(+)-Catechin	Sigma Chemical Co.	C-1251
Trypsin	No.1 Whatman filter paper	Fisher Scientific	1001-090
	BAPNA (N∝-Benzoyl-DL-Arginine p-Nitroanilide)	Sigma Chemical Co.	B-4875
	Trypsin – bovine pancreas	Sigma Chemical Co.	T-1426
General Use Chemicals	Methanol	Fisher Scientific	A 452-4
	Hydrochloric acid	Fisher Scientific	A-144C-212
	Sodium hydroxide	Fisher Scientific	BP359-212
	Sodium chloride	Fisher Scientific	S271-3
	Acetic acid, glacial	Fisher Scientific	A38S-212

2.2. Methods

2.2.1. Tempering

Three and a half kilograms of navy beans was weighed into a 18.5 L plastic bin. A pre-determined amount of distilled water was added (pre-heated to

a specific temperature if required), to raise the moisture content of the navy beans to the desired level. The following equation was used to determine the amount of distilled water to add:

$$\text{Weight of } H_20 = \frac{\text{weight of sample x [target \% moisture – actual \% moisture]}}{\text{[100 – target \% moisture]}}$$

(Arntfield et al., 1997)

The mixture was then shaken every 10 min for the first hour to evenly distribute the water over the seeds. The mixture was then left to equilibrate to the desired moisture content under the specified time and temperature conditions.

2.2.2. Micronization

The tempered navy beans were micronized using a 4 lamp pilot scale MR2 Micronizer from the Micronizing Company (UK), FramLingham, England. Initial experiments by Bellido (2003) indicated that micronizer parameters should be set at a slope of 5.0, gas mixture of 9, and feeder rate of 60 to yield an output temperature of 95-100°C. The output temperature was read with an infra-red thermometer (emissivity was assumed to be 0.95). These conditions were used for the preliminary microbiological analysis of navy beans.

Further research done by colleagues indicated that a residence time distribution (RTE) of 160 ± 4.0 s produced the most uniform treatment with an output temperature of 98-105°C (Cinq-Mars et al., 2003) when navy beans were

tempered to 25% for 16-24h (Cinq-mars et al., 2003). All further experimentation was done using these conditions.

Great northern (a variety of *Phaseolus vulgaris*) bean samples were collected from a commercial micronization operation for microbial analysis. Facilities at Infra-Ready Foods Ltd. in Saskatoon, Saskatchewan, were used. The beans were tempered to 13 % moisture overnight, and micronized in a 16 lamp micronizer (Micronizing Company (UK), FramLingham, England). The outlet temperature was approximately 124°C.

2.2.3. Chemical assays

Sample preparation

Following micronization, navy bean samples were dispersed on a tray in a single layer and dried for 27 h at room temperature (19-21°C) at a relative humidity of 51-55%. If analysis could not be conducted the same day, samples were sealed in Ziploc bags and stored at 5°C until analysis could be done (up to 4 months). All samples were ground for 2 min using a Stein Laboratory Mill (Fried Stein laboratories, Inc. Atchison, Kansas) and passed through an 80 mesh sieve, unless otherwise stated, and the assays listed in the following sections were conducted.

2.2.3.1. Determination of trypsin inhibitor

Trypsin inhibitor determinations were done following the method of Smith et al., (1980) in conjunction with the AACC Method 22-40, 1999.

Sample Preparation

Navy bean samples were ground (using the Stein Laboratory Mill), without generating heat, to pass through a 140 mesh sieve. One gram of the sieved sample was then gradually dispersed in 50 mL of 10 mM NaOH under continuous stirring. The solution was adjusted to a pH of 9.4-9.6 with 1 M NaOH or 1 M HCl and left overnight at 4°C. The following day, the sample was stirred for approximately one hour at which time the sample had reached room temperature. Samples were then diluted with deionized water so that 1 mL of sample inhibited 40-60% of the trypsin. If the resulting values were not in this range, the samples were re-diluted and analyzed accordingly.

Solution Preparation

A trypsin standard solution (20 mg of bovine trypsin in 1 L of 0.001N HCl), which remained stable for 2 weeks, was prepared. A BAPNA solution was prepared fresh daily by dissolving 40 mg of benzoyl-DL-arginine-p-nitroanilide hydrochloride in 1mL of dimethyl sulphoxide and diluting to 100 mL with pre-warmed tris buffer at 37°C. The tris buffer was prepared by adding 6.05 g of hydroxymethyl methylamine and 2.94 g of $CaCl_2$ $2H_20$ in 900 mL of deionized water and adjusting to a pH of 8.2 with 1M HCL. All solutions were pre-heated to 37°C.

Assay

A reagent blank, a sample blank, and a standard solution were prepared in duplicate for each sample. Two mL of deionized water was placed in a test tube for the reagent blank. Sample blanks contained 1 mL of deionized water

and 1 mL of diluted sample extract. The standard was prepared with 2 mL of deionized water and 2 mL of standard trypsin solution. The navy bean samples were prepared by adding 1 mL of deionized water, 2 mL of standard trypsin solution, and 1 mL of diluted sample extract in a test tube. All test tubes were vortexed and preheated to 37°C for approximately 10 min. Five mL of BAPNA was then added to all test tubes at 20 s intervals. Each test tube was then vortexed and incubated for exactly 10 min. One mL of 30% acetic acid was then added to all test tubes to stop the reaction. Two mL of standard trypsin solution was then added to the reagent and sample blanks, to bring each test tube volume up to 4 mL. The solutions were vortexed and filtered with No. 1 Whatman filter paper and absorbance read at 410 nm. The color was stable for several hours. Analysis was done in duplicate and the following equations were used to calculate the mg of trypsin inhibited/g of sample:

a) abs change = (standard trypsin solution abs – reagent blank abs) – (abs of sample – abs of sample blank)

b) % inhibited = $\frac{\text{abs change}}{\text{(standard trypsin solution abs – reagent blank abs) *100}}$
(must be 40-60%)

c) Trypsin inhibited (mg/g sample) = $\frac{\text{(2.632 * dilution * abs change)}}{\text{Weight (g)}}$

Note: abs = absorbance

2.2.3.2. Determination of tannins

The tannin content of navy beans was analyzed using the procedure outlined by Price et al. (1978) and Barampama and Simard (1994). Within 24 h of grinding, 200 mg of the sample was placed in a test tube and shaken for 20 min with 10 mL of methanol. The shaken sample was then centrifuged for 10 min at 653 x g. One mL of the supernatant was then placed in a test tube at 30°C and 5 mL of the vanillin reagent (1% vanillin in methanol with 8% concentrated HCl), which was pre-warmed to 30°C, was added. The test tube was incubated at 30°C for exactly 20 min and the absorbance was read at 500 nm. A blank was prepared for each sample by adding 5mL of 4% HCl in methanol instead of the vanillin reagent.

A catechin equivalent (vanillin is a catechin) standard curve of absorbance versus catechin concentration was prepared ranging from 0.05 to 0.3 mg/mL. Sample absorbance was subtracted from the blank and catechin concentration was determined using the standard curve. This value was multiplied by a dilution factor to determine the mg of catechin equivalents per 200 mg of sample. Tannin levels were reported on a dry weight basis (d.w.b.) and all measurements were done in duplicate.

2.2.3.3. Determination of phytic acid

The procedure by Latta and Eskin (1980) was used to determine phytic acid. A 0.5 g sample was weighed into a 25 mL flask with 10 mL of 2.4% HCL

and mixed for 1 h. The sample was centrifuged at 7800 x g for 10 min and 1 mL of the supernatant was diluted with distilled water to 25 mL.

Chromaflex columns were prepared by packing 0.5g of 200-400 mesh AG-1X8 chloride anion exchange resin into the column, adding 15 mL of 5% HCl, and rinsing with 20 mL of deionized water. Ten mL of the diluted sample was then pipetted into the prepared column. Following the elution of unbound materials, 15 mL of 0.1N NaCl was added. After this solvent had eluted from the column, 15 mL of 0.7M NaCl was added and the eluant was collected in a 25 mL volumetric. This eluant was then diluted to 25 mL with distilled water. Three mL of the sample was placed in a centrifuge tube with 1 mL of Wade reagent (0.03% $FeCl_3(6H_20)$ and 0.3% sulfosalicylic acid in distilled water). The mixture was vortexed and centrifuged for 10 min at 7800 x g. A blank was prepared by mixing 3 mL of water and 1 mL of Wade reagent. The absorbance was read at 500 nm, after zeroing with distilled water. The amount of phytic acid was then determined using a standard curve for phytic acid and reported on a d.w.b. The standard curve was prepared using a stock solution of 200 ug/mL of phytic acid in distilled water diluted to 10, 20, 30, and 40 ug/mL. From each solution, 3 mL was withdrawn and vortexed with 1 mL of Wade reagent, before centrifuging for 10 min at 7800 x g. The samples were read at 500 nm to produce a standard curve of absorbance versus phytic acid concentration. All measurements were done in duplicate.

2.2.3.4. Determination of gelatinized starch

To determine the percent of gelatinized starch in navy beans, the procedure by Chiang and Johnson (1977) was used.

A standard curve was produced using a stock solution of 250 ug/mL of glucose in distilled water. From the stock solution, volumes were withdrawn and diluted appropriately to produce concentrations ranging from 25 to 250ug/mL. Half a mL of each solution was vortexed in a test tube with 4.5 mL of o-toluidine and boiled for 10 min. The test tubes were cooled and 5 mL of glacial acetic acid was added. The samples were vortexed immediately before reading the absorbance at 630 nm. A standard curve of absorbance versus glucose concentration was generated.

To ensure solutions were thoroughly mixed, solutions were vortexed for a minimum of 7-10 s. All measurements were done in duplicate.

2.2.4. Microbial enumeration

Preparation:

Twenty five grams of beans were aseptically added to 225 mL of sterilized 0.1% peptone in a blender jar. The sample was blended for 1 min, stopping at 30 s to allow for better mixing. The blended sample sat undisturbed for 2 min to allow the particles to settle out.

Enumeration of total and sporeforming bacteria, and total and sporeforming yeast and mould followed procedures outlined by Wilkie (1988) and

Vanderzand and Splittstoesser (1992) with minor modifications as noted. All plating was done aseptically and in duplicate.

2.2.4.1. Total bacteria

Ten-fold dilutions of the blended sample were made using 0.1% peptone. The plates were poured plated with tempered (47-50°C) standard methods agar (SMA) and incubated at 32 - 35°C for 48 h aerobically.

2.2.4.2. Bacterial spores

Ten mL of the blended sample was pipetted into sterilized test tubes and incubated in a water bath for exactly 10 min at 80°C. The test tubes were cooled rapidly in ice and pour plated using SMA. The samples were then incubated at 37°C aerobically and anaerobically for 48 h.

2.2.4.3. Yeast and mould

The blended samples were diluted appropriately and pour plated with potato dextrose agar acidified (PDA) to a pH of 3.5 with sterile 10% tartaric acid. The plates were incubated aerobically at room temperature (R.T.) for 5 days.

2.2.4.4. Yeast and mould spores

Ten mL of the blended sample was pipetted into sterilized test tubes and heated in a water bath at 70°C for exactly 10 min. Test tubes were cooled rapidly

with ice, diluted appropriately, and pour plated with PDA. The plates were incubated at R.T. aerobically and anaerobically for 5 days.

2.2.4.5. Coliforms

The blended samples were serially diluted to 3 levels. One mL of each of the 3 dilution levels was placed into 3 test tubes, each containing 10 mL of lauryl sulfate tryptone broth (LST). The 9 test tubes were incubated at 35°C for 48 h and checked for gas production. If gas was present, 1 loopful of the positive dilution series, was transferred into 10 mL of brilliant green lactose bile broth (BGLB). These test tubes were then incubated at 35°C for another 48 h and any test tubes positive for gas production were recorded. The MPN/g of confirmed coliforms in the sample was determined by comparison to published tables (Prescott et al., 1990).

2.2.4.6. Tempering bin swabs

The technique and method for environmental swabbing used is outlined by Wilkie (1998). Once the area was swabbed, serial dilutions were made, and the sample was pour plated with SMA, plates were incubated for 48 h at 32 - 35°C. This procedure was done to ensure tempering bin sanitation.

2.2.5. Presumptive bacterial identification

Preparation:

Colonies for testing were prepared by picking random individual colonies from the SPC agar (of micronized navy beans) and incubating them for 2 days at 32-35°C in nutrient broth. The number of colonies picked from the SPC was determined by the square root of the total number of colonies on a plate with > 100 colonies. This broth was then streaked onto SPC agar and incubated at 32 – 35°C for 48 h. These colonies were then used in the tests described below.

2.2.5.1 KOH test

The KOH (potassium hydroxide) test is a commonly used rapid method for the distinction between gram-positive and gram-negative organisms. A colony was picked off the plate with an inoculating loop and stirred on a glass slide with 1 to 2 drops of 3% KOH. After several sonds, if a reaction had occurred, the solution became viscous and a slime or stringy texture was seen when pulling the loop away from the slide. This was a positive reaction, and thought to be the result of cell wall destruction (lipopolysaccharide) and liberation of the DNA. A positive reaction indicated a gram-negative organism. If the suspension remained watery and no slime developed, no

reaction occurred, and this was indicative of gram-positive bacteria (Gregersen, 1978).

2.2.5.2. Catalase test

The catalase test differentiates between organisms based on the presence of catalase. When catalase is present, the organism catalytically converts hydrogen peroxide into oxygen and water (Prescott, et al., 1990). Two drops of 3% H_2O_2 was placed on a microscope slide. A colony was then picked off of the plate, with a platinum loop, and mixed in the 3% H_2O_2. The production of bubbles (oxygen) indicated the presence of catalase, and the organism was classified as catalase positive. If no bubbles were produced, the organism was catalase negative (Harrigan and McCance, 1976; Prescott et al., 1990).

2.2.5.3. Oxidase test

The oxidase test identifies organisms that contain cytochrome c and the enzymes capable of oxidizing it (the presence of iron-containing enzymes that are able to reduce O_2). A filter paper was first moistened with a few drops of 1% tetramethyl-p-phenylenediamine. With a transfer loop, a colony was then picked from the SPC plate and smeared on the moistened paper. The formation of a purple color on the filter paper within 1 min was indicative of a positive test. (Prescott et al., 1990).

2.2.5.4. Identification of endospore-forming bacteria

A loopful of the organisms grown in nutrient broth was streaked onto nutrient agar plates and incubated with a moist towel, in a plastic bag, for 10 days at 37°C. Colonies were then prepared using the wet mount technique and

viewed under phase contrast using a Zeiss microscope at 1000X magnification. An organism was deemed spore-forming if their shape and refractivity was representative of spores.

2.2.6. Texture determinations

2.2.6.1. Peak force evaluations for cooking time

Navy beans were cooked for 20, 27.5, 35, 38.75, 42.5, 50, and 57.5 min at a slow boil in a 1:10 ratio of beans to water. They were then drained and cooled for 1 - 1.5 h before evaluating. Texture measurements were done as outlined by Arntfield et al. (1997). Thirty gram samples were weighed out and compressed through a 10 cm^2 extrusion bar using the Lloyd Materials Testing Instrument model L1000R (Lloyd Instruments Ltd, Farmham, U.K.). The peak force measurements obtained were based on the maximum force required to pass through the extrusion. All measurements were done in triplicate with a maximum load cell of 1000 N.

2.2.6.2. Sensory evaluation of navy beans

Raw navy beans were soaked for 18 h and cooked under a slow boil for 27.5, 35, 38.75, 42.5, and 50 min., in a 1:10 ratio of beans to water. The samples were then cooled for approximately 1 h and 30 g of each sample was placed in randomLy numbered cups. An expert panel of 6 members evaluated each sample for initial hardness, mouth feel in terms of smoothness, and seed

coat presence on a structured line scale of 1 to 5. Each panelist evaluated the samples in a randomLy assigned order. The panelists were also asked to make comments on these or any other attributes they noticed. Results were discussed and recorded for analysis.

2.2.7. Water activity determination

Whole samples were analyzed for their water activity using the Novasina SPRINT Water Activity Meter model TH500 (Novasina, Axair, Ltd). Measurements were done in triplicate.

2.2.8. Moisture Determination

Moisture was determined by the AACC Method 44-15A (1983). All measurements were done in triplicate.

2.2.9. Design and Statistical Analysis

Preliminary microbiological data was collected to understand and minimize the large increases in bacterial counts during tempering. Various tempering treatments of shorter times and different temperatures were tested. These tempering times and temperatures were chosen in conjunction with ideal conditions to induce endogenous enzymatic activity. These conditions aimed to maximize the breakdown of anti-nutritional factors such as phytic acid and raffinose oligosaccharides. The primary experiment done in this study used a 2 x 4 full factorial. Two moisture contents of 20 and 25% and 4 different tempering

times and temperatures were chosen, based on bacterial activity and optimum enzyme activity. Samples were evaluated to determine the pre-treatment's effect on several antinutritional factors, starch gelatinization, cooking time, microbial changes, water activity, and end moisture content. Other experiments involved looking at microbial comparisons between industrial tempering conditions to those of sterile conditions. Additionally, commercially produced great northern beans were examined for their microbial responses to micronization. A comparison of microbial reduction between the original and optimally chosen micronization parameters for the most even flow treatment was also done. All data were analyzed with Statistical Analysis Software (SAS) computer program, Version 7.1 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

3.1 Textural evaluations

3.1.1. Determination of the ideal cooking time

3.1.1.1. Peak force

Figure 4 illustrates the cooking time of raw navy beans soaked for 18 h and then cooked for times of 20, 27.5, 35, 38.75, 42.5, 50, and 57.5 minutes. The point at which the graph levels off indicates the point at which further cooking will not reduce hardness. According to this graph, the peak force of cooked navy beans levels off after 38.75 min. Yet, due to the high variability between samples, using Tukey's statistical comparison, it was determined that after 27.50 min of cooking, there was no significant difference in peak force. It is apparent that this is not the point where the force values plateau. To further clarify the time required for ideal cooking, an expert sensory panel was used to analyze navy beans cooked for most of the same length of times used for instrumental analysis. The extreme times were not part of the sensory evaluation as they were not necessary for evaluation.

3.1.1.2. Expert panel findings

The sensory panel determined that the ideal end use of micronized navy beans would be a baked bean. The expert panel identified and outlined the ideal textural characteristics of typical baked beans in Table 4. Panelists were asked to evaluate 5 different samples cooked for different times ranging from 27.5 to

50.0 min. They were asked to write comments on each sample and rate each sample

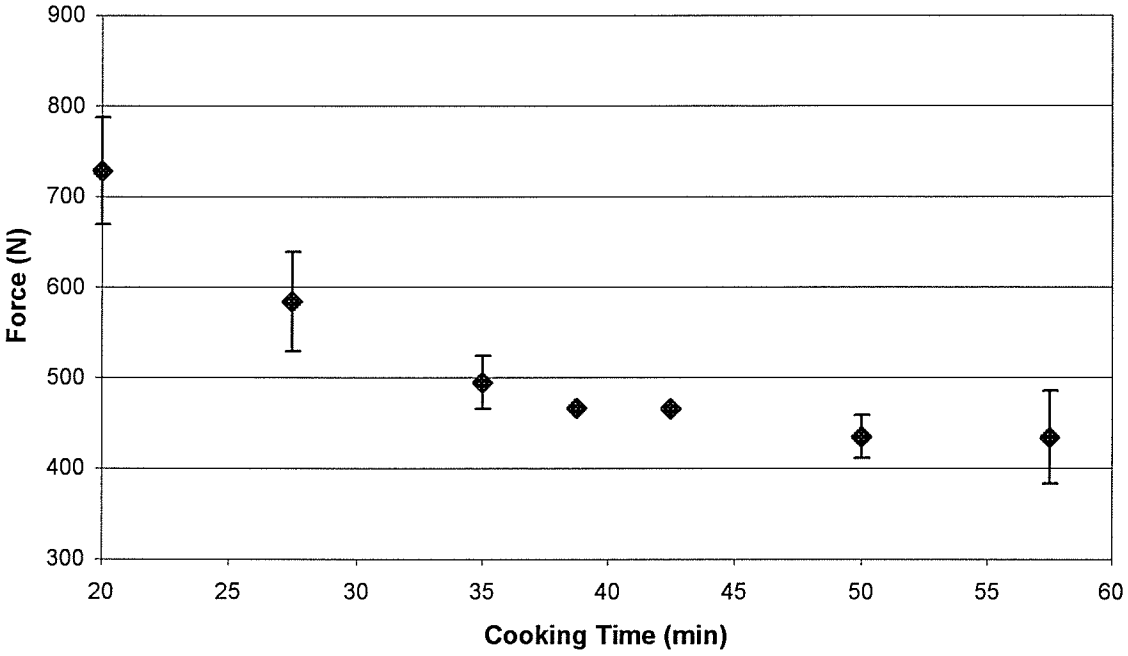

Figure 4. The compression force for presoaked (18h) raw navy beans following cooking for various times. Standard deviation is indicated by the error bars.

based on a scale of 1 to 5, with 3 being just right. Additionally, panelists were asked to identify the cooking time that produced the beans most like the ideal characteristics outlined in Table 4. Based on frequency, the "just right" scale identified 50 min as the target cooking time that would produce beans with textural attributes closest to our target bean texture for hardness, smoothness, and seed coat (Table 5). Despite these "just right" results, the panelists chose 42.5 min as the time that produced the bean with the most ideal baked bean characteristics.

Upon discussion, the group determined that there was high variability and inconsistencies within each cooking time. Several panelists expressed that two beans, within the same sample, were drastically different. They found it very difficult to accurately evaluate the samples. Table 6 lists the panelists' comments for each cooking time. An outline of the experiment including ballot sheet and instructions are presented in Appendix I – The Sensory Evaluation of Navy Bean Texture: Instructions and Ballots.

Table 4. Important sensory characteristics of a baked bean.

Navy Bean Characteristics
Shape - apparent oval shape - appear individually and not squished together - cotyledons remain within the seed
Initial bite - should provide slight resistance, but further mastication should require very little force.
Cotyledons - Smooth with no particulate matter or inconsistencies in smoothness within the seed - Minimal residual in the mouth after swallowing
Seed Coat - Present, but not chewy

Table 5. Panelist results of the "Just Right" scale of raw navy beans soaked for 18h and cooked for various time periods.

Rating	Hardness					Smoothness					Seed Coat Presence				
	Cooking Time (min)														
	27.5	35	38.75	42.5	50	27.5	35	38.75	42.5	50	27.5	35	38.75	42.5	50
1.0	4	2				4	4	1		1	1	2			
1.5				1										1	
2.0	2	4	3	1		2	2	2	3		5	4	3	3	2
2.5			1	2	1			1	2	1				1	
3.0			1	1	4			1	1	4			2	1	4
3.5															
4.0				1											
4.5															
5.0					1										

Note: The number within each box represents the number of panelists that chose that rating for the corresponding sample.

Table 6. Individual and group comments on navy beans soaked for 18h and cooked for different time periods.

Cooking Time (min)	Comments
27.5	Very inconsistent in all attributes Too hard
35	Inconsistent quality Undercooked Seeds not swollen
38.75	Very inconsistent One panelist could not rate due to numerous inconsistencies Interior is coarse Hard at first bite
42.5	Inconsistent Best appearance Slightly particulate
50	Very inconsistent Prominent seed coat because the cotyledon is so soft Seed coat removed, fallen off Seed coat too chewy

General Comments: 42.5 min was chosen as the ideal texture

All 4 analyses presented different results for optimum cooking time. The just right scale indicates that 50 min is the ideal time (Table 6), while the panel chose 42.5 min as the ideal cooking time. The peak force statistical analysis identified that after 27.5 min there is no significant change in force values, while visual interpretation of the graph indicates 38.75 min is an adequate cooking time. Although the raw cooking curve for navy beans plateaus at 38.75 min for peak force, this does not reflect seed coat presence, cotyledon smoothness, or indicate the overall consumer acceptance. Although the just right scale indicated 50 min, 5 of the 6 sensory panel members chose 42.5 min as the ideal bean texture cooking time. Since the ultimate deciding factor for product acceptability is consumer opinion, the ideal cooking time of 42.5 min was chosen and the corresponding force value of 465 N was used for comparison to the micronized navy beans in the experiments to follow.

3.1.2. The effects of residual moisture on peak force

Preliminary experiments were done to determine if the amount of residual moisture following micronization would impact peak force values. Results tabulated in Table 7 indicate that moisture content had a significant impact on peak force when cooked for 27.5 and 35.0 min. Navy beans with residual moisture contents of 20.8% and 21% had significantly lower peak force values than samples with lower moisture content. Scanlon et al. (1998) found that lentils also exhibit reduced cooking times (i.e. lower peak force values) as tempering level and consequently residual moisture increased. To control this factor, from

this point forward samples were air dried at room temperature for 27h, to yield moisture contents ranging from 10.8% – 12.5%.

3.1.3. The effects of pre-treatment conditions on the peak force of micronized navy beans

Peak force values of navy beans were evaluated as a function of tempering treatment and moisture level. Tempering moistures of 20% and 25%, were not significantly different. There was no significant interaction between moisture content and tempering treatment (Table 8). However, when examining the tempering treatment alone, there was a significant difference ($p = 0.0305$). In Figure 5, based on Tukey's test, no treatment was significantly different from the raw, but the tempering treatment of 18h at room temperature and 4.5h at 45°C were significantly different from each other. Perhaps the difference is due to the difference in phytic acid levels. A possible negative trend between phytic acid levels and tempering conditions is discussed in the following section.

3.2. Anti-nutritional Factors

3.2.1. Phytic Acid

The phytic acid concentration of raw navy beans was $1.47 \pm 0.26\%$ which was similar to the literature value of 1.29 – 1.58% (Lolas and Markakis, 1975). Based on the statistics in Table 8, the % of phytic acid in navy beans is

Table 7. The statistical evaluation of residual moisture content and its effect on the peak force (N) of navy beans cooked for different time periods.

Cooking Time (min)	Residual Moisture Following Micronization(%)				
	17.1	18.7	19.7	20.8	21.0
27.5[1]	710.1 ± 5.2 [A]	653.5 ± 29.9 [AB]	N/d	612.7 ± 39.5 [B]	539.4 ± 24.3 [C]
35[1]	511.33 ± 12.6 [A]	542.1 ± 34.6 [A]	508.7 ± 55.2 [A]	474.9 ± 18.3 [AB]	398.6 ± 34.5 [B]
38.75[1]	468.90 ± 8.1 [A]	468.8 ± 20.2 [A]	462.0 ± 36.5 [A]	444.8 ± 37.3 [A]	408.8 ± 23.7 [A]
42.5[1]	447.5 ± 24.8 [A]	440.1 ± 43.4 [A]	431.7 ± 12.5 [A]	427.0 ± 4.7 [A]	374.0 ± 34.5 [A]

[1] Same letters within the same row are not significantly different at $P \leq 0.05$.
Micronization conditions: slope = 5, feeder rate = 60, and gas mixture = 9.
N/d = not determined

Table 8. The statistical probabilities of dependant variables being influenced by independent variables and their interaction.

Dependent Variables	Independent Variables		
	Tempering Treatment	Moisture Level (after tempering)	Tempering Treatment * Moisture Level
% Gelatinized Starch	0.3314	0.9692	0.3490
% Phytic Acid	0.0020	0.3158	0.5314
Trypsin Inhibitor Activity (mg TIA/g)	0.0064	0.6381	0.7164
Tannin (mg cat eq./g)	0.5540	0.3998	0.1611
Peak Force at 42.5 min (N)	0.0305	0.1972	0.7438
Water Activity (Tempered)	0.3284	0.0048	0.2614
Water Activity (Micronized)	0.9160	0.2458	0.4560
Tempered % Moisture	0.7948	0.0011	0.1987
Micronized % Residual Moisture	0.6358	<0.0001	0.3932

* represents the interaction effects

significantly ($P \leq 0.05$) affected by the tempering treatment applied prior to micronizing. According to Tukey's test results in Figure 6, tempering for 1.5h at 60°C retained, and even increased phytic acid to a significantly higher concentration than 4.5h at 45°C and unprocessed navy beans. No treatment significantly reduced phytic acid concentration compared to raw navy beans.

These results were not expected. Toews (2001) showed that tempering green peas at all combinations of 20%, 25%, and 30% moisture for 6h and 20h,

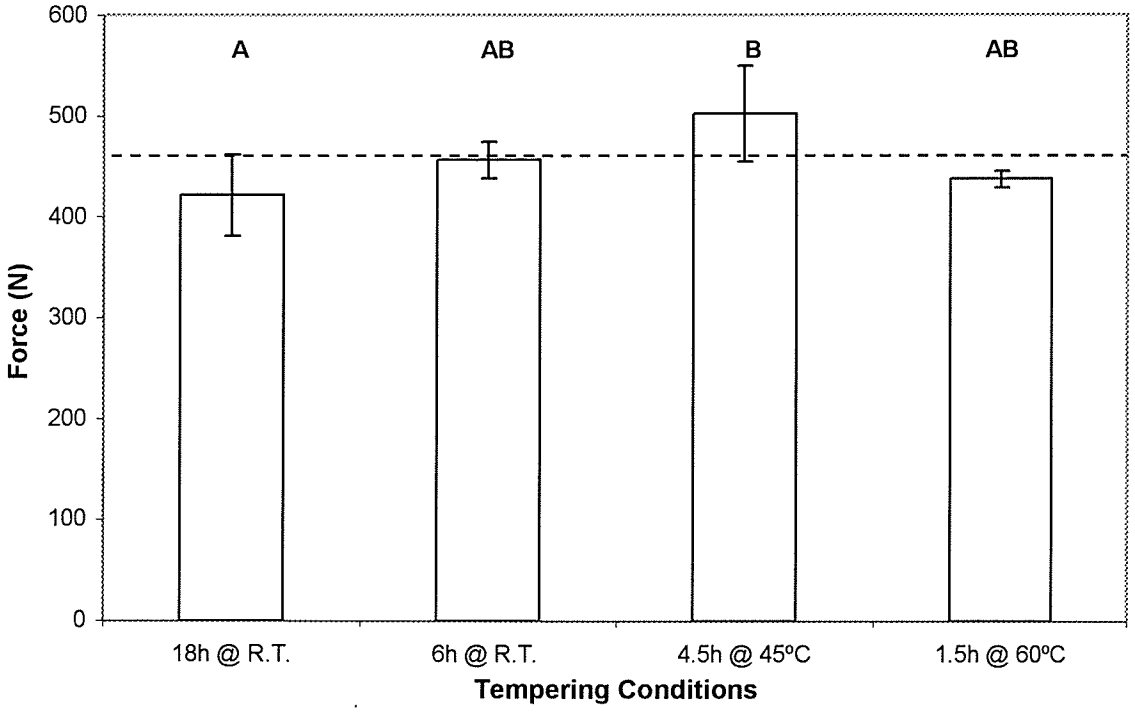

Figure 5. The response of peak force to different tempering treatments when cooked for 42.5 minutes. Different capital letters above the tempering levels indicate significant differences ($P \leq 0.05$). Standard deviation is indicated by the error bars. The dotted line represents the peak force for optimally cooked non-micronized beans ($465 \pm 2N^{AB}$)

followed by micronization, had phytic acid levels lower than the raw samples. Tempering in water at 45°C and 60°C for 4.5 and 1.5 h respectively were chosen to enhance the activation of phytase, which breaks down phytic acid, without increasing the cooking time (Kon, 1979). Although leaching is the major source of phytic acid loss during pulse preparation, experiments by Chang (1977) have shown that 33% of phytic acid can be hydrolyzed by phytase in a moist environment at 60°C. Literature has also indicated that at temperatures above

room temperature, phytase activity is stimulated (Chang, 1977; Becker et al., 1974). Although the tempering chamber was set at 60°C, perhaps the time period of 1.5 h was not long enough for the internal beans to reach 60°C,

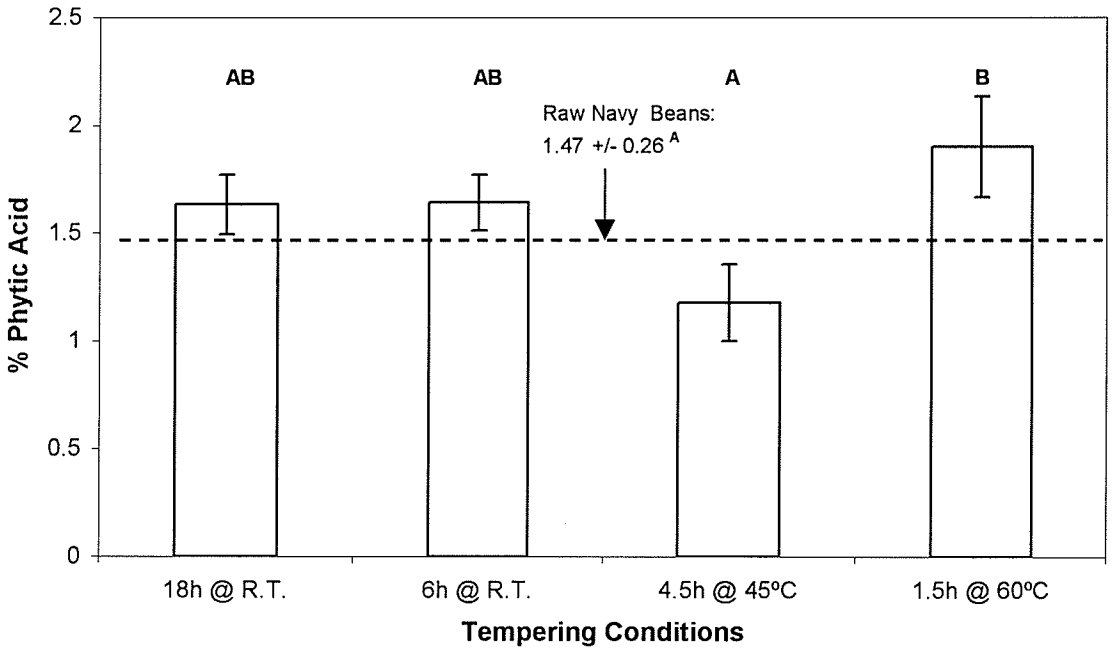

Figure 6. The effects of different tempering conditions on the amount of phytic acid remaining in navy beans after micronization. Different letters indicate significant differences between the tempering conditions at $p \leq 0.05$.

resulting in no destruction of cell membranes and no phytase release and stimulation.

There is no plausible reason for phytic acid to increase when micronizing. This significantly large increase in phytic acid when tempering for 1.5h at 60°C was likely a result of seed variability.

Although there is no significant correlation between the % phytic acid and bean hardness (Table 9), there appears to be a negative trend between the two.

Looking at Figures 5 and 6 and the negative correlation coefficient, there appears to be an inverse relationship between peak force and % phytic acid. Literature has proven that when divalent cations are removed by phytic acid chelation, pectic substances in the middle lamella cross-link and reduce dissolution and cell separation, which leads to longer cooking times (Stanley and Aguilera, 1985; Jones and Boulter, 1983; Moscoso et al., 1984). Although the correlation is not significant (Table 9), it is suspected that the high variability in samples prevented us from obtaining statistically significant data.

3.2.2. Tannins

Due to the light color of navy beans we did not expect a large amount of tannin to be present, but in fact there were 39.94 mg catechin equivalents/g (3.99 %) in raw navy beans. This was high compared to the 1.3 mg of tannic acid/g that literature suggests is present in navy beans (Elias et al., 1979). Perhaps this difference could be attributed to the methods used. The vanillin assay of Price et al. (1978) uses catechin equivalents, which exhibit different reaction kinetics from pure tannin. The root relationship of catechin equivalents, compared to the direct linear relationship of pure tannin, may lead to the overestimation of tannin.

Micronizing navy beans did not significantly change tannin concentrations, regardless of the pre-treatment employed (Table 8). This could be expected considering the high heat resistance of tannins. Cooking white field beans required 20 minutes at 121°C to lose 52.6% of their tannin concentration (Elias et al., 1979), while faba beans retain 50% of their tannin after 12 h at 100°C.

Literature indicates that most tannin losses occur through leaching during cooking and soaking (Barampama and Simard, 1994). Considering this, no significant differences after micronization could be expected.

Table 9. Correlation coefficients of the physiochemical characteristics of micronized navy bean (n = 16)

	% Starch Gelatinized	% Phytic Acid	Trypsin (mg TIA/g)	Tannin (mg cat eq./g)
% Gelatinized Starch	X			
% Phytic Acid	-0.092	X		
Trypsin (mg TIA/g)	0.614[A]	0.155	X	
Tannin (mg cat eq./g)	0.061	-0.067	-0.131	X
Force (N)	-0.091	-0.310	-0.118	0.072
Water Activity (Tempered)	-0.034	0.157	-0.329	-0.130
Water Activity (Micronized)	-0.063	-0.002	0.064	-0.161
% Moisture (Tempered)	0.054	0.131	-0.034	-0.044
% Residual Moisture (Micronized)	0.116	0.221	0.124	-0.263

[A] significant at $p \leq 0.01$

Note: not all correlations are presented due to different conditions for treatments

3.2.3. Trypsin inhibitor activity

The probabilities related to the effects of the independent variables on trypsin inhibitor activity (TIA) are shown in Table 8. The p value of 0.0064 indicates that tempering treatments significantly affected TIA. Navy beans treated for 18h at R.T. and 6h at R.T. had significantly higher TIA ($p \leq 0.05$) than unprocessed beans, and those treated for 1.5h at 60°C (Figure 7).

There has been controversy about the heat stability of trypsin inhibitors in the literature. Most researchers use bean flour to identify the effects of heat and moisture on TIA. Research has also shown that temperature, time and moisture play integral roles in trypsin inhibitor inactivation. Buera et al. (1984) found that when increasing the heat treatment applied to navy bean flour, from 70°C to 100°C, for times ranging from 10 to 50 minutes, trypsin inhibitor reduction increased by a minimum of 40%. Increasing the moisture content of bean flour from 14% to 30%, also increased TIA loss at 90°C and 100°C by factors of 6.5 and 4.5 respectively (Buera et al., 1984). Heat cannot penetrate whole beans as quickly as it can flour, and so the data on trypsin inhibitor inactivation is quite variable. Raw soybeans required 110°C for 40 minutes to destroy all of the TIA (Wilson et al., 1972), while Nitsan (1971) found that autoclaving field beans did not destroy any trypsin inhibitors. Perhaps the internal temperature of the whole beans used in the current investigation did not reach the temperature and time required to inactivate TIA.

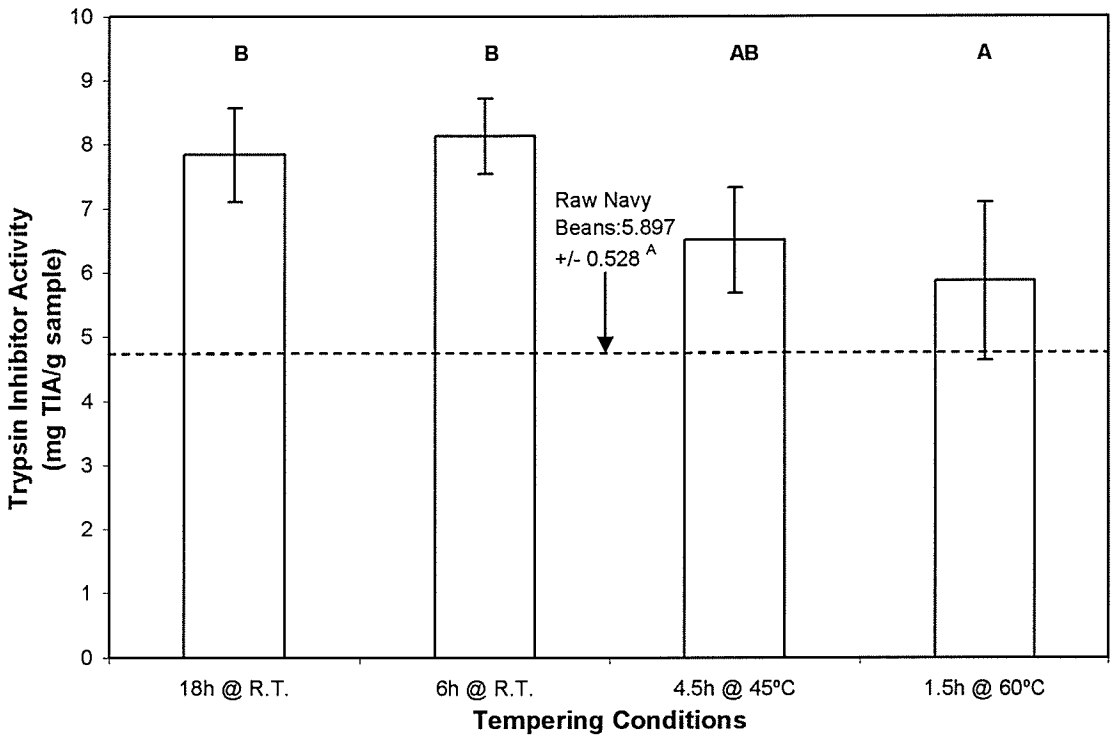

Figure 7. The effects of different tempering conditions on the amount of trypsin inhibitor activity remaining in navy beans after micronization. Micronization conditions yield a residence time distribution of 160 s. Different letters above different column indicate significant differences at $P \leq 0.05$.

A colleagues navy beans micronized under the same conditions to temperatures of 119°C and tempered for 18h at 25% moisture had TIA reduced to 1.39 mg/g compared to 6.03 mg/g when micronized with an output temperature of 98-105°C. This supports the concept that micronization has the capability to reduce TIA, but the lack of sufficient heat during micronization resulted in no TIA loss in this experiment.

Trypsin inhibitor units were not expected to increase. Perhaps the low moisture in the raw samples allowed for a larger amount of heat to be created during grinding, which may have inactivated some of the TIA, to yield a lower raw TIA value than actually present.

3.3. Starch gelatinization

The effect that tempering level and various time/temperature tempering conditions have on the degree of gelatinized starch in micronized navy beans are shown in Figure 8. No tempering level or time/temperature combination had any significant impact on the degree of starch gelatinized during micronization (Figure 8). Research studies have shown that micronizing pulse crops such as lentils (Arntfield et al., 1997; Arntfield et al., 2001; Zhao, 2000), beans (Bellido, 2002), and peas (Cenkowski and Sosulski, 1998; Toews, 2001) increases the amount of gelatinized starch. For starch to gelatinize, moisture must migrate and absorb into the seed's starchy endosperm (amylose and amylopectin). When the swollen granules are heated to the appropriate temperature, the starch gelatinizes and the granules soften (Lineback and Inglett, 1982; Lee et al., 1995). With the high probability that many of the seeds were hard-to-cook, as determined in the sensory and peak force experiments, it is possible that only a limited amount of moisture reached the cotyledon. Without moisture, the temperature required for gelatinization would have increased above the expected 66°C to 77°C (Schooch and Maywald, 1968; Biliaderis, 1979). With higher temperatures required, an outlet temperature of 98-105°C was not likely sufficient to gelatinize starch granules regardless of the tempering treatment or moisture level. If the required amount of water was fully absorbed into the seed, then perhaps cell separation was inhibited by lignification of the cell wall (Stanley, 1992) and/or crosslinking in the middle lamella occurred (de Valle and Stanely, 1985; Jones and Boulter, 1983). The extreme variabilities in the amount of

starch gelatinized, as seen in Figure 8, could also be accounted for by the presence of hard-to-cook seeds.

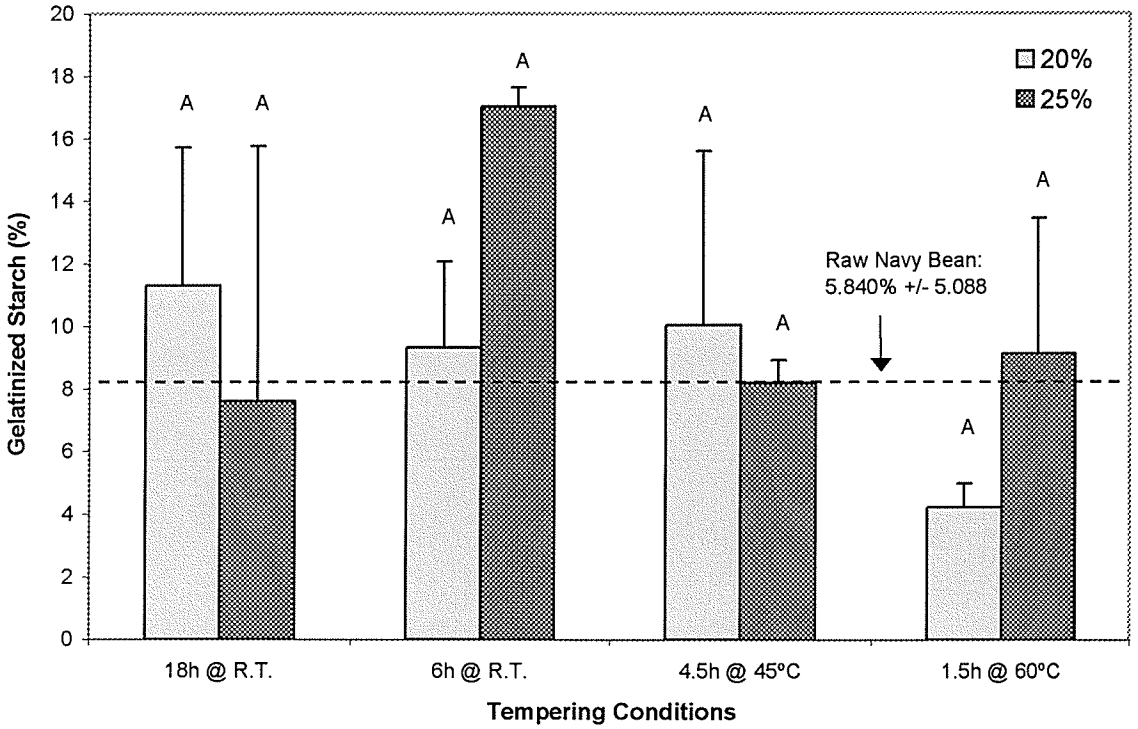

Figure 8. The percent of gelatinized starch in micronized navy beans as a result of various pre-treatment time/temperature and moisture combinations. Different letters above each set of columns indicate significant differences ($p \leq 0.05$). Error bars indicate the standard deviation of duplicate micronization runs.

Toews (2001) determined that micronized yellow and green peas, tempered to 20% moisture, did not have a significant effect on the amount of gelatinized starch compared to raw peas. Yet, increasing the moisture at tempering to 30% and 35% for green and yellow peas respectively, significantly increased the degree of starch gelatinization to 23.9% – 25.1% and 31.1% - 32.1% respectively. Bellido (2002) used the same micronization parameters, as in the present study, and found large increases in the degree of starch gelatinized after

micronizing navy and black beans that were tempered to 28.0% (for 11.5h) and 25.8% (for 32h) moisture, respectively. Contrary to this, no significant differences in the amount of gelatinized starch was found between raw and micronized navy beans tempered to 20% and 25% moisture in this experiment (Figure 8). The only explanation for this difference in gelatinization is the beans themselves. Those used by Bellido (2002) were from a different crop year and did not exhibit the same variability in texture after cooking as was seen in this study.

The gelatinization of starch granules results in granule softening (Lineback and Inglett, 1982; Lee et al., 1995). Bellido (2002) determined that there was a highly significant, negative correlation between peak force and the amount of gelatinized starch. It was therefore expected that the amount of gelatinized starch would impact the peak force values for micronized navy beans, but this did not occur (Table 9). The lack of consistency, and hence variability in the amount of gelatinized starch, resulted in no correlation. However, there was a significant positive correlation between the % gelatinized starch and TIA (Table 9). Bellido et al. (2002) showed that navy beans tempered with water, followed by micronization, have greater intercellular pore sizes than raw beans. Applying this, perhaps when "regular" beans absorb moisture during tempering, they swell and allow for micronization to open up the internal microstructure. Therefore, the more available the moisture, the more starch is gelatinized and more pores are created for a possible increase in heat penetration to inactivate trypsin inhibitors. During the micronization of hard-to-cook beans, there is no cellular swelling and

opening of the microstructure, which will lead to no starch gelatinization and perhaps minimal TIA destruction by heat.

3.4. Micronized Moisture and Water Activity

3.4.1. Moisture

The final moisture content of micronized navy beans for all treatments are shown in Table 10. All treatments at 20% moisture yielded significantly lower residual (after micronization) moisture than those tempered to 25% ($p \leq 0.05$) (Table 9).

As seen in Table 10, there is a larger standard deviation between replicate samples of micronized navy beans at a tempering level of 25% compared to those at 20%. Perhaps the source for this variability is related to the variability in seed hardness that the sensory panel identified. Hard to cook beans exhibit variable hydration. Therefore, if there is more moisture to be absorbed (i.e. 25% tempering), beans not affected by the hard to cook phenomenon will absorb more than 25%, and the HTC beans will absorb the same minimal amount. With some beans absorbing more moisture, there would be greater variability between the amounts of water within each bean. This will affect two factors. One factor is the temperatures reached during micronization, hence the amount of moisture loss by higher temperatures vaporizing the water. It will also affect the size of the bean. Hence, the speed and path the bean will travel down the micronizer will differ and the degree of infra-red exposure each bean receives will not be uniform. With less moisture to absorb (i.e. 20% tempering), the size and shape

of hard-to-cook beans and non-hard-to-cook beans remain closer, allowing for a more consistent exposure time and output temperatures during micronization, and ultimately a more even moisture loss.

Table 10. The residual moisture of micronized navy beans pre-treated under various conditions followed by micronization at a residence time distribution of 160 s.

Tempering Level	Tempering Conditions [1]				Cumulative Average [1,2]
	18 h at R.T.	6 h at R.T.	4.5h at 45°C	1.5 h at 60°C	
20 %	18.46 ± 0.54	17.92 ± 0.78	18.68 ± 0.10	17.73 ± 0.74	18.20 [A] ± 0.62
25%	20.43 ± 1.65	20.97 ± 1.80	19.43 ± 2.00	21.07 ± 0.38	20.61 [B] ± 1.55

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

The variability in residual moisture may also relate to the variability during tempering. The actual moisture content when tempering to target moisture contents of 20% and 25% are illustrated in Table 11. It can be seen that 25% moisture yielded a much larger standard deviation than 20%.

Navy beans treated to 25% moisture lost 4.4% moisture during micronization while 20% moisture lost only 1.8%. It can be theorized that there was more water driven off in navy beans tempered to 25% because the water was more available than that in 20% moisture beans. Lower moisture resulted in less "available" moisture and a higher percent of bound water. Greater energy is

required to remove bound water from lower moisture and lower water activity foods.

Table 11. The moisture content of navy beans tempered to 20 and 25% prior to micronizing.

Tempering Level (%)	Mean ± standard deviation[1] (%)
20	21.38 ± 0.57 [A]
25	25.64 ± 1.47 [B]

[1] different letters in the same column indicate significant differences at $P \leq 0.05$

Both tempering and residual moisture exhibited a significant, positive correlation with water activity at tempering, water activity in micronized beans, and % moisture between each other (Table 12). These results are expected, based on the positive relationship between moisture and water activity as well as the drying effects of micronization.

3.4.2. Water Activity

3.4.2.1. Pre-micronization

Raw navy beans had an average water activity of 0.680, which can be considered a relatively stable water activity for a food product. Interestingly, the water activity after tempering is above 0.86 at 25% moisture (Table 13). This is a concern for the growth of *Staphylococcus aureus* depending on the duration and temperature of tempering. An estimation of the microflora composition on navy beans after micronization is examined later, but further studies and investigations

would need to address the direct or indirect influence I.R. has on the various organisms able to grow at these water activities in Table 13.

Table 12. Correlation coefficients between moisture and water activities of tempered and micronized navy beans (N = 16)

	Water activity (Tempered)	Water Activity (Micronized)	% Moisture (Tempered)	% Moisture (Micronized)
Water Activity (Tempered)	X	0.603 [B]	0.805 [C]	0.521 [B]
Water Activity (Micronized)		X	0.792 [C]	0.594 [B]
% Moisture (Tempered)			X	0.727 [C]
% Moisture (Micronized)				X

[A] significant at $p \leq 0.10$
[B] significant at $p \leq 0.05$
[C] significant at $p \leq 0.01$

3.4.2.2. Post-micronization

In the present experiment, the water activity of micronized navy beans was taken after the micronized product sat overnight at room temperature, in a sealed container, to allow for full moisture recovery and absorption. Like residual moisture, micronized navy bean water activity was significantly different between 20% and 25% tempering levels (Table 13).

The water activity of the final product tempered to 20% moisture is below 0.86, while beans tempered to 25% moisture are at 0.86. Regardless of spoilage by bacterial, yeast, or mold growth, the problem with water activities ≥ 0.86 is the

Table 13. Water activity during tempering and post-micronization

Tempering Level	Tempering Water Activity [1, 2]	Post-Micronization Water Activity [1, 2]
20 %	0.85 ± 0.02 [A]	0.82 ± 0.01 [A]
25%	0.89 ± 0.02 [B]	0.86 ± 0.04 [B]

[1] Mean ± standard deviation
[2] different letters in the same column indicate significant differences at $P \leq 0.05$

possibility for the growth of *S. aureus*. To inhibit all bacteria and most yeast and mould growth, a water activity below 0.68 is a good guideline (Francis, 2000). Ideally, the water activity should be as low as possible without affecting cooking time or quality. Our experimental results suggest that without a post-micronization drying step, micronized navy beans would have a very short shelf-life. To utilize the advantages of this value added product, a drying process needs to be implemented as a post-process treatment to micronization.

3.5. Microbiological changes in response to the micronization process

A preliminary experiment was performed using a tempering condition of 25% moisture for 18h at R.T. to determine the effects of the micronization process on navy bean microflora. As seen in Table 14, tempering navy beans at 25% moisture for 18h significantly increased bacteria, while the subsequent micronization step significantly decreased bacteria ($p \leq 0.01$). Yeast and mould did not significantly change during tempering, but micronization significantly reduced yeast and mould to close to 0 log CFU/g. These trends can be seen in

Table 14. The statistical probabilities of preliminary experiments to determine the response of navy bean microorganisms to the micronization process.

Microorganism	Statistical probabilities of microbial increases as a result of tempering	Statistical probabilities of microbial decreases as a result of micronization
Bacteria	0.005	0.006
Yeast and mould	0.746	0.0012

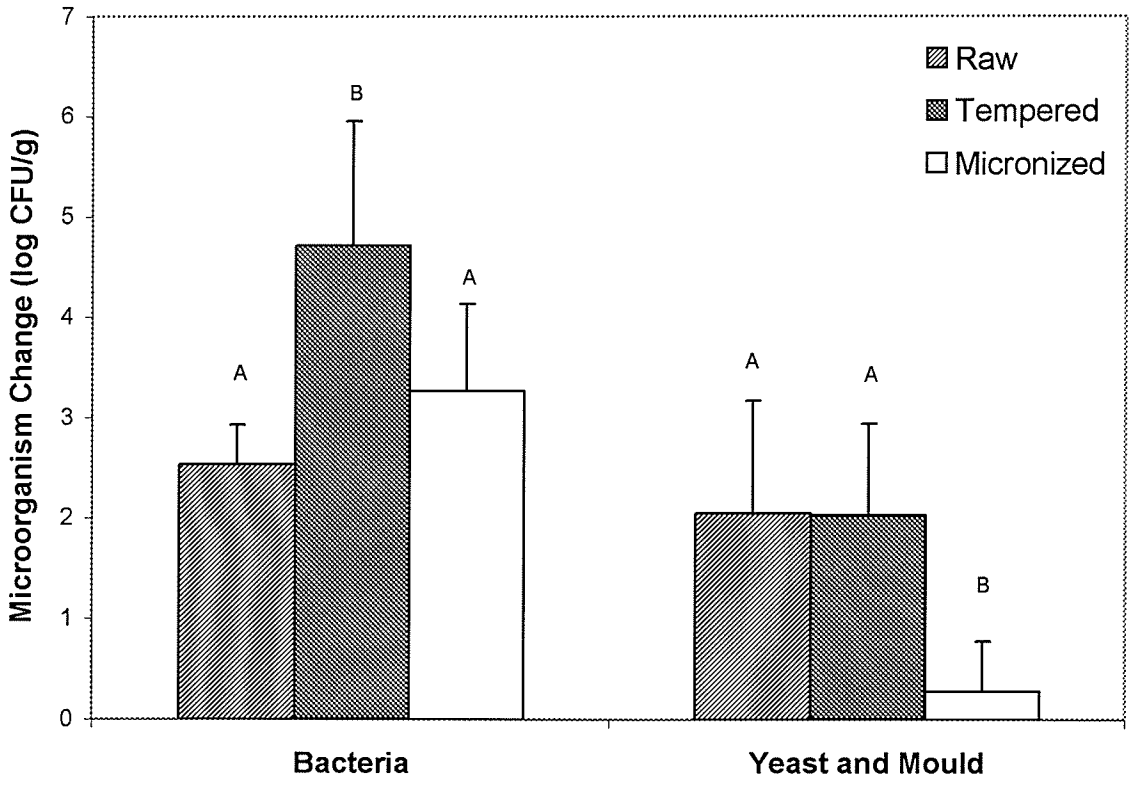

Figure 9. Microbial changes on navy beans at 3 stages of the micronization process. Beans were tempered for 18h at R.T. and 25% moisture. The navy beans were micronized at a slope of 5.0, vibration of 65, and an outlet temperature of 95-100°C. Different letters within the same grouping are significantly different. Error bars indicate standard deviations. Note that the tempering bins were not sanitized prior to tempering.

Figure 9. Additionally, spores were enumerated to determine if spores were the only organisms remaining after micronization. As seen in Figure 10, although the amount of organisms present is not large, the total amount of bacteria on navy beans after micronization, was significantly larger than the amount of spore-formers. This indicates that other organisms may have survived the micronization process. Identification of the bacteria remaining after micronization was done and will be discussed later.

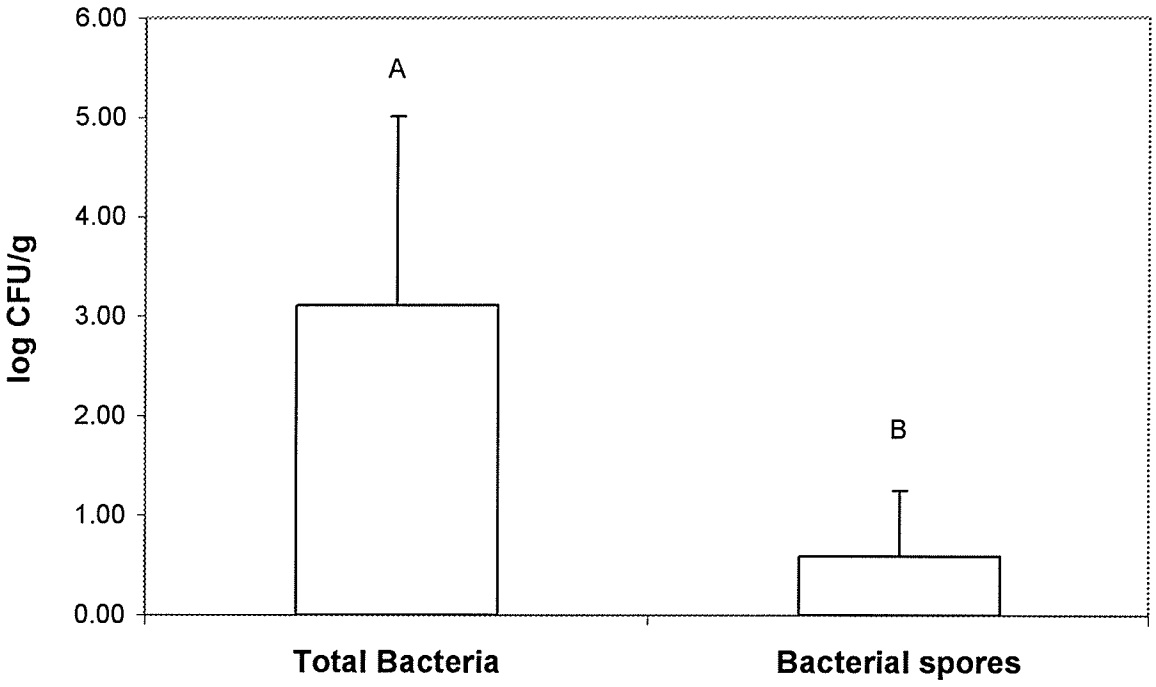

Figure 10. A comparison of the total and spore-forming bacteria on navy beans after micronization. Beans were tempered for 18h at R.T. and 25% moisture. They were micronized at a slope of 5.0, vibration of 65, and outlet temperature of 95-100°C. Different letters within the same grouping are significantly different. Error bars indicate standard deviations.

Due to the significant effects of tempering and micronization on navy bean microflora, changing any aspect of the micronization process will need

subsequent microbiological examination. The effects of different tempering conditions on navy bean microflora are examined in the following section.

3.6. The effects of pre-treatments on the microflora of navy beans

3.6.1. Bacteria

3.6.1.1. Tempering Effects

As shown in Table 15, tempering treatment had a significant impact on the amount of bacterial growth during tempering ($p \leq 0.05$). Tempering for 18h at R.T. exhibited significantly greater bacterial growth compared to 6h at R.T. and 4.5h at 45°C (Figure 11). Although beans tempered for 1.5h at 60°C were not

Table 15. The statistical probabilities of independent variables and their effects on microbial changes

Dependent Variables	Independent Variables		
	Tempering Treatment	Tempering Level	Tempering Treatment * Tempering Level
Bacterial Increase R-T [1]	0.0084	0.7975	0.0253
Bacterial Reduction T-M [2]	0.0645	0.6897	0.1228
Yeast and Mould Increase R-T [1]	0.1144	0.6535	0.5477
Yeast and Mould Decrease T-M [2]	0.0517	0.6401	0.6201

[1] R-T = the difference in microbial counts from raw to tempered stages

2 T-M = the difference in microbial counts from tempered to micronized stages

3 * represents the interaction of the 2 variables

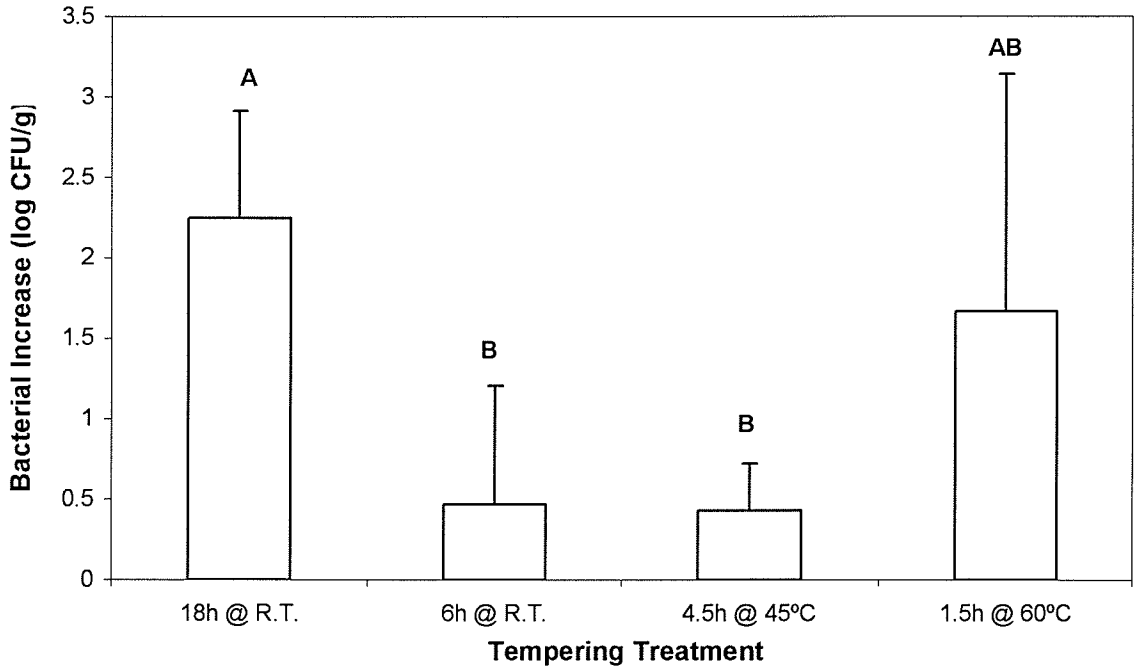

Figure 11. The response of bacteria to various tempering treatments during the tempering stage at $p \leq 0.05$. Error bars indicate the standard deviation.

significantly different from any other tempering treatment, the mean increase compared to beans tempered for 6h at R.T. and 4.5h at 45°C, was > 1 log CFU/g. Considering that conventional microbiological practices indicate that any change ≥ 1 log is considered a significant difference, tempering for 1.5h at 60°C produced significantly higher bacterial growth during tempering than 6h and 4.5h at R.T. and 45°C respectively. Factors impeding the ability to detect statistical significances are the high variability between samples from the same batch and the low number of replicates. Consequently, tempering for 6h at R.T. and 4.5h at 45°C yielded the best conditions for limiting microbial growth during tempering.

Environmental conditions are the primary factors governing the ability of bacteria to metabolize and reproduce. Available moisture and temperature are

two key factors with the ability to limit or enhance microbial growth (Prescott et al., 1990). At temperatures which allow for microbial viability, a rise in temperature can double the velocity of enzyme catalyzed reactions every 10°C (Prescott et al., 1990). As reactions increase within the cell, the whole metabolism of a cell increases and cells multiply faster (Prescott et al., 1990). Therefore, increasing environment temperatures closer to their optimum growth temperature (35-37°C) by incubating at 60°C, can promote rapid growth. Consequently, bacteria held at sub-optimum temperatures will require longer incubation periods for growth (18h at R.T.). For this same reason, we might have expected navy beans tempered for 4.5h at 45°C to produce similar results to 1.5h at 60°C. Preliminary data indicated that tempering at 60°C for 1.5h resulted in an air temperature of 43°C after 1 h and 47°C after 1.5h, while tempering at 45°C for 4.5h had an air temperature of only 35°C after 1 h and 38°C after 1.5h. Incubation temperatures of 45°C may not have been sufficient to increase navy bean temperatures to 35°C – 37°C. It is, therefore, suspected that tempering at 60°C increased bean temperatures closer to 35-37°C more quickly and more rapidly than 45°C, resulting in a greater increase in bacterial growth.

Contrary to what we expected, increasing the moisture at tempering did not have a significant effect on the amount of bacterial growth during this step (Table 15). Perhaps the tempering levels of 20% and 25% were too similar to yield significant increases in bacteria.

Interestingly, in Table 15 there is a significant interaction between tempering treatment and tempering level ($P = 0.025$). Further investigation

determined that tempering treatments for 18h at R.T. were significantly affected ($p \leq 0.057$) by tempering level at $p \leq 0.1$. Samples tempered to 25% moisture had significantly greater (> 1 log CFU/g) bacterial growth occurring during tempering compared to 20% moisture.

Additionally, large variability within replicates under the same tempering conditions can be seen in Figure 11, especially when tempering for 1.5h at 60°C. Perhaps this could be attributed to the high incubation temperatures employed. Having to open the chamber and shake the bins every 10 - 15 min for the first hour could have affected the uniformity of heat applied during tempering, more so than treatments at lower temperatures.

3.6.1.2. Micronization Effects

Statistically, tempering treatments did not significantly effect the amount of bacteria inactivated during micronization at $p \leq 0.05$, but they did at $p \leq 0.1$ (Table 15). Micronizing after tempering for 18h at R.T. and 1.5h at 60°C yielded significantly greater bacterial reductions than navy beans tempered for 6h at R.T. and 4.5h at 45°C by inactivating more than 1 log CFU/g of bacteria (Figure 12). Bacteria are more susceptible to heat treatments when in the exponential growth phase of their life cycle (Ray, 1996). Considering that bacteria grew more rapidly and to greater amounts during tempering for 18h at R.T. and 1.5h at 60°C (Figure 11), it follows that they would be most susceptible to heat destruction and have larger amounts of bacteria available to be destroyed during micronization (Figure 12). This can be further substantiated by the highly significant correlation ($p \leq$

0.01) between bacterial increases during tempering and reductions during micronization (Table 16).

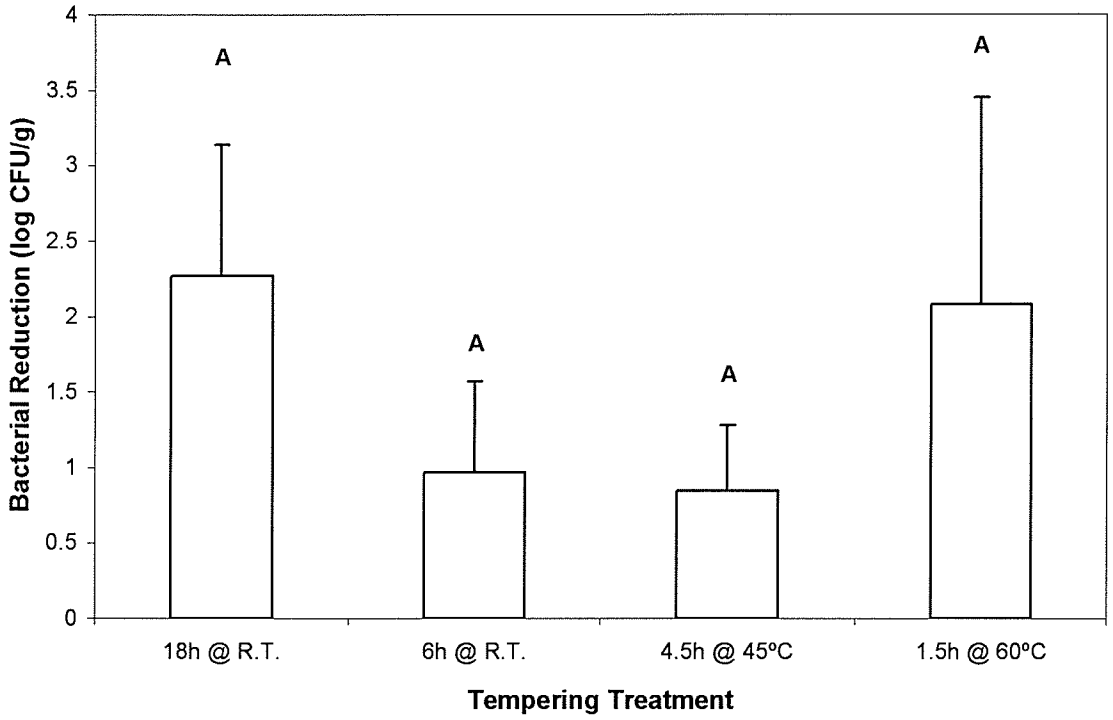

Figure 12. The effect of tempering treatments on the amount of bacterial reduction during micronization at $p \leq 0.05$. Error bars indicate standard deviations.

As seen during the tempering stage, tempering level did not play a significant role in the reduction of bacteria during micronization (Table 15). Based on the concept that high water activity enhances cell destruction upon heating (Nickerson and Sinskey, 1972; Ray, 1996), we expected beans tempered to 25% moisture to exhibit higher bacterial destruction during micronization. As suggested with tempering effects, the difference between the two moisture levels may not have been large enough to cause a significant difference.

Table 16: The correlations coefficients of navy bean microbial changes and moisture and water activity at tempering.

	Bacterial Increase R-T	Bacterial Reduction T-M	Yeast and Mould Increase R-T	Yeast and Mould Reduction T-M
Water Activity (Tempered)	0.308	0.192	0.323	0.010
% Moisture (Tempered)	0.355	0.177	0.297	-0.013
Bacterial Increase R-T*	X	0.926 [C]	-0.295	-0.516 [B]
Bacterial Reduction T-M**	X	X	-0.252	-0.543 [B]
Yeast and Mould Increase R-T*	X	X	X	0.159
Yeast and Mould Decrease T-M**	X	X	X	X

[A] significant at $p \leq 0.10$
[B] significant at $p \leq 0.05$
[C] significant at $p \leq 0.01$
* R-T represents the change in microbial counts from the raw to the tempered navy beans.
** T-M represents the change in microbial counts from the tempered to the micronized navy beans.

3.6.2. Yeast and mould

3.6.2.1. Tempering effects

As seen in Table 15, yeast and mould did not significantly increase during tempering regardless of the pre-treatment employed. For all tempering conditions, the amount of yeast and mould did not significantly change compared to raw navy beans (Figure 13). This could be expected considering the amount of

bacteria present on raw navy beans. With bacteria present in amounts equal to or exceeding that of yeast and mould, yeast and mould growth is often suppressed by bacterial growth.

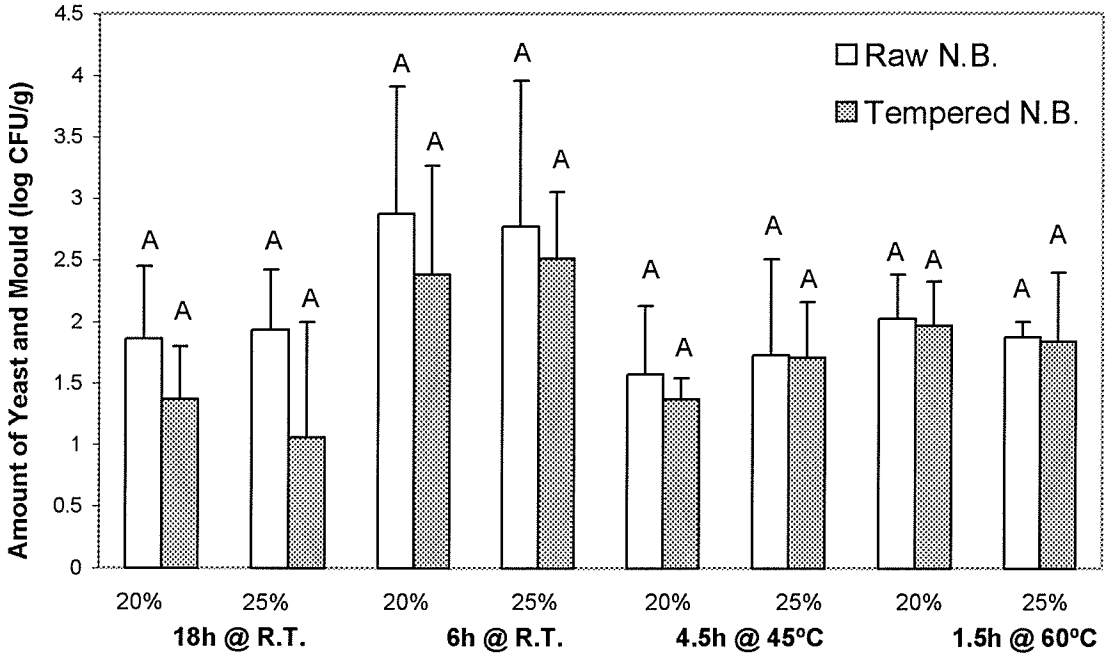

Figure 13. The relationship of yeast and mould between raw and tempered navy beans at $p \leq 0.05$. The same letters within the same grouping are not significantly different. Error bars indicate the standard deviations.

3.6.2.2. Micronization effects

Although there was no significant change in yeast and mould during tempering, yeast and mould were reduced during the micronization step (Figure 14). Tempering for 6h at R.T. resulted in a significantly greater reduction in yeast and mold than tempering for 18h at R.T, with other treatments being not significantly different from either of these treatments. Unfortunately, this statistical analysis does not accurately reflect the true effect of micronization on yeast and mould. In fact, all yeast and mould were completely eliminated during

micronization (except in one case where 2 CFU/g were found). These statistics are simply a reflection of the amount of yeast and mould present prior to micronization. Hence, there is no proof that any tempering treatment can have a significantly greater affect on the amount of yeast and mould killed by micronization. It can be concluded that yeast and mould are destroyed by micronization when present in quantities ≤ 3.61 log CFU/g under any of the tempering treatments in this experiment.

A significant, negative correlation was found between bacterial increases during tempering and yeast and mould reductions during micronization (Table 16). Recalling that the rapid bacterial growth during tempering is theorized to

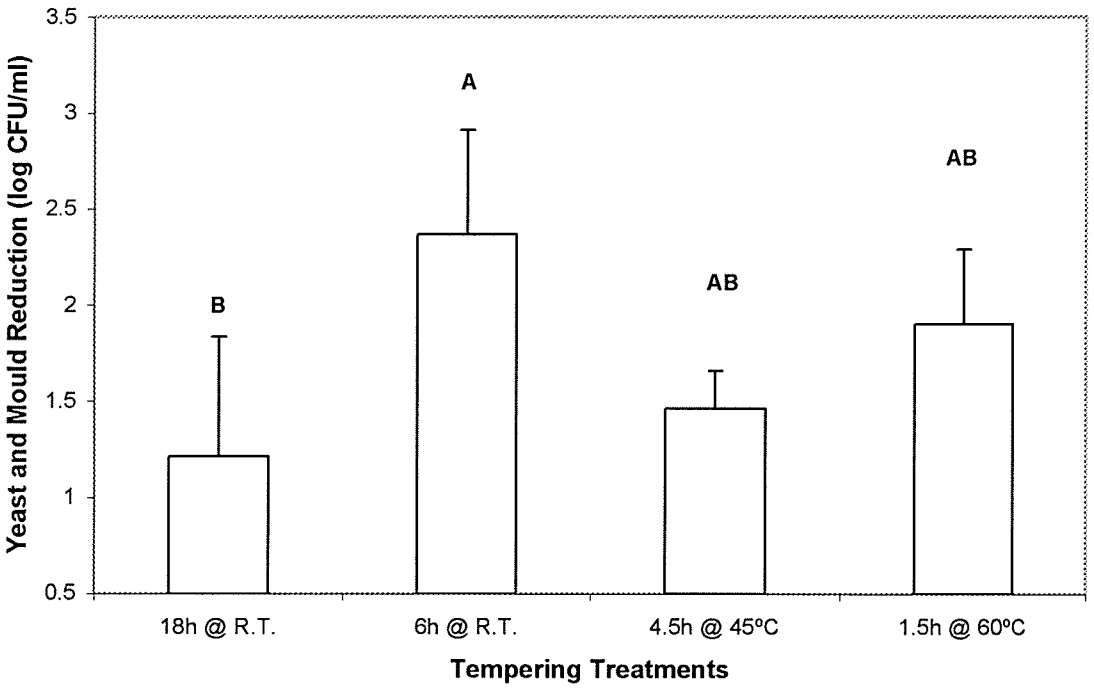

Figure 14. Yeast and mould reductions by micronization at $p \leq 0.05$. Error bars indicate the standard deviations.

outgrow and limit the growth of yeast and mould, and that all yeast and mould are destroyed upon micronization, the lower the yeast and mould levels prior to micronization, would explain the smaller reduction obtained. The same holds true for the negative, significant correlation between bacterial reductions and yeast and mould reductions (Table 16). Greater bacterial reduction indicates greater bacterial increases at tempering, and hence less yeast and mould present at tempering. Therefore, the yeast and mould reductions during micronization were less.

3.6.3. Spore-formers

3.6.3.1. Micronization's effect on bacterial spores

By conducting a paired t-test between the amount of spores present on raw navy beans and those on micronized navy beans, it was determined that micronization significantly reduced the amount of spore-formers when tempered to 20% moisture for 4.5h at 45°C and 25% moisture for 1.5h at 60°C (Table 17). Spores make up an important part of the bacterial microflora on navy beans. In this experiment, depending on the sample, they can represent 23% to 100% of the total bacteria present after micronizing. From this, the question of what other organisms constitute the remaining microflora arises. As the time/temperature profile of navy beans during micronization is not well established, the identity of the remaining aerobic bacteria is a concern that will be examined in a subsequent section.

3.6.3.2. Micronization's effect on yeast and mould spores

Yeast and mould spores were not present on raw navy beans, except for one of the 16 samples (3 CFU/g), and no spores were present on post-micronized beans, regardless of the pre-treatment employed.

Table 17. A comparison of the amount of bacterial spores present on raw and micronized navy bean samples.

Tempering Treatment	Moisture level (%)			
	20		25	
	Raw (log CFU/g)	Micronized (log CFU/g)	Raw (log CFU/g)	Micronized (log CFU/g)
18h at R.T. [1]	2.28^{A}	2.35^{A}	2.42^{A}	2.31^{A}
6h at R.T. [1]	3.00^{A}	2.35^{A}	2.81^{A}	2.46^{A}
4.5h at 45°C [1]	2.28^{A}	1.45^{B}	2.20^{A}	2.20^{A}
1.5h at 60°C [1]	2.39^{A}	2.30^{A}	2.62^{A}	1.89^{B}

[1] The same letters within the same row and moisture level are not significantly different at $P \leq 0.05$.

3.7. Identification of the bacterial microflora on micronized navy beans

Tempering for 18h at R.T. provided the best environment for bacterial growth (Figure 11). According to preliminary research, a stable water activity is reached after tempering navy beans for 6h at R.T. and 25% moisture (Aw = 0.88). Therefore, tempering for 18h at R.T. to a moisture content of 25%, as in our study, provided a suitable environment for bacterial growth. Considering this

tempering treatment has the highest probability of causing a food safety concern, it was chosen for use in the identification of the remaining bacterial microflora.

Navy beans tempered for 18h at R.T. and 20% and 25% moisture, followed by micronization, were examined (Table 18 and 19) to determine which aerobic bacteria survived micronization. The bacterial cells identified when tempering for 18h at R.T. and 20% moisture, were endospore-forming *bacilli* (Table 18) (Sneath, 1986). *Bacillus* organisms are sporulating, aerobic organisms that predominate in the soil, which make them likely to be a predominant organism on navy beans. With the exception of one gram negative organism, endospore-forming *Bacillus* were also identified as the only bacteria surviving micronization following tempering to 25% for 18h at R.T. (Table 19). The vegetative cells of *Bacillus* appear to be very resilient to the lethal effects of micronization.

Although the identification of bacteria present at tempering was not done, since the bacteria surviving micronization are primarily *Bacillus* spore-formers, it is suspected that *Bacillus* would dominate navy bean microflora during tempering as well. One organism in the *Bacillus* genera that has the ability to cause food poisoning is *Bacillus cereus*. *B. cereus* needs a population greater than 10^6 CFU/g to be hazardous to human health (U.S. Food and Drug Administration, 1994). However, the fact that *B. cereus* requires a water activity of 0.95 to grow (Troller and Christian, 1978) and the average water activity for beans tempered

Table 18. Bacterial cell characteristics of colonies selected from navy beans tempered for 18h at R.T. and 20% moisture, followed by micronization at an outlet temperature of 98-103°C.

Sample #	Gram	Catalase	Oxidase	Morphology	Spore-forming
1	+	+	+	Rod	Yes
2	+	+	+	Rod	Yes
3	+	+	+	Rod	Yes
4	+	+	+	Rod	Yes
5	+	+	+	Rod	Yes
6	+	+	n/d	Rod	Yes
7	+	+	n/d	Rod	Yes
8	+	+	+	Rod	Yes
9	+	+	+	Rod	Yes
10	+	+	+	Rod	Yes
11	+	+	+	Rod	Yes
12	+	+	+	Rod	Yes
13	+	+	+	Rod	Yes
14	+	+	-	Rod	Yes
15	+	+	-	Rod	Yes
16	+	+	+	Rod	Yes

+ = positive,
- = negative
n/d = not determined

Table 19. Bacterial cell characteristics of colonies selected from navy beans tempered for 18h at R.T. and 25% moisture, followed by micronization at an outlet temperature of 98-103°C.

Colony Sample # [1]	Gram	Catalase	Oxidase	Morphology	Spore-forming
1	-	+	-	Rod	No
2	+	+	n/d	Rod	Yes
3	+	+	n/d	Rod	Yes
4	+	+	+	Rod	Yes
5	+	+	+	Rod	Yes
6	+	+	+	Rod	Yes
7	+	+	+	Rod	Yes
8	+	+	-	Rod	Yes
9	+	+	+	Rod	Yes
10	+	+	+	Rod	Yes
11	+	+	+	Rod	Yes
12	+	+	+	Rod	Yes
13	+	+	+	Rod	Yes
14	+	+	+	Rod	Yes

+ = positive
- = negative
n/d = not determined

to 25% moisture, regardless of tempering treatment was 0.89 (Table 13), this organism is not a concern. *Staphylococcus aureus*, which only requires a water activity of 0.86 to grow (Troller and Christian, 1978), could be a problem if growth during tempering gets out of control. At levels of 10^6 CFU/g *S. aureus* can produce a heat stable enterotoxin (Troller and Christian, 1978), which can survive the high temperatures and times, such as those reached during canning (Vanderzant and Splittstoesser, 1992). Therefore, if produced, these toxins would survive micronization. In our current investigation, the total bacterial count did not reach 10^6 CFU/g, but to keep this problem from arising, heavily soiled samples should not be used and *S. aureus* levels should be checked.

The presence of a gram negative bacterium from the *Enterbacteriaceae* family surviving micronization pin points the possibility of pathogenic bacteria surviving micronization. According to Prescott et al. (1990), the gram negative, oxidase negative, rod that was found on micronized navy beans could belong to the genera *Salmonella, Citrobacter, Enterobacter, Proteus, Escherichia, Serratia, Shigella, Yersinia, or Klebsiella*. During preliminary experiments, micronized samples contained no coliform which eliminates *Escherichia coli* as a concern. While further identification of the gram negative bacterium was not pursued, the presence of this *Enterobacteriaceae* indicates that pathogenic bacteria may survive micronization. Future work with micronization should develop and use parameter conditions of the unit that remove all possibility for pathogen survival.

Eliminating the survival of gram negatives, avoiding heavily soiled samples, and drying after micronization to a water activity ≤ 0.68, would yield a

microbiological stable bean (Francis, 2000). This would in turn protect micronized navy beans from any food safety implications and create easy transport and storage.

3.8. The bacterial microflora of commercially micronized great northern beans

Great northern beans were tempered to 13% moisture overnight (approximately 18h) and micronized under a 16-lamp commercial micronizer at Infra-Ready Foods Inc., Saskatoon, Sask. It was suggested that the beans were hard-to-cook, considering the lack of absorption that occurred during tempering (target moisture = 25%, actual moisture = 13%). Regardless of the moisture content during tempering, bacteria still increased from 3.48 log CFU/g to 5.53 log CFU/g (Table 20). The high levels of bacteria reached during tempering are suspected to be a result of the non-sanitized tempering chamber used.

As suggested by Bellido et al. (2003), the output temperature is a good parameter for monitoring the effectiveness of the micronization process. To give a target temperature of 124°C on the commercial micronization unit, the slope of the vibrating trough can be adjusted. The adjustments done on the commercial micronizer to obtain this temperature varied from no slope (flat) to a slightly negative slope. Under these conditions, micronization decreased the bacterial counts on great northern bean by 4 log CFU/g to a micronized count of 1.53 log CFU/g (Table 20). This large decrease in bacterial count is likely directly related to the higher temperature of heat applied compared to the experimental unit. In

addition, the fact that the slope was level or negative, and not positive as with the pilot scale unit, resulted in a much more even infra-red treatment amongst the great northern beans, and a greater bacterial reduction with less variability between replicates.

As outlined in Table 20, no yeast, mould, or coliforms survived micronization. Commercially micronized great northern beans exhibited slightly greater spore reduction capabilities by reducing spores by 0.81 log CFU/g compared to 0.38 log CFU/g by the pilot scale unit. It could be concluded that the higher outlet temperatures obtained during micronization inactivated more bacterial spores.

Table 20. Microbial evaluation of great northern beans processed commercially at Infra-Reddy Food Inc., Saskatoon, Sask. (n = 1)

Test	Great Northern Bean Sample (log CFU/g)		
	Raw	Tempered	Micronized
Total Bacteria	3.48	5.53	1.53
Aerobic Bacterial Spores	1.11	n/d	0.30
Anaerobic Bacterial Spores	0	n/d	0
Yeast and mould	0.30	1.08	0
Yeast and mould spores	0	0	0
Coliforms	0	0	0

n/d = not determined

Additionally, the end moisture content of micronized great northern beans was 8.9%, which is below the suggested 12-15% moisture that is required to inhibit microbial growth and yield a microbiologically safe dried food (Beuchat, 1989).

3.9. The impact of tempering bin cleanliness on microbial growth during tempering

Changes in the microflora of navy beans tempered for 18h to 25% moisture at R.T. were compared under three different tempering conditions. Tempering in bins sanitized with chlorine, tempering in bins cleaned with soap and water, and tempering in containers not cleaned at all (commercial application) were examined. Since there was no uniformity in contamination of the bins prior to tempering, table 21 compares these tempering conditions on the basis of actual bacterial increases. It can be seen that the amount of bacterial growth was not significantly different among any of the three tempering conditions. However, considering that beans in the uncleaned tempering bin reached only 13% moisture, rather than the original target of 25%, these bacterial counts may be much lower than expected. From these results (Table 21) it can be seen that cleaning and sanitizing the bins was just as effective as only cleaning. Unfortunately no conclusions regarding the uncleaned, commercial production bins could be drawn due to the low moisture content obtained during tempering.

Table 21. A comparison of microbial changes[1] during tempering between cleaned and sanitized, cleaned, and unclean tempering bins when navy beans are tempered for 18h at room temperature and 25% moisture.

Tempering Conditions	Bacterial increase during tempering (log CFU/ 10cm^2) ($P \leq 0.821$) [2]
Cleaned and Sanitized (n = 2)	2.79 ± 0.32 [A]
Cleaned (n = 7)	2.19 ± 1.35 [A]
Unclean (n = 1) [3]	2.06 [A]

[1] mean $\pm$ standard deviation
[2] different letters indicate significant differences ($P \leq 0.05$)
[3] sample taken from commercial operation

3.10. The Effects of Micronizer Parameters on Micronized Navy Bean Microflora

Experiments by Bellido (2003) determined that a slope of 5.0 and gas setting of 9.0, with all other parameters held stable, were the ideal settings for micronization based on the outlet temperature and accompanying physiochemical properties of navy beans. Later determinations by Cinq-Mars et al. (2003) indicated that micronizing with a slope of 2.75, gas of 9.0, distance of 11mm, and feeder rate of 60, produced the most even infra-red heat treatment with a residence distribution time of 160 s for navy beans tempered for 18h at R.T. and 25% moisture. A comparison of the two parameters, as seen in Table 22, reaffirms the Cinq-Mars et al. (2003) investigation (Table 22). The lower variability and higher bacterial reduction indicated a more even heat treatment.

Table 22. A comparison of microbial reduction [1] when navy beans tempered for 18h at R.T. to 25% moisture are micronized under different conditions

Conditions	Bacterial Reduction in log CFU/g By Micronization ($P \leq 0.206$)[3]
Slope = 5.0, Gas = 9.0, Feeder vibration rate = 65 [2] (n = 10) Outlet temp = 95–100°C	1.50 ± 1.35 [A]
Slope = 2.75, Gas = 9.0, Distance = 11mm, Feeder vibration rate = 60 (n = 2) *RTD = 160 s, Outlet temp = 98-105°C	2.91 ± 0.69 [A]

[1] mean ± standard deviation

[2] previous research determined that distance did not significantly impact output temperatures and was therefore not measured

[3] different letters indicate significant differences ($P \leq 0.05$)

*RTD represents residence time distribution

CONCLUSIONS

The hard-to-cook phenomenon is suspected to have existed throughout the navy bean samples used in this study. The presence of the hard-to-cook phenomenon affected absorption characteristics, which ultimately affected the ability of micronization to impact the anti-nutritional factors, starch gelatinization, and cooking time of navy beans. Conclusions drawn regarding these characteristics are to be taken cautiously.

The micronization process involved significant bacterial increases during tempering and significant bacterial decreases during micronization when navy beans were tempered for 18h at R.T. and 25% moisture. The tempering treatments and moisture levels chosen to minimize this bacterial growth during tempering and still maintain the potential benefits of reducing phytic acid, tannins, trypsin inhibitors, cooking time, and increasing the amount of gelatinized starch, did not give the desired results. No tempering treatment (time or temperature) or tempering level reduced phytic acid, tannin, or trypsin concentrations in micronized navy beans compared to raw navy beans. It was suspected that the outlet temperature of 98-105°C and/or moisture levels of 20% and 25% were not high enough to inactivate these components, or activate compounds such as the enzyme phytase, which would promote the breakdown of phytic acid.

Micronization did not gelatinize navy bean starch. The presence of hard-to-cook beans did not allow for water penetration and cell separation, which is needed to swell starch granules. This inhibits the gelatinization process.

The high variability in hardness due to the presence of hard-to-cook beans was magnified during cooking tests with a sensory panel. Although difficult to determine, an optimum cooking time of 42.5 min corresponding to a force of 465 N was chosen. The various tempering treatments and moisture levels employed, followed by micronization, did not reduce cooking time, but did eliminate the soaking step.

Due to the lack of full moisture absorption during tempering, the microbial growth during tempering presented in this thesis is likely to be the worse case scenario for microbial problems. The less water absorbed, the more available water on the surface for microbial growth, and more growth occurs than if normal absorption were to take place.

Reducing moisture to decrease the amount of bacterial growth during tempering had no effect on most tempering treatments. Tempering for 18h at R.T. was the only tempering treatment that showed significantly less bacterial growth at 20% moisture compared to 25%.

Tempering time and temperature impacted bacterial growth during tempering. Decreasing the tempering time from 18h at R.T. to 6h at R.T. significantly lowered bacterial growth. Yet, increasing the tempering temperature to 60°C and reducing the time to 1.5h was suggested to have brought bacteria closer to their optimum growth temperatures, which induced rapid growth, resulting in significantly greater populations than tempering for 6h at R.T. and 4.5h at 45°C. Between 4.5h at 45°C and 6h at R.T., tempering for 6h at R.T. is more energy efficient and should be used for future studies.

Yeast and mould did not change significantly during tempering regardless of the tempering treatment or moisture level. Yeast and mould were almost completely eliminated by micronization regardless of the tempering treatment or moisture level.

Spore-forming *Bacillus spp.* represented 100% of the surviving bacteria on micronized navy beans tempered for 18h at R.T. and 20% moisture. When navy beans were tempered for 18h to 25% moisture at R.T., gram-negative organisms also survived.

Tempering environment cleanliness did not significantly affect bacterial growth during tempering. If the target moisture of 25% was reached when field beans were tempered in the unclean conditions of the commercial operation, there may have been a significant difference in the results. For that reason the impact of using clean and unclean tempering bins should be re-evaluated in future studies.

A slope of 0 and higher output temperatures during commercial micronization lead to a more even heat treatment, and hence, a larger bacterial reduction compared to pilot scale micronization.

Although not statistically different, when micronizer parameters that promoted more uniform heat distribution were used (slope = 2.75, gas = 9.0, distance = 11mm, and feeder rate = 60, and residence time distribution = 160s), the amount of bacteria reduced during micronization is over 1 log CFU/g greater, with half the variability, than navy beans micronized using parameters with a slope of 5 and gas of 9.

RECOMMENDATIONS

The presence of the hard-to-cook phenomenon is a major factor limiting the applicability of micronization. The presence of moisture in navy beans is one of the primary factors impacting the potential for micronization to reduce anti-nutritional factors, gelatinize starch, and reduce cooking time. For future experimental work with micronization to be accurate and conclusive, a method must be developed to identify hard-to-cook navy beans. The natural occurrence of the hard-to-cook phenomenon in raw navy beans indicates that measures must be taken to properly store beans at low temperatures and relative humidities for micronization to be an effective process.

Microbiologically, future work with navy beans should involve tempering for short periods of time (6h), at low moisture (20%), and at room temperature to control bacterial growth during tempering. Lower moistures during tempering will also lead to significantly lower post-micronization moisture. Still, a post-micronization drying step must be implemented for increased storage stability. Further investigations must also address the food safety implications of gram-negative bacteria surviving micronization (when tempering for 18h at R.T. and 25% moisture) and identify the micronization conditions that are needed to kill all gram-negative and pathogenic organisms.

REFERENCES

Abdul-Kadir, R., Bargman, T.J., and Rupnow, J.H. 1990. Effect of infrared heat on rehydration rate and cooking of *phaseolus vulgaris* (var. pinto). J. Food Sci. 55:1472-1473.

AACC. 1983. AACC Approved method, method 44-15A, 8th ed. Minneapolis, MN. American Association of Cereal Chemists, Inc.

AACC. 1999. AACC Approved method, method 22-40, 10th ed. Minneapolis, MN. American Association of Cereal Chemists, Inc.

[AAFC] Agriculture and Agri-Food Canada 2002a. Agri-Food Trade Service. Suppliers and products Fact Sheets. URL: http://ats.agr.ca/ (January 10/03)

[AAFC] Agriculture and Agri-Food Canada 2002b. Market Analysis Division. Canada: Pulse and special crops outlook. URL: www.agr.gc.ca/mad-dam/ (January 10, 2003)

Abdel-Gawad, A.S. 1993. Effect of domestic processing on oligosaccharide content of some dry legume seeds. Food Chem. 46:25-31.

Aguilera J.M. and Rivera, R. 1992. Hard-to-cook defect in black beans: hardening rates, water imbibition and multiple mechanism hypothesis. Food Res. Intl. 25:101-108.

Aguilera, J.M. and Stanley, D.W. 1985. A review of textural defects in cooked reconstituted legumes – the influence of storage and processing. J. Food Process. Preserv. 9:140-169.

Anderson, J.W., Gustafson, N.J., Spener, D.B., Tietyen, J., and Bryant, C.A. 1990. Serum lipid response of hypercholesterolemic men to single and divided doses of canned beans. Amer. J. Clin. Nutr. 51:1013-1019.

Antunes, P.L. and Sgarbieri, V.C. 1980. Effect of heat treatment on the toxicity and nutritive value of dry bean (*Phaseolus vulgaris* var. Rosinha G2) proteins. J. Agric. Food Chem. 28:935-938.

Arntfield, S.D., Scanlon, M.G., Malcolmson, L.J., Watts, B., Ryland, D., and Savoie, V. 1997. Effect of tempering and end moisture content on the quality of micronized lentils. Food Res. Intl. 30:371-380.

Arntfield, S.D., Scanlon, M.G., Malcolmson, L.J., Watts, B., Ryland, D., and Savoie, V. 2001. Reduction in lentil cooking time using micronization: comparison of 2 micronization temperatures. J. Food Sci. 66: 500-505.

Banks, W. and Greenwood, C.T. 1975. Starch and Its Components. Halsted Press, New York, NY. 342pp.

Banwart, G. 1989. Basic Food Microbiology. Van Nostrand Reinhold, New York, NY. 773pp.

Barampama Z. and Simard, R.E. 1994. Oligosaccharides, antinutritional factors, and protein digestibility of dry beans as affected by processing. J. Food Sci. 59:833-837.

Batra, L.R. and Millner, P.D. 1976. Asian fermented foods and beverages. Developments in industrial microbiology. 17:117-128.

Becker, R., Olson, A.C., Frederick, D.P., Kon, S., Gumbmann, M.R., and Wagner, J.R. 1974. Conditions for the autolysis of alpha-galactosides and phytic acid in California small white beans. J. Food Sci. 39:766-769.

Bellido, G.G. 2002. Effects of pretreatment and micronization on the cookability, chemical components and physical structure of navy and black beans (*Phaseolus vulgaris* L.). M.Sc. Thesis, University of Manitoba, Winnipeg, MB.

Biliaderis, C.G., Grant, D.R., and Vose, J.R. 1979. Molecular weight distributions of legume starches by gel chromatography. Cereal Chem. 56:475-480.

Blenford, D.E. 1980. Potential applications of micronizing in food processing. Food Trade Review. 50:6-8.

Buera, M.P., Pilosof, A.M.R., and Bartholomai, G.B. 1984. Kinetics of trypsin inhibitory activity loss in heated flour from bean, *phaseolus vulagaris*. J. Food Sci. 49: 124-126,136.

Burr, H.K., Kon, S., and Morris, H.J. 1968. Cooking rates of dry beans as influenced by moisture content and temperature and time of storage. Food Technol. 22(3):236-238.

Butler L.G., Riedl, D.J., Lebryk, D.G., and Blytt, H. J. 1984. Interaction of proteins with sorghum tannin: Mechanism, specificity and significance. J. Amer. Oil Chem. Soc. 61:916-920.

Carvalho, M.R.B. and Sgarbieri, V.C. 1997. Heat treatment and inactivation of trypsin-chymotrypsin inhibitors and lectins from beans (*phaseolus vulgaris* L.). J. Food Biochem. 21:219-233.

Cenkowski, S. and Sosulski, F.W. 1997. Physical and cooking properties of micronized lentils. J. Food Proc. Eng. 20:249-264.

Cenkowski, S. and Sosulaksi, F.W. 1998. Cooking characteristics of split peas treated with infrared heat. Trans. Am. Soc. Agric. Eng. 41(3):715-720.

Chang, R. 1977. Phytate: Removal from whole dry beans by enzymatic hydrolysis and diffusion. J Food Sci. 42:1098-1101.

Chernick, A. and Chernick, B.A. 1963. Studies of factors affecting cooking quality of yellow peas. Can. J. Plant Sci. 43:174-183.

Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Crit. Rev. Food Sci. Nutr. 12:296-335.

Chiang, B.Y. and Johnson, J.A. 1977. Measurement of total and gelatinized starch by glucoamylase and o-toluidine reagent. Cereal Chem. 54(3):429-435.

Cinq-Mars, C.D., Arntfield, S.D., and Parsons, R.V. 2003. Quantification and optimization of the residence time distribution of navy beans (*Phaseolus vulgaris*) during pilot scale micronization. Food Res. Intl. 36:685-693.

de Lumen, B.O. and Salamat, L.A. 1980. Trypsin inhibitor in winged bean (*Psophocarpus tetragonolobus*) and the possible role of tannin. J. Agric. Food Chem. 28:533-536.

del Valle, J.M. and Stanley, D.W. 1995. Reversible and irreversible components of bean hardening. Food Res. Intl. 28:455-463.

Deshpande, S. S. and Cheryan, M. 1984. Effects of phytic acid, divalent cations, and their interactions on alpha-amylase activity. J. Food Sci. 49:516-519,524.

Deshpande, S.S., Sathe, S.K., Salunkhe, D.K. 1984. Dry beans of *Phaseolus vulgaris.* J. Food Sci. 42:1094-1098.

Deshpande, S.S. and Damodaran, S. 1990. Food legumes: chemistry and technology. Pages 147-151 in: Chemistry and Technology in Advances in Cereal Science and Technology. Pomeranz, Y., American Association of Cereal Chemists, Inc. St. Paul, MN.

Dhurandhar, N.V. and Chang, K.C. 1990. Effect of cooking on firmness, trypsin inhibitors, lectins and cystine/cysteine content of navy and red kidney beans (*phaseolus vulgaris*). J. Food Sci. 55:470-474.

Drumm, T.D., Gray, J.I., and Hosfield, G.L. 1990. Variability in the major lipid components of four market classes of dry beans. J. Sci. Food Agric. 50:485-497.

Durigan, J.F., Sgarbieri, V.C., and Almeida, L.D. 1987. Antinutritional factors and toxicity in raw dry beans (*Phaseolus vulgaris*, L.) of 12 brazilian cultivars. J. Food Biochem. 11:185-200.

El-Tabey Shehata, A.M. 1992. Hard-to-cook phenomenon in legumes. Food Res. Intl. 8:191-221.

Elbert, E.M. and Witt, R.L. 1968. Gelatinization of starch. J. Home Econ. 60:186-188.

Elias, L.G., De Fernandez, D.G., and Bressani, R. 1979. Possible effects of seed coat polyphenolics on the nutritional quality of bean protein. J. Food Sci. 44:524-527.

[FAO] Food and Agriculture Organization of the United Nations 2002. FAOSTAT Agriculture Data. Food Balance Sheets. URL: http://apps.fao.org/. (August 10, 2002 and December 10,2002)

[FAO] Food and Agriculture Organization of the United Nations. 1996. World food summit. November. 13-17pp.

Fleming, S.E. 1981. A study of relationships between flatus potential and carbohydrate distribution in legume seeds. J. Food Sci. 46:794-798.

Fontana, A.J. Jr. 2000. Understanding the importance of water activity in food. Cereal Foods World, 45 (1):7-10.

Francis, F.J. 2000. Wiley Encyclopedia of Food Science and Technology. John Wiley and Sons Inc., New York, NY. pp. 1631, 2635-2640.

Fujimura, T. and Kugimiya, M. 1994. Gelatinization of starches inside cotyledon cells of kidney beans. Starch, 10:374-378.

Garruti R.D.S. and Bourne, M.C. 1985. Effect of storage conditions of dry bean seeds (*phaseolus vulgaris* L.) on texture profile parameters after cooking. J. Food Sci. 50: 1067-1071.

Giese, J. 1997. Water activity: The new critical measurement. Food Technol. 51(10): 93-94.

Gregersen, T. 1978. Rapid method for distinction of gram-negative bacteria. Europ. J. Appl. Microbiol. Biotechnol. 5:123-127.

Griffiths, D.W. 1979. The inhibition of digestive enzymes by extracts of field beans (*Vicia faba*). J. Sci. Food Agric. 30:458-462.

Griffiths, D.W. and Moseley, G. 1980. The effect of diets containing field beans of high or low polyphenolic content on the activity of digestive enzymes in the intestine of rats. J. Sci. Food. Agric. 31:255-259.

Gubbels, G.H., Chubey, B.B., Ali-Khan, S.T., Stauvers, M. 1985. Cooking quality of field pea matured under various environmental conditions. Can. J. Plant Sci. 65:55-61.

Harrigan, W.F. and McCance, M.E. 1976. Laboratory Methods in Food and Dairy Microbiology. Academic Press Inc., New York, NY. 452pp.

Haytowitz, D.B., Marsh, A.C., and Matthews, R.H. 1981. Content of selected nutrients in raw, cooked, and processed legumes. Food Technol. 35(3):73-74.

Hohlberg, A.I. and Stanley, D.W. 1987. Hard-to-cook defect in black beans. Protein and starch considerations. J. Agric. Food Chem. 35: 571-576.

Iyer, V., Salunkhe, D.K., Sathe, S.K., and Rockland, L.B. 1980. Quick-cooking beans (*phaseolus vulgaris* L.): II. Phytates, oligosaccharides, and antienzymes. Qual. Plant Plant Foods Hum. Nutr. 30:45-52.

Jay, J.M. 1996. Modern Food Microbiology. Chapman and Hall, New York, NY. 661pp.

Jayaprakasha, H.M., Rao, K.J., and Kumar, W.A.L. 1997. Studies on the influence of water activity (Aw) on the stability of foods – A critical appraisal. J. Food Sci. Technol. 34:273-285.

Jones, P.M.B. and Boulter, D. 1983. The cause of reduced cooking rate in *Phaseolus vulgaris* following adverse storage conditions. J. Food Sci. 48:623-626,649.

Jood, S., Mehta, U., and Singh, R. 1986. Effect of processing on available carbohydrates in legumes. J. Agric. Food Chem. 34:417-420.

Kadam, S.S. and Salunkhe, D.K. 1989. Minerals and vitamins. Pages 117-121 in: Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology , and Utilization. Vol. 1. Salunkhe, D.K. and Kadam, S.S. CRC Press Inc., Boca Raton, Fl.

Kakade, M.L. 1974. Biochemical basis for the differences in plant protein utilization. J. Agri. Food Chem. 33(4):550-555.

Kakade, M.L., Arnold, R.L., Liener, I.E., and Waibel, P.E. 1969. Unavailability of cystine from trypsin inhibitors as a factor contributing to the poor nutritive value of navy beans. J. Nutr. 99:34-42.

Kamat, A.S., Nerkar, D.P., and Nair, P.M. 1989. Bacillus cereus in some Indian foods, incidence and antibiotic, heat and radiation resistance. J. Food Safety, 10:31-41.

Kereliuk, G.R. and Kozub, G.C. 1995. Chemical composition of small white (Navy) beans. Lebensm.-Wiss. U.-Technol. 28:272-278.

Koehler, H.H., Herrick, H., and Burke, D.W. 1986. Differentiating lectin activity in twenty-four cultivars of dry beans (*Phaseolus vulgaris* L.). J. Food Sci. 51:1471-1475.

Koehler, H.H., Chang, C., Scheier, G., and Burke, D.W. 1987. Nutrient composition, protein quality, and sensory properties of thirty-six cultivars of dry beans (*Phaseolus vulgaris* L.). J. Food Sci. 52:1335-1340.

Kon, S. 1979. Effect of soaking temperature on cooking and nutritional quality of beans. J. Food Sci. 44:1329-1334,1340.

Kon, S. and Sanshuck, D.W. 1981. Phytate content and its effects on cooking quality of beans. J. Food Proc. Pres. 5:169-178.

Kon, S., Olson, A.C., Frederick, D.P., Eggling, S.B., and Wagner, J.R. 1973. Effect of different treatments on phytate and soluble sugars in California small white beans (*phaseolus vulgaris*). J. Food Sci. 38:215-217.

Labuza, T.P., 1980. The effect of water activity on reaction kinetics of food deterioration. Food Technol. 33(4):36-41,59.

Latta, M. and Eskin, M. 1980. A simple and rapid colorimentric method for phytate determination. J. Agric. Food Chem. 28(6):1315-1317.

Lee, J.J., Uebersax, M.A., Zabik, M.E., and Steffe, J.F. 1995. Gelatinization properties of navy bean starch. Starch/starke, 9:329-333.

Lineback, D.R. and Inglett, G.E. 1982. Food Carbohydrates. Avi Publishing Inc., Westport, Conn. 494pp.

Liu, K. 1995. Cellular, biological, and physicochemical basis for the hard-to-cook defect in legume seeds. Crit. Rev. Food Sci. Nutr. 35(4):263-398.

Lolas, G.M. and Markakis, P. 1975. Phytic acid and other phosphorus compounds of beans (*Phaseolus vulgaris* L.). J. Agr. Food Chem. 23:13-15.

Lolas, G. M. and Markakis, P. 1977. The phytase of navy beans (*phaseolus vulgaris*). J. Food Sci. 42:1094-1097, 1106.

Mafuleka, M.M., Ott, D.B., Hosfield, G.L., and Uebersac, M.A. 1993. The role of phytase and lignin in decorticated dry bean (*phaseolus vulgaris*) hardening during storage. J. Food Proc. Pres. 17:1-20.

Maga, J.A. 1982. Phytate: Its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. J. Agric. Food Chem. 30:1-9.

McCurdy, S.M. 1992. Infrared processing of dry peas, canola, and canola screenings. J. Food Sci. 57:941-944.

McNab J.M. and Wilson, B.J. 1974. Effects of micronising on the utilization of field beans (*Vicia faba* L.) by the young chick. J Sci. Food Agric. 25:395-400.

Moscoso, W., Bourne, M.C., and Hood, L.F. 1984. Relationship between hard to cook phenomenon in red kidney beans and water absorption, puncture force, pectin, phytic acid, and minerals. J. Food Sci. 49:1577-1583.

Moseley, G. and Griffiths, D.W., 1979. Varietal variation in the anti-nutritive effects of field beans (*Vicia faba*) when fed to rats. J. Sci. Food Agric. 30: 772-778.

Murray, J. 1987. Unlocking new product opportunities with infra-red processing. Paper given at Food Plants/87, Chicago, IL, Sept. 23-25. in McCurdy, 1992.

Nickerson, J.T. and Sinskey, A.J. 1972. Microbiology of Foods and Food Processing. American Elsevier Pub Co., New York, NY. 306pp.

Nitsan, Z.J. 1971. Vicia faba beans vs. soyabean meal as a source of protein. J. Sci. Food Agric. 22:252-255.

NSW Agriculture, 2002. Queensland Deparment of Primary Industries and Bean Growers, P4.2.20 Navy Bean. URL: www.agric.nsw.gov.au/reader/15420 (December 10, 2002)

Occena, L.G., Bennink, M.R., Uebersax, M.A., and Chung, Y.S. 1997. Evaluation of drum-dried meals prepared from split beans (*phaseolus vulgaris* L.): protein quality and selected antinutritional factors. J. Food Proc. Preserv. 21:335-344.

Ockerman, H.W. 1991. Food Science Sourcebook. Van Nostrand Reinhold, New York, NY. 63-68, 1473pp.

Paredes-López, O., Maza-Calvino, E.C., and Montes-Rivera, R. 1988. Effect of the hard-to-cook phenomenon on some physicochemical properties of bean starch. Starch/starke 40:205-210.

Paredes-López, O., Maza-Calvino, E.C., and Gonzalez-castaneds, J. 1989a. Effect of the hardening phenomenon on some physicochemical properties of common bean. Food Chem. 31:225-236.

Paredes-López, O., Reyes-Moreno, C., Montes-Rivera, R., and Carabez-Trejo, A. 1989b. Hard-to-cook phenomenon in common beans-influence of growing location and hardening procedures. Int. J. Food Sci. Tech. 24:535-542.

Plhak, L.C., Caldwell, K.B., and Stanley, D.W. 1989. Comparison of methods used to characterize water imbibition in hard-to-cook beans. J Food Sci. 54:326-329,336.

Prescott, L.M., Harley, J.P., and Klein, D.A. 1990. Microbiology. Wn.C. Brown Publishers, Dubuque, IA. 882pp.

Price, M.L., van Scoyoc, S., and Butler, L.G. 1978. A critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. J. Agric. Food Chem. 26(5):1215-1218.

Proctor, J.P. and Watts, B.M. 1987. Effect of cultivar, growing location,moisture,a nd phytate content on the cooking times of freshly harvested navy beans. Can. J. Plant Sci. 67:923-926.

Quenzer, N.M., Huffman, V.L., Burns, E.E. 1978. Some factors affecting pinto bean quality. J. Food Sci. 43:1059-1061.

Ray, B. 1996. Fundamental Food Microbiology. CRC Press Inc., Boca Raton, Fl. 516pp.

Narasinga Rao, B.S. and Prabhavathi, J. 1982. Tannin content of foods commonly consumed in India and its influence on ionizable iron. J. Sci. Food Agric. 33:89-96.

Reddy, N.R., Pierson, M.D., and Salunkhe, D.K. 1984. Chemical, nutritional, and physiological aspects of dry bean carbohydrates: a review. Food Chem. 13:25.

Reddy, N.R., Pierson, M.D., Sathe, S.K., and Salunkhe, D.K. 1985. Dry bean tannins: a review of nutritional implications. J. Am. Oil Chem. Soc. 62:541. 1985.

Robinson, R.K., Batt, C.A., and Patel, P.D. 2000. Encyclopedia of Food Microbiology. Academic Press, San Diego, Cal. 2372pp.

Ruperez, P. 1998. Oligosaccharides in raw and processed legumes. Z. Lebensm. Unters. Forsch. A. 206:130-133.

Ryan, C.A., 1973. Proteolytic enzymes and their inhibitors in plants. Ann. Rev. Plant Physiol. 24:173-196.

Ryas-Duarte, P. Bergeron, D., and Nielsen, S.S. 1992. Screening of heat-stable trypsin inhibitors in dry beans and their partial purification from great northern beans (*phaseolus vulgaris*) using anhydrotrypsin-sepharose affinity chromatography. J. Agric. Food Chem. 40:32-42.

Sakai, N. and Hanzawa, T. 1994. Applications and advances in far-infrared heating in Japan. Trends in Food Sci. Tech. 5:357-362.

Salunkhe, D.K. 1990. Dietary Tannins: Consequences and Remedies. CRC Press Inc., Boca Raton, Fl. 200pp.

Salunkhe, D.K. and Kadam, S.S. 1989. Introduction. Pages 1-4 in: CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization. Vol. 1. CRC Press Inc., Boca Raton, Fl.

Sarantinos, J. and Black, R. 1996. Effects of micronisation on the chemical and functional properties of chickpeas. Food Aust. 48:39-42.

Sayeed, S. and Njaa, L.R. 1985. Effect of a Bangladeshi home-cooking procedure on the amino acid content, trypsin inhibitor activity and in vitro digestibility of some legume seeds. Qual. Plant Pl. Foods Hum. Nutr. 35:379-388.

Scanlon, M.G., Malcolmson, L.J., Arntfield, S.D., Watts, B., Ryland, D., and Prokopowich, D.J. 1998. Micronization pretreatments for reducing the cooking time of lentils. J. Sci. Food Agric. 76:23-30.

Schoch, T.H. and Maywald, E.C. 1968. Preparation and properties of various legume starches. Cereal Chem. 45:564-573.

Shomer, I., Paster, N., Lindner, P., and Vasiliver, R. 1990. The role of cell wall structure in the hard-to-cook phenomenon in beans (*phaseolus vulgaris* L.) Food Struct. 9:139-149.

Silva, H.C. and Braga, G.L. 1982. Effect of soaking and cooking on the oligosaccharide content of dry beans (*Phaseolus vulgaris*, L.). J. Food Sci. 47:924-925.

Simpson, H.C.R., Lously, S., Geekie, M., Simpson, R.W., Carter, R.D., Hockaday, T.D.R., and Mann, J.I. 1981. A high carbohydrate leguminous fibre diet improves all aspects of diabetic control. Lancet 1:1-5.

Singh, K.B., Erskine, W., Robertson, L.D., Nakkhoul, H, and Williams, P.C. 1988. Influence of pretreatment on the cooking quality parameters of dry food legumes. J Sci. Food Agric. 44:135-142.

Smith, C., Van Megen, W., Twaalfhoven, L. and Hitchcock, C. 1980. The determination of trypsin inhibitor levels in foodstuffs. J. Sci. Food Agric. 31:341-350.

Srikantia, S.G. 1976. Chemistry and nutritive value of sorghum, maize, and millets, in Proc. Symp. Prod. Process. Util. Maize, sorghum other millets, Central Food Technological Research Institute, Mysore, India, 31.

Stanley, D.W. 1992. A possible role for condensed tannins in bean hardening. Food Res. Intl. 25:187-192.

Stanley, D.W. and Aguilera, J.M. 1985. A review of textural defects in cooked reconsistuted legumes-The influence of structure and composition. J. Food Biochem. 9:277-323.

Swanson, B.G., Hughes, J.S., and Rasmussen, H.P. 1985. Seed microstructure: Review of water imbibition in legumes. Food Microstr. 4:115-124.

Toews, R. 2001. Effects of pretreatment and micronization on the cookability and chemical components of green and yellow field peas. M. Sc. Thesis, University of Manitoba, Winnipeg, MB

Troller, J.A. and Christian, J.H.B. 1978. Water activity and food. Academia Press. New York, NY. 235pp.

Uebersax, M.A. and Bedford, C.L. 1980. Navy bean processing: Effect of storage and soaking methods on quality of canned beans. Michigan State University Agricultural Experiment Station. 410 Agricultural Business, File: 35.34:2-11.

USDA, Economic Research Service. Briefing Rooms 2002a. URL: www.ers.usda.gov/briefing/drybeans/background.htm (December 10, 2002)

USDA, Economic Research Service. Government Publications 2002 b. URL: www.ers.usda.gov\publications\sb965\data\pulsed.xls (December 10, 2002)

U.S. Food & Drug Administration. Center for food safety and applied nutrition. 1994. Food borne pathogenic microorganisms and natural toxins handbook. Bad bug book. *Bacillus cereus* and other *Bacillus* spp. URL: www.cfsan.fda.gov/~mow/chap12.htmL (February 24, 2002)

Uzogara, S.G., Morton, I.D., and Daniel, J.W. 1990. Influence of various salts in the cooking water on pectin losses and cooked texture of cowpeas (*vigna unguiculata*). J Food Biochem. 14:283-191.

van der Poel, T.F.B., van Zuilichem, D.J., and van Oort, M.G. 1990. Thermal inactivation of lectins and trypsin inhibitor activity during steam processing of dry beans (*phaseolus vulgaris*) and effects on protein quality. J. Sci. Food Agric. 53:215-228.

Vanderzand, C. and Splittstoesser, D.F. 1992. Compendium of Methods for the Microbiological Evaluation of Foods. Corporate American Public Health Association, Washington, D.C. 1219pp.

Varriano-Marston, E. and Jackson, M.G. 1981. Hard-to-cook phenomenon in beans: structural changes during storage and imbibition. J. Food Sci. 46:1379-1385.

Vidal-Valverde, C., Frias, J., and Valverde, S. 1993. Changes in the carbohydrate composition of legumes after soaking and cooking. J. Amer. Diet. Assoc. 93:547-550.

Wilkie, F.H. 1998. Laboratory Methods in Food Microbiology. Academic Press, San Diego CA. 552pp.

Wilson, B.J., McNab, J.M., and Bentley, H. 1972. Trypsin inhibitor activity in the field bean (*Vicia faba* L.) J. Sci. Food Agric. 23: 679-684.

Zhao, Y. 2000. The effects of pretreatment and micronization on the quality and cookability of lentils. M.Sc. Thesis, University of Manitoba, Winnipeg, MB.

Ziena, H.M., Youssef, M.M., and El-mahdy, A.R., 1991. Amino acid composition and some anti-nutritional factors of cooked faba beans (*Medammis*): Effects of cooking temperature and time. J. Food Sci. 56 :1347-1349, 1352.

APPENDIX I
The Sensory Evaluation of Navy Bean Texture: Instructions and Ballots

Objective:

To determine the optimum cooking time, based on hardness, smoothness, and seed coat presence, for raw navy beans soaked for 18 h and cooked for 27.5, 35, 38.75, 42.5, and 50 min in distilled water.

Panelists:

The expert panel consisted of 6 members

Methods:

Initially the group discussed the typical uses and ways that navy beans are consumed. Among these were re-fried beans, chili, bean soups, cold bean salads, Chinese deserts, and baked beans.

The panelists identified the baked bean or chili type bean texture as the ideal texture for micronized navy beans to be. The panelists agreed that the targeted texture should be softer than those used in salads. The shape should remain apparent, appear individually, and not be squished together.

Discussions of the type of bean characteristics to be examined resulted in identification of hardness, smoothness, and the presence of the seed coat as important attributes. The panel decided that the initial bite through the bean should provide slight resistance, but further mastication should require very little force. The cotyledons should be smooth, with no particulate matter or inconsistencies in smoothness within the seed. Additionally, there should minimal residual in the mouth after swallowing. The seed coat was determined to be ideal if it was present, but not chewy. The intactness of the seed was also examined and determined visually. Beans were ideally whole, with cotyledons remaining within the seed, making the bean individual and not squished together.

The panel used a structured line scale from 1 – 5, with just right = 3 to determine the 3 textural characteristics. Each panelist evaluated the samples in random orders and recorded their results and comments individually. The group discussed their results and commented on the overall acceptability of the beans.

Based on the discussion and results, the optimal cooking time of navy beans was determined by the panel.

APPENDIX I

The Sensory Evaluation of Navy Bean Texture: Instructions and Ballots

Sample Preparation:

Approximately 600 g of navy beans stored at 4°C were removed at place into a chlorine sanitized bin at a ratio of 1:3 beans to distilled water and soaked for approximately 18 h. Pyrex glass pots were used to boil 900 g of soaked beans in distilled water at a 1:10 ratio. The beans were boiled at a constant rate for 27.5, 35, 38.75, 42.5, and 50 min. The samples were drained and cooled in plastic containers for approximately 1 h prior to sensory evaluations. Approximately 30 g of each sample was placed in 125 mL Styrofoam cups, pre-coded with 3 digit random numbers. Each panelist received 5 samples, and was assigned a random order of evaluation. Filtered water and soda crackers were available for rinsing and cleansing the palate between samples.

APPENDIX I
The Sensory Evaluation of Navy Bean Texture: Instructions and Ballots

Ballot used for the textural evaluation of navy beans

Evaluation of Navy Beans

Instuctions:

1. Place one teaspoon of beans in your mouth, move to the molars and evaluate the force required to bite through the sample.
2. Chew the sample slowly and evaluate the way the sample is perceived in the mouth throughout the chewing and just prior to swallowing.
3. Repeat this procedure taking another teaspoon of sample.
4. Note your observations in the space provided and grade them on a structured line scale of 1 to 5.

Evaluate each sample in the order listed.

Code No. _____

Code No._____

Code No._____

Code No._____

Code No. _____

APPENDIX I

The Sensory Evaluation of Navy Bean Texture: Instructions and Ballots

Structured line scale used by panelists for navy bean evaluations

Textural Characteristics

A) Hardeness

1 3 5

1. Too Hard
3. Just Right
5. Too Soft

B) Smoothness

1 3 5

1. Granular
3. Just Right
5. Too smooth (pasty)

C) Seed Coat Presence

1 3 5

1. Chewy
3. Just Right
5. Not detected – too soft

APPENDIX II

The raw physiochemical data of micronized navy beans after tempering under different conditions.

	Tempering Conditions							
	18h at R.T.		6h at R.T.		4.5h at 45°C		1.5h at 60°C	
	20%	25%	20%	25%	20%	25%	20%	25%
% Phytic Acid	1.54 ± 0.03	1.73 ± 0.15	1.66 ±0.06	1.63 ± 0.22	1.21 ± 0.06	1.15 ± 0.30	1.82 ± 0.27	1.98 ± 0.26
Trypsin (mg TIA/g)	8.23 ± 0.76	7.46 ± 0.67	8.06 ± 0.67	8.20 ± 0.77	6.81 ±0.28	6.20 ± 1.27	4.83 ± 0.09	6.90 ± 0.50
Tannin (mg cat eq./g)	38.02 ± 4.71	42.64 ± 3.00	41.37 ± 2.46	38.60 ± 1.22	43.24 ± 0.19	41.60 ± 4.25	42.16 ±1.26	42.14 ± 5.60
% Gel. Starch	11.31 ± 4.41	7.6 ± 8.18	9.32 ± 2.76	17.03 ± 0.62	10.05 ± 5.55	8.2 ± 0.72	4.23 ± 0.75	9.12 ± 4.34
Peak Force at 42.5 min (N)	416 ± 37	427 ± 58	449 ± 15	464 ± 24	476 ± 8.0	530 ± 62	436 ± 14	439 ± 3.2
Tempered Aw	0.83 ± 0.00	0.87 ± 0.04	0.85 ± 0.02	0.90 ± 0.01	0.841 ± 0.00	0.89 ±0.00	0.88 ± 0.03	0.88 ± 0.02
Micronized Aw	0.83 ± 0.02	0.83 ± 0.08	0.82 ±0.01	0.86 ± 0.01	0.82 ± 0.00	0.87 ±0.02	0.81 ± 0.02	0.86 ± 0.01
Tempered % Moisture	21.07 ± 0.49	24.72 ± 2.88	21.14 ± 0.33	26.73 ± 0.09	21.11 ± 0.11	26.01 ± 0.20	22.21 ± 0.18	25.10 ± 1.33

APPENDIX III

Raw data of the microbial increases on navy beans during tempering

	Tempering Conditions							
	18h at R.T.		6h at R.T.		4.5h at 45°C		1.5h at 60°C	
	20%	25%	20%	25%	20%	25%	20%	25%
Bacteria (log CFU/g)	1.70 ± 0.21	2.79 ± 0.32	0.13 ± 0.13	0.81 ± 1.06	0.38 ± 0.08	0.48 ± 0.48	1.06 ± 1.22	2.27 ± 1.90
Yeast and Mould (log CFU/g)	-0.50 ± 0.15	-0.87 ± 0.45	-0.49 ±0.16	-0.27 ±0.63	-0.20 ± 0.39	-0.02 ± 0.32	-0.06 ±0.00	-0.04 ± 0.43

APPENDIX IV

Raw data of the microbial decreases on navy beans as a result of micronization

	Tempering Conditions							
	18h at R.T.		6h at R.T.		4.5h at 45°C		1.5h at 60°C	
	20%	25%	20%	25%	20%	25%	20%	25%
Bacteria (log CFU/g)	1.37 ± 0.43	1.06 ± 0.94	2.38 ± 0.88	2.36 ± 0.33	1.37 ± 0.17	1.55 ± 0.24	1.97 ± 0.35	1.84 ± 0.56
Yeast and Mould (log CFU/g)	1.37 ± 0.43	1.06 ± 0.94	2.38 ± 0.88	2.36 ± 0.33	1.37 ± 0.17	1.55 ± 0.24	1.97 ± 0.35	1.84 ± 0.56