## HYDRATION PROPERTIES AND NOVEL PROCESSING TO PRODUCE

## WHOLE PEA SNACK FOOD

By

Da An

## A Thesis

Submitted to the Faculty of Graduate Studies in Partial Fulfilment of the

Requirements for the Degree of

## MASTER OF SCIENCE

Department of Food Science

University of Manitoba

Winnipeg, Manitoba

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Of

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#### ABSTRACT

Hydration and nutritional properties of peas, as well as different processing methods to produce a whole pea snack food were investigated in this study. Different varieties of peas from different locations grown in 2005 and 2006 were evaluated for hydration properties including hydration capacities and imbibing rates. Those varieties which absorbed water continuously to an acceptable level and contained lower levels of phytic acid and higher levels of total phenolics and antioxidant activities were subjected to two different processes: micronization and superheated steam system (SHS). The processed peas were assessed for the texture, phytic acid, phenolic acid and antioxidant levels. Results showed water absorption ability of peas was directly related to the initial moisture content and inversely related to the percent of stone seeds. The climatic, especially temperature differences, in the two years of the study influenced pea quality including hydration, antinutritional factors and antioxidant activities. Different processing conditions were explored for both micronization and superheated steam processes. Results showed the effects of tempering level and processing conditions on maximum force to break the processed seed were significantly different (P< 0.05) for all types of pea products. Pea products from SHS had significantly higher maximum force (up to 95.0 N) than the commercial peas (32.8 N) (P<0.0001) which is a pea snack fried in oil. However, the micronized peas had significant lower maximum forces at break (as low as 18.4 N) than the commercial peas (P<0.05). Micronization also decreased the phytic acid level and increased the total phenolics level. From the antioxidant

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#### INTRODUCTION

Pulses are one of the world's most important food supplies, especially in developing countries. (Reyes Moreno and Paredes-Lopez, 1993). Pea (Pisum sativum) is one of the most commonly grown food pulses in the world and it has been widely used in the human diet for a long time. Peas are excellent source of proteins, vitamins, minerals, and other nutrients while being low in fat, high in fiber and containing no cholesterol (Sharareh, 2006). Along with the increasing awareness of the nutritional value of peas, more and more pea products have been added to the North American diet. P. sativum is an annual plant, with a lifecycle of one year. It is a cool season crop grown in many parts of the world, and planting can take place from winter through to early summer depending on location (Wikipedia, 2007). Canada's cool climate provides pulse crops with natural protection against insects and disease, and Canada also has a well established infrastructure to store and move pulses from the producing regions to the port terminals (Agriculture and Agri-Food Canada, 2006). Pulse crops have seen a noticeable increase in production in the last decade. According to Agriculture and Agri-Food Canada (2006), there was a 320% increase in pea production between 1993 and 2003. Since then, the production of peas has levelled off. Utilization of peas for human consumption involves whole seed processing, as well as pea flour utilization, and requires promotion of the nutritional benefits of pea products.

Micronization can improve the cookability of cereals, legumes or oil seeds. It is an intensive heat treatment that can be used to cook foods through relatively short exposure times to electromagnetic wavelengths in the infrared (IR) region of spectrum (Arntfield et al., 2004). It will cause rapid internal heating and a rise in water vapour pressure inside the heated sample (Fasina et al., 1997). Kadam et al. (1987) reported that micronization can be used to increase the digestibility and nutritional quality of cereals and legumes for human food. Preparation of pulses with infrared heat to reduce their cooking time has been the subject of a number of recent studies. Infrared heating in processing of legume seeds has been reported to increase the starch gelatinization and decrease the protein solubility of the products (Blenford, 1980). Arntfield et al. (1997<sup>b</sup>) reported that higher moisture content in the seed during micronization resulted in a higher degree of starch gelatinization. The gelatinized starch softens the texture of the seeds and thus reduces their cooking time. They found that micronization could reduce the cooking time of lentils. They also indicated that the decrease in protein solubility by micronization is due to the heat-induced denaturation of proteins. Protein denaturation increases the digestibility of legume proteins and also helps to produce soft texture in the seeds.

Superheated steam (SHS) technology is another technique which can be used to process particulate foods. The use of superheated steam has many benefits to the consumer and industry. First of all, it can lead to energy savings as high as 50-80% over the use of hot air (Pronyk et al., 2005). SHS dehydrators

are designed as closed systems where the exhaust may be collected and condensed (Pronyk et al., 2005).

Marrow fat peas, green peas, and yellow peas from different locations have been studied in this project. The first objective of this project is to determine the physical and structural factors that affect moisture absorption and establish the moisture distribution and hydration rate in the seed which are necessary for effective processing of whole seeds. The hydration determination is crucial because only those varieties that can absorb water consistently to an acceptable level can be effectively used in whole pea processing. A number of investigations have indicated that hydration capacities of pulses should be adjusted prior to processing (Scanlon et al., 1998; Zhao, 2000; Toews, 2001). Another aim is to develop new food products, especially snack foods of whole peas with crunchy texture, which can be eaten directly by using some processing techniques based on superheated steam, infrared micronization, roasting and boiling. This will make pulse crop more readily available to consumers. In addition, some chemical compounds including phytic acid, total phenolics and antioxidant properties which influence the nutritional gualities of peas will be determined to see the effects of processing.

#### LITERATURE REVIEW

#### 2.1. Background

#### 2.1.1. Production and Consumption of Peas

Pea is among the oldest crops in the world as it was first cultivated as early as 9000 years ago. It is a cool-season legume crop that is grown on over 25 million acres worldwide. The major pea producing areas are Russia and China, followed by Canada, Europe, Australia and the United States. Europe, Australia, Canada and the United States raise over 4.5 million acres and are major exporters of peas (NDSU, 2003).

The Canadian pulse industry has grown tremendously over the past twenty years to become a major player in global pulse production and trade, with a strong base of Canadian processing and exporting companies. Over this time, Canada has emerged to become the world's largest exporter of lentils and peas, and a top five bean exporter (Pulse Canada, 2007).

Canadian pulse production peaked in 2005 at more than 4.8 million tonnes. Pulse production is normally in the range of 4 to 4.5 million tonnes per year. The value of Canadian pulse exports alone exceeded \$1 billion in 2006 (Pulse Canada, 2007). Quebec and Ontario produce bean crops, Manitoba produces white and colored beans, as well as peas and lentils (Pulse Canada, 2007). Saskatchewan is the largest producer of peas, lentils and chickpeas with a small

bean industry, and Alberta produces beans under irrigation as well as peas, lentils and chickpeas (Pulse Canada, 2007).

Early in the 20<sup>th</sup> century, first Ontario and then Manitoba led Canadian pea production. Since the mid-1980's, Saskatchewan has produced the majority of Canadian peas with significant acreage also being grown in Alberta and Manitoba (SPG, 2007). Ontario is no longer a large scale producer. In Saskatchewan, pea yields average 1800 lb/ac (2018 kg/ha), but yields as high as 3500 lb/ac (3900 kg/ha) have been reported (SPG, 2007). About 60% of the Canadian pea crop is exported to Europe, South America, and Asia. The large European livestock feed market is the major pea market. South Asia is also a significant market for yellow peas. An increasing amount is being used in Saskatchewan for livestock feed, with a small amount of the pea crop processed into pea fibre, pea protein and pea starch (SPG, 2007). Increasing amounts of pea are exported to South America and the Indian subcontinent for food use. A small portion of the Canadian pea crop is used domestically as food.

## 2.2. Characteristics and Quality of Peas

## 2.2.1. Seed Characteristics and Structure

There are three types of pea: Marrowfat (Figure 1); Yellow (Figure 2), and Green (Figure 3). Both yellow and green peas are round seeded and glossy. Color ranges from yellow to white or green to white. Marrowfat peas are bigger than yellow and green peas, and have a wrinkled skin and are green in color.

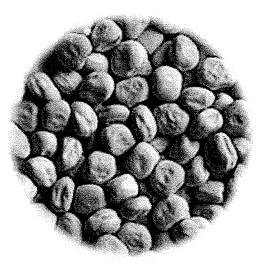


Figure 1. Marrowfat Pea

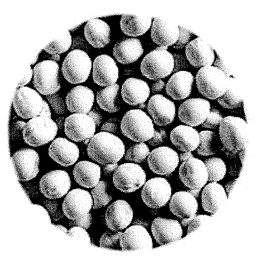


Figure 2. Yellow Pea

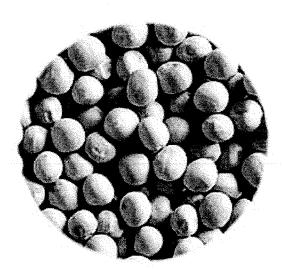


Figure 3. Green Pea

In the study of Kadam et al. (1989), mature legume seeds were shown to have three major components: embryonic axis; seed coat or hull and cotyledons. The embryonic axis and seed coat contribute only 1-2% and 8-15% of the total seed weight, respectively. Even though the embryonic axis is rich in nutrients, it does not contribute much to the overall nutritional value of the food because it only constitutes such a small amount of the whole seed.

Seed coats are described as being permeable or impermeable. A permeable (normal) seed imbibes water readily when available while an impermeable seed does not take up water for days or even longer. Impermeable seeds are often referred to as "hard" or "stone seeds". Hardseededness is undesirable by the food processing industry. Ideally, seeds should take up water quickly and at a constant rate. This trait is particularly critical when whole seeds are processed. Seed coat permeability is important to both the scientific and industrial communities. The permeability property of a seed coat has been related to its structure (Ma et al., 2004; Zeng et al., 2005). A typical legume seed coat is chemically made up of cellulose, hemicellulose, lignin, pectin, and calcium (Kadam et al., 1989). These components provide structure and support to cells (Hincks and Stanley, 1987). Since the seed coat constitutes 80-90% of the total dietary fibre of the pea seed, removal of the seed coat is undesirable from a nutritional perspective (Kadam et al., 1989).

Seed coat characteristics of peas play an important role in water imbibition properties. Anatomically, the seed coat contains several specialized areas,

i.e. hilum, micropyle, and raphe, which are also involved in the water absorbing capacity of seeds. Koning (2007) reported that the raphe is a ridge on the seed coat, and the micropyle is a hole that goes through the seed coat. Hyde (1954) concluded that the helium acts as a hygroscopic valve, opening to imbibe moisture when the environmental humidity is high, and closing to eliminate moisture when the environmental humidity is low. Therefore, the microstructure plays an important role in water imbibition characteristics during soaking and processing. Determination of hydration is crucial before whole pea processing because only those varieties that can absorb water consistently to an acceptable level can be used for processing.

#### 2.2.2. Nutritional Quality and Health-Promoting Benefits of Peas

Peas are an excellent food choice with health-promoting benefits. Their nutritional composition includes complex carbohydrates (e.g. fibre, resistant starch, oligosaccharides), protein, important vitamins and minerals as well as antioxidants and only very small amounts of fat (Sharareh, 2006). All support a healthy diet although some antinutrients contain in filed peas such as trypsin inhibitors and phytic acid. Field peas are palatable, contain over 20% protein and also contain a substantial amount of starch. These are desirable characteristics for supplying available nutrients.

Pulses have about twice the protein content of cereal grains and are high in complex carbohydrates including fibre. In fact, a one-cup serving of pulses provides almost half of the daily amount of fibre recommended for Canadians

(Pulse Canada, 2007). Increased dietary intake of high fibre foods like pulses is important for North Americans who currently consume less than half of the recommended amount. Pulses also have a low glycemic index which results in slower carbohydrate digestion (Pulse Canada, 2007). This makes pulses a valuable food choice for people with diabetes as well as those at risk for both diabetes and cardiovascular disease.

Research suggests that regular dietary intake of pulses can reduce serum cholesterol and triglycerides and can also reduce the risk of developing nutrition-related health problems including obesity, diabetes, heart disease and cancer. (Committee on Diet and Health, 1989; Scientific Review Committee, 1990).

#### 2.2.2.1. Carbohydrates

The proportion of carbohydrates in peas ranges from 29.3% to 58.5% (Reddy et al., 1989; Deshpande and Damodaran, 1990). Carbohydrates can be classified into starch, soluble sugars, and pectin. Each fraction varies within different types of peas and appears to be related to seed size. Some small seeded peas such as yellow and green peas have less than 30% starch in the dry matter, while the larger seeded varieties such as marrowfat peas contain closer to 50% starch (Christensen and Mustafa, 1999). Starch is the predominant carbohydrate in field peas, and is present as granules embedded in the cotyledon's protein matrix (Kadam et al., 1989; Joseph et al., 1993). Each starch granule has its own structure and properties, but the granules are made up of two glucose polymers: amylose and amylopectin. These two glucose polymers

form crystalline and amorphous regions within starch granules that are visible under polarized light (Bogracheva et al., 1998). Amylose is mainly responsible for the starch gelatinization temperature and pasting properties, which are important in the heat processing of peas (Bogracheva et al., 1998; Deshpande and Damodaran, 1990). Starch gelatinization during legume processing can give greater starch availability and digesbility (Arntfield et al., 1997), thus it is a critical parameter during the heat treatments of legumes.

Field peas also contain a relatively high proportion of soluble sugars which ranges from 4% to 15% of total carbohydrate content (Reddy et al., 1989). This high proportion of soluble sugars will provide readily available carbohydrate (Christensen and Mustafa, 1999). Soluble sugars in peas include reducing sugars such as glucose and fructose, as well as some oligosaccharides in the raffinose series such as raffinose, stachyose and verbascose (Table 1) (Wang and Daun, 2004). The raffinose sugars that make up 30-80% of the soluble sugars (Reddy et al., 1984), are only digested by anaerobic microorganisms living in the colon.

Pectin is another carbohydrate found in peas mainly in the cell walls of the seed coat and in the middle lamellar region and intercellular layers of cotyledons (Toews, 2001). It is mainly responsible for maintaining seed structure integrity by associating with other intercellular components including other pectin molecules (Talbot and Ray, 1992). Pectin in peas can be divided into a water soluble fraction, an ethydiamine tetra acetic acid (EDTA) soluble fraction and a residual fraction. During soaking and cooling, the water soluble pectins leach out of the

seed (Uzogara et al., 1990), a phenomenon which is enhanced when sodium salts are present. Sodium ions displace the divalent cations such as calcium and magnesium in the EDTA and residual fractions of pectins thereby increasing the pectin solubility (Liu et al., 1993).

Sugars (g/100g dry matters)	Mean	Range
Sucrose	2.8	2.1 - 4.3
Raffinose	0.7	0.5 - 0.9
Stachyose	2.7	1.9 – 3.9
Verbascose	1.0	0.5 – 1.5
Oligosaccharides <sup>1</sup>	4.4	3.5 - 5.5

Table 1. Sugar Fraction in Canadian Field Peas. (adapted from Wang and Daun,2004)

<sup>1</sup>Sum of raffinose, starchyose and verbascose

## 2.2.2.2. Proteins

Peas, like other legume seeds are characterized by having highly digestible protein. The protein level in peas ranges from 17.5% to 33.0% (Kadam, et al., 1989; Bastianelli et al., 1998; Mustafa, et al., 1998). Most of the proteins in peas are storage proteins (Bora et al., 1994). Gueguen and Barbot (1988) found that the concentrations of specific amino acids in pea proteins (Table 2) depend on the storage proteins, of which albumins and globulins are the main types (Bora et al., 1994). Included in the albumin proteins are the protease inhibitors, which are antinutritional factors that reduce the nutritive value of peas if they are not denatured during processing (Deshpande and Damodaran, 1990).

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During heat processing of legumes, the nutritional values of proteins is improved by inactivating antiphysiological factors, particularly trypsin protease inhibitors and haemagglutinin, and unfolding the storage protein structures, thus making them more susceptible to attack by digestive enzymes (Sathe, et al., 1984). There are many factors which influence the extent of protein denaturation such as the initial moisture level, heating temperatures and time.

Amino acid (g/16 g N)	Mean	Range
Alanine	4.1	3.8 – 4.6
Arginine	8.5	6.8 – 12.6
Aspartic Acid	12.5	10.4 -14.8
Cystine	1.2	1.0 -1.9
Glutamic Acid	15.6	14.1 -17.6
Glycine	4.3	3.6 - 5.2
Histidine	2.3	1.9 - 2.9
Isoleucine	3.3	2.4 - 4.4
Leucine	6.5	5.3 - 7.4
Lysine	6.3	3.9 – 7.6
Methionine	1.1	0.9 – 1.4
Phenylalanine	4.4	3.1 – 5.0
Proline	4.8	4.0 - 6.9
Serine	5.6	5.0 – 6.2
Threonine	4.4	3.8 – 5.1
Tryptophan	0.8	0.7 – 0.9
Tyrosine	2.9	1.7 – 4.1
Valine	4.0	3.2 – 5.0
Cys + Met	2.3	1.9 – 5.0
Tyr + Phe	7.2	6.1 – 8.7

Table 2. Amino Acid Composition of Canadian Field Peas (adapted from Wang and Daun, 2004)

#### 2.2.2.3. Lipids and Other Nutritional Components

The range of lipids in field peas is 0.7-3.2% (Deshpande and Damodaran, 1990; Daveby et al., 1993; Bastianelli et al., 1998). It was reported that this value varies with the type of field peas, where wrinkled peas have the highest and coloured peas have the least (Bastianelli et al., 1998). Kosson et al. (1994) reported that the distribution of lipids varies with cultivar, location, climate, season and environmental conditions.

Field peas are also a good source of certain minerals and vitamins (Table 3 and Table 4). Singh et al. (1968) reported that almost all of the minerals are in the cotyledons, but 40% of calcium is in the seed coat.

Minerals	Mean	Range
		(mg/100 g dry matter)
Calcium (Ca)	82.3	59.6 -106.9
Copper (Cu)	0.7	0.5 - 00.9
Iron (Fe)	5.6	4.1 – 7.9
Potassium (K)	1047.2	687.4 – 1473.2
Magnesium (Mg)	142.4	115.4 – 172.3
Manganese (Mn)	1.2	0.2 - 2.4
Phosphorus (P)	436.7	226.5 – 950.5
Zinc (Zn)	3.9	2.5 - 6.4

Table 3. Mineral Composition of Canadian Field Peas (adapted from Wang and Daun, 2004).

Vitamin	Mean	Range (mg/100 g)
Retinol (A)	ND <sup>1</sup>	ND
C	0.55	0.28 – 0.96
D	ND	ND
Thiamin (B1)	0.51	0.34 – 0.98
Riboflavin (B2)	0.18	0.12 – 0.40
Niacin (B3)	1.55	0.85 – 2.29
Panthothenic acid (B5)	1.18	0.72 – 2.98
Pyridoxine (B6)	0.05	0.01 – 0.10
Cyanocobalamin (B12)	ND	ND
Biotin	ND	ND
γ-tocopherol	6.89	5.28 – 8.51
a-tocopherol	0.01	0.00 – 0.16
Folic acid (µg/100 g)	ND	ND

Table 4. Vitamins in Canadian Field Peas (adapted from Wang and Daun, 2004).

<sup>1.</sup> ND = not detectable

### 2.2.3. Phytochemicals

Research recommendations (Pulse Canada, 2007) suggest that the intake of legumes should be increased for better health and management of chronic diseases, such as cardiovascular disease, diabetes and cancer. However, there are concerns that high intakes of these foods may also increase the intake of certain phytochemicals with antinutrient effects such as phytic acid and trypsin inhibitors (Chavan, et al. 2003). Legumes contain several antinutrients which may hinder efficient utilization, absorption or digestion of nutrients and thus reduce their nutrient bioavailability and nutritional quality (Liener, 1975). There is currently much interest in phytochemicals as bioactive components in foods (Dillard and German, 2000). Many iatrical data as well as in vitro studies strongly suggest that foods containing phytochemicals with antioxidant potential have strong protective effects against major disease risks including cancer and cardiovascular diseases (Steinberg, 1991; Block et al., 1992; Kaur and Kapoor, 2002). Legumes are a rich source of polyphenols, which have high antioxidant activities (Cardador-Martinez, et al., 2002; Heimler, et al., 2005; Madhujith and Shahidi, 2005; Xu and Chang, 2007).

#### 2.2.3.1. Phytic Acid

Phytic acid (phytate), myoinositol 1,2,3,4,5,6 – hexakis (dihydrogen phosphate), is often present in legume seeds (Urbano, et al. 2000). It is the major source of total phosphorus in legumes. The antinutritional effects of phytic acid primarily relate to the strong chelating associated with its six reactive phosphate groups. It complexes with protein and particularly with minerals (Urbano, et al. 2000). The phytate-protein complex decreases the solubility of proteins and thus affects their functional properties and the phytate-mineral complex causes the minerals to be biologically unavailable for absorption (O'Dell, 1979). The amount of phytic acid in dry peas varies with variety, growing conditions and irrigation conditions (Marzo, et al., 1997).

There are several methods of decreasing the inhibitory effect of phytic acid on mineral absorption such as soaking, germination, fermentation, and cooking (Urbano, et al. 2000).

2.2.3.2. Phenolics

Phenolic compounds, such as phenolic acids, flavonols, flavones, isoflavones, anthocyanins, and condensed tannins, have been identified and characterized in legumes (Champ, 2002; Chang, 2002; Madhujith, et al. 2004; Xu, et al. 2007). Some of the polyphenolic compounds seemed to be bound to a pea superoxide dismutase that could act as a carrier (Nice, et al. 1995). Alonso et al. (1998) analysed polyphenols in peas and found that their concentrations varied according to the treatment of the peas. Numerous studies have shown that the majority of the antioxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocuamin, catechin and isocatechin rather than from Vitamin C, E and  $\beta$ -carotene (Wang et al., 1996; Kahkonen et al., 1999). Most of the current research focuses on the antioxidant action of phenolics. The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen guenchers and metal chelators (Rice-Evans et al., 1995). According to the research findings from Tawaha et al. (2007), the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. These results were also consistant with the findings of many research groups who reported positive correlation between total phenolic contents and antioxidant activity (Cai et al., 2004; Zheng and Wang, 2001).

#### 2.2.3.3. Antioxidants

Many research studies found antioxidants were present in peas (Nilsson et al. 2004). Dietary antioxidants protect against reactive oxygen species in the human body by several mechanisms. An increased intake of antioxidants may, therefore; have a number of health effects, such as reducing the incidence of cancer and cardiovascular diseases (Diplock et al., 1998). Due to the detection of many new bioactive compounds in peas with possible antioxidant activity, and the increased interest in the relationship between antioxidants and disease risks and mechanisms, numerous studies have been conducted on the measurement of antioxidants in peas. It has been proven that antioxidant properties of peas may highly relate to phenolic contents by many researchers (Nice et al. 1995).

#### 2.3. Superheated Steam (SHS)

#### 2.3.1. History

Superheated steam technology was originally introduced in the twentieth century as a technique for paper drying and is currently used for drying and processing of food materials. Drying is often a necessary operation to preserve products for consumption. Reducing moisture content of a food product helps to prevent or decrease microbial and enzymatic reactions in the food. Due to the mass reduction, it improves the economics of storage and transportation of foods (Chou and Chua, 2001). However, drying can also have an adverse effect on

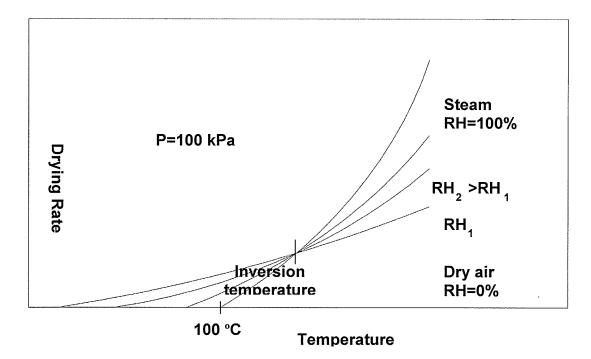
chemical, physical, and nutritional properties of food products (Chou and Chua, 2001) because of the degradation of food components during dehydration process. The quality of many food products degrades during dehydration above room temperature. Increased heat and exposure time of the product at elevated temperatures increase the rate of nutrient quality degradation (Chou and Chua, 2001).

To optimize the current drying technology, a few things can be improved. Firstly, a reduction in energy consumption during drying would improve the economics and reduce the environmental impact. In addition, product quality can be improved by minimizing chemical, physical and nutritional degradation (Chou, and Chua, 2001).

Drying as a method of preservation has been used for thousands of years; however, many of the dryers being used nowadays were developed in the late 19<sup>th</sup> century (Pronyk et al., 2004). In the early 20<sup>th</sup> century, SHS was hypothesized to be used as a drying medium instead of air; nevertheless, this technology was not developed for another 50 years (Pronyk et al., 2004). There was very little research on SHS until the 1970s. The increased interest in the field of SHS drying was probably brought about by the energy crisis of the 1970s as energy efficiencies were achieved with SHS (Pronyk et al., 2004). In the last 25 years many companies have come forward to offer industrial SHS dryers and a fertile worldwide research community has formed.

#### 2.3.2. Principles

The ability of SHS to dry material is due to the addition of sensible heat which raises the temperature above the corresponding saturation temperature at a given pressure (Pronyk et al., 2004). Unlike saturated steam, a drop in temperature will not result in condensation of the steam as long as the temperature is still greater than the saturation temperature at the processing pressure. The moisture evaporated from the product becomes part of the drying medium and does not need to be exhausted unless the pressure increases beyond a set point, at which time the excess steam may be released (Pronyk et al., 2004). This allows for the recycling of the drying medium, provided that additional sensible heat is added. The drying rate for SHS will be greater than for hot air if the temperature is above the inversion temperature. At the inversion temperature, the evaporation rates for pure superheated steam and completely dry air are equal (Figure 4). Subsequently, above the inversion temperature the rate of evaporation will be greater for SHS than dry air.

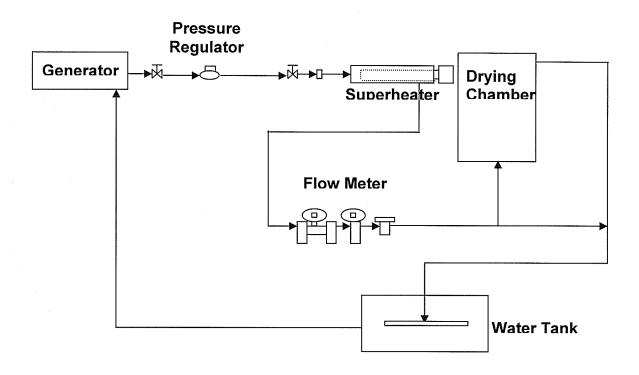


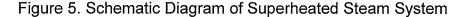
#### **RH: Relative Humidity**

## Figure 4. Drying Rate vs. Temperature

The superheated steam system consists of a steam generator (boiler), steam conveying pipelines, a drying chamber, a superheater, heating tapes, a water supply system, and data acquisition and control systems (Figure 5). The superheated steam system used for experiments at University of Manitoba is located in the Biosystem Engineering Department (Figure 6).

Saturated steam is produced by a generator at 0.58 MPa and 158°C. When the steam goes through the steam pressure regulator, its pressure drops to 0.13MPa and then the superheated steam is generated. The pressure regulator ensures a steady flow of steam goes through the pipelines and the drying chamber. After the steam passes through the electrical superheater, where its temperature is adjusted to the desired level, the flow rate is adjusted by the flow meter, and the steam goes through the drying chamber (Figures 7 and 8). After it goes into the drying chamber from side, the steam will spiral around the sample holder, where there is a stainless steel pipe underneath to heat the sample thoroughly and evenly. Finally, the steam goes to the water tank and is condensed. The whole system is a closed system with no oxygen involved.





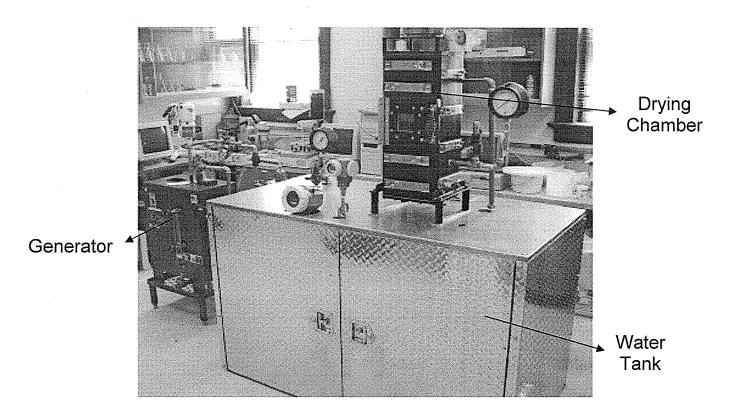


Figure 6. Superheated Steam System

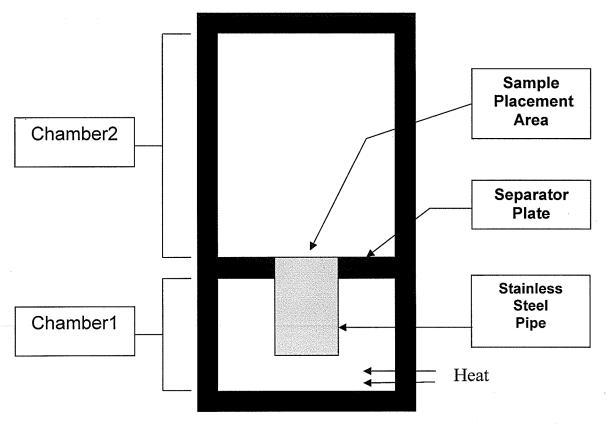


Figure 7. Schematic Diagram of Drying Chamber

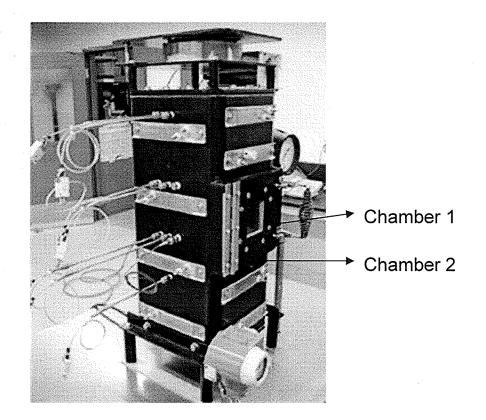


Figure 8. Drying Chamber

# 2.3.3. Applications of SHS

There are hundreds of superheated steam drying systems being used by industry with most suppliers located in Europe (Bruce and Hulkkonen, 1998). Even though it has proven benefits, utilization of SHS is not widely spread due to lack of the knowledge about the process and the effects of SHS on product quality (Pronyk et al., 2004). Therefore, use of SHS as a drying method or even as a processing system needs more research.

About five years ago, a Dutch research institute began to develop SHS for use in the food industry (de Jong, 2006). Experiments in their pilot plant have shown that SHS can be successfully used for many food applications, including

drying, sterilization, and pasteurization and toasting, and recently the technique has proved to be a good alternative to frying and baking. In recent trials, SHS has been used successfully to replace the final frying step for French fries, battered meat or seafood and expanded snacks. For French fries, the SHS treatment reduces calorie levels by over 25% without loss of crispiness and with an improved flavour (de Jong, 2006), compared to other frying alternatives, such as heating in the oven or microwaving. In addition, using SHS reduces the presence of trans fatty acids and the other negative impacts of using fat as a processing medium (de Jong, 2006).

Currently, there are many universities (Table 5) conducting research on this technique. In Canada, Dr. Cenkowski is the leader of SHS research at University of Manitoba. Since 1995, the University of Manitoba in Canada has conducted systematic research on the drying and processing characteristics of a variety of food products while ensuring the quality of the dried products, which is seen as an inseparable issue in processing with SHS (Pronyk et al., 2005).They have applied SHS as a drying system on such products as sugar-beet pulp, potato, Asian noodles, and spent grains. They are also trying to use SHS as a processing technique on pulses which is part of this study.

Table 5. Universities Currently Conducting Research or with Experiments in Superheated Steam Drying and Processing (Pronyk, 2007)

Principal	University	Location
Researchers		
S. Cenkowski	University of Manitoba	Winnipeg, Canada
S. Devahastin	Universtity of Technology Thonburi	Bangkok, Thailand
S. Soponronnait,		
S. Prachayawarakorn		
J. Fitzpatrick	University College Cork	Cork, Ireland
S. Heinrich	University of Magdeburg	Magdeburg, Germany
R.Moreira	Texas A&M University	Texas, USA
A. Mujumdar	National University of Singapore	Singapore
Z. Pakowski	Technical University of Lodz	Lodz, Poland
S. Pang	University of Canterbury	Christchurch, New Zealand
R. Renström, J.Berghel	Karlstad University	Karlstad, Sweden
M.J. Urbicain	PLAPIQUI(UNS-Conicet)	Bahia Blanca, Argentina
R. Wimmerstedt	Lund University	Lund, Sweden

2.3.4. Advantages and Potential of SHS for Further Research

The use of superheated steam has many benefits to the consumer and industry. First of all, SHS can lead to energy savings as high as 50-80% over hot air (Pronyk et al., 2004). These savings can be achieved due to higher heat transfer coefficients and increased drying rates in the constant and falling periods if the steam temperature is above the inversion temperature. The constant rate drying period is also longer in SHS drying thus providing high drying rates for longer periods of time. These higher rates will increase the efficiency of the processing operation potentially leading to a reduction in equipment size and capital costs or an increase in output (Pronyk et al., 2004). Use of SHS as the drying medium instead of hot air means that there is an oxygen free environment during drying and therefore no oxidative or combustive reactions. The oxygen free environment also produces improved product quality, such as reduced scorching.

SHS dehydrators are designed as closed systems where the exhaust may be collected and condensed (Pronyk et al., 2004). In this way toxic or expensive compounds are removed and collected before they reach the environment thus reducing air pollution.

SHS processing allows concurrent blanching, pasteurization, sterilization, and deodorization of food products during drying (Pronyk et al., 2004). Products also become partially cooked with potential beneficial changes in textural properties.

Therefore, based on these advantages of SHS, investigators should further expand the research on utilizing SHS and its effects on product quality. Those food products which are sensitive to high temperature have a high potential to be dried and processed with SHS. This also creates a challenge in equipment design that needs to be solved (Cenkowski et al., 2005).

# 2.4. Micronization

Micronization is an intensive heat treatment that can be used to cook foods by relatively short exposure times to electromagnetic wavelengths in the infrared (IR) region of spectrum (Cenkowski et al., 2004). Key to this processing system is the energy generated by the infrared heaters. The energy penetrates the entire sample, heating it quickly and thoroughly. Fast preparation, increased heating efficiency, lower cost, and better quality products characterize the advantages of micronization.

# 2.4.1. The Electromagnetic Spectrum

The electromagnetic spectrum includes gamma rays, X-rays, ultraviolet, visible, infrared, microwaves and radio waves. The difference between the various types of light or radiation is their wavelength, frequency and energy. Infrared light lies between the visible and microwave sectors of the electromagnetic spectrum (Figure. 9). Wavelength increases, while frequency and energy decrease as one moves from gamma rays to radio waves. From Figure 9, we can see that the IR region of the electromagnetic spectrum contains wavelength from 0.75  $\mu$ m to 1000  $\mu$ m, which can be further divided into three regions: near IR (0.75  $\mu$ m to 1.4  $\mu$ m), mid IR (1.4  $\mu$ m to 3  $\mu$ m) and far IR (3 $\mu$ m to 1000  $\mu$ m).

## 2.4.2. Principles

IR radiation can be produced artificially with electrical and gas-fired generators (Ginzburg, 1969). The electrical generators are easy to use and control, and are designed for small-scale laboratory studies. The gas-fired micronizer is more appropriate for large-scale operations because of the low operational cost. Some fundamental differences in the way of electric and gas-fired generators have been discussed by Cenkowski and Sosulski (1998). Sufficient IR energy to micronize foods in electric generators is obtained at temperatures around 2200°C, with a peak wavelength of 1.2  $\mu$ m, while for gas-fired micronizers, the working temperature is between 400 and 750°C, with wavelengths from 1.8  $\mu$ m to 3.4  $\mu$ m (Cenkowski and Sosulski, 1998). They also indicated that, while shorter wavelengths supply more energy, the higher degree of penetration for the longer wavelength radiation makes it more suitable for materials such as large-seeded legumes.

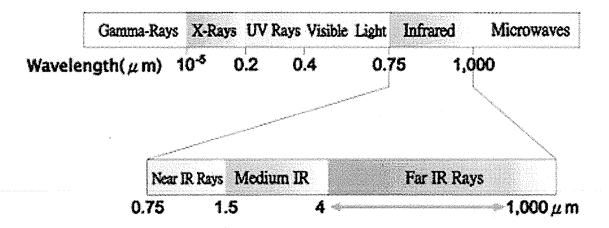


Figure 9. Infrared Region in Electromagnetic Spectrum

The electrical generators of infrared radiation are based on a spiral tungsten filament in a quartz envelope (Cenkowski and Sosulski, 1998). The infrared radiation is generated by the tungsten wire filament as a result of electrical resistance and the quartz allows good transmission of radiation from the ultraviolet to microwave range (Cenkowski and Sosulski, 1998).

The processing temperatures developed in products exposed to IR are influenced by the intensity of the IR rays, which can also be controlled by controlling the temperature of the IR source and the distance between the sample and the rays (Blenford, 1980; Driscoll, 1992). The temperature reached in the sample is also influenced by the time of exposure to the IR source.

During the micronization process, infrared rays strike an absorbent material and cause the molecules within a micromized product to vibrate at a frequency of 80-170 million megacycles per second (Ginzburg, 1969; Lawrence, 1973; Bellido, 2002). As a result, there is rapid internal frictional heating and a rise in water pressure vapour (Lawrence, 1973). The internal temperature of products such as grains and pulses can reach 90 °C to 124 °C after 50 s to 60 s of processing (Bellido, 2002). Kouzeh-Kanani et al. (1981) also reported that the temperature of legumes can reach over 100 °C in 60 seconds by micronization.

# 2.4.3. Applications in Food Industry

Since micronization is an efficient, heat process that provides advantages not available with other heat treatments, its popularity in the food industry is increasing. IR was initially used in the early 1970's by the cocoa bean industry to facilitate bean shell separation and reduce yeast, mold, insect, and microbial counts (Anonymous, 1981). Currently, the micronization process is being used in food and feed applications for improving the nutritional quality.

Micronization has been used for improving the value of animal feed, such as pulses (Arntfield, et al., 2004; Chubb, 1982; Igbasan and Guenter, 1996; Metussin et al., 1992), sorghum (Shiau and Yang, 1982), barley (Lawrence, 1973), maize (Lawrence, 1973) and maize germ (Kouzeh-Kanani et al., 1984). Micronization also has been applied to pulses including soybeans (Kouzeh-Kanani et al., 1981; McCurdy, 1992) to improve the nutritional value, lentils to improve the cookability (Zhao, 2000; Scanlon, et al., 1998), field peas to improve their quality by decreasing the bitter flavour (McCurdy, 1992; Igbasan and Guenter, 1996; Toews, 2001), and canola and canola screenings to improve the dehulling characteristics (McCurdy, 1992). In addition, micronization has been used to precook rice, barley (Blendford, 1980), and lentils (Scanlon, et al., 1998), and also to improve nutritional quality of soymilk (Metussin et al., 1992).

# 2.4.4. The Effects of Micronization on Food Products

Since heat during the micronization process is transferred directly to a processed product by radiation, it is simple and efficient. Micronization involves simultaneous heat and mass transfer, and complex chemical changes take place during processing (Cenkowski, et al., 2003). Processing efficiency depends on

the intensity of the radiant energy from the source of radiation. It has been proven that micronization can be used for animal feed, including cereals, legumes, and oil seeds. This process increased starch gelatinisation, resulting in greater starch availability and digestibility, and contributed to the removal of enzymes (e.g. Lipase in oil seed) and other inhibitors (e.g. trypsin and goitrogenic factors). While affecting these changes, available lysine is not reduced (Hildon, 1980). Lawrence (1973) found that micronization increased starch availability of barley and maize, and when flaking followed micronization, starch availability was further improved. In a later study, he demonstrated that micronization can improve the nutritional value of wheat for the growing pig (Lawrence, 1975).

# 2.4.4.1. Effects of Micronization on Legumes

A heat treatment is necessary before legumes can be used in the human diet because protein availability is improved by inactivating antiphysiological factors, particularly trypsin inhibitor and haemagglutinin, and by unfolding the storage proteins, making them more susceptible to attack by digestive enzymes (Sathe, et al., 1984). Micronization, as a heat treatment, likely performs this function. In addition, it can improve the cookability of legumes, by gelatinizing starch, thereby increasing the availability and digestibility (Arntfield et al., 2004). Use of micronization in legume processing is still in the early stages of development, but studies have indicated that it has high potential (Abdul-Kadir et al., 1990).

# 2.4.4.1.1. Effects on Cooking Quality of Legumes

Micronization has been proven to reduce the level of antinutritional factors in oil seeds, cereals and pulses, including myrosinase in canola seeds and canola screenings (McCurdy, 1992), as well as lipoxygenase and trypsin inhibitors in pulses (Kouzeh-Kanani et al., 1981; Kadam, et al., 1987). Infrared heating in the processing of legume seeds also has been reported to increase the starch gelatinization and decrease the protein solubility of the products (Blenford, 1980; Zheng, et al., 1998). Arntfield et al. (1997) reported that higher moisture content in the seed during micronization resulted in a higher degree of starch gelatinization. The gelatinized starch softens the texture of the seeds, and reduces cooking time. Scanlon et al. (1998) found that micronization can reduce the cooking time of lentils from 30 to 15 min if the seeds were tempered to 25% moisture content before micronization. The decrease in protein solubility by micronization is due to the heat-induced denaturation of proteins. Protein denaturation increases the digestibility of legume proteins and also helps to produce a soft texture in the seeds.

Adbul-Kadir et al. (1990) reported that increased hardness in the seeds following micronization resulted if the initial moisture content was low. Other studies also indicated that infrared heating might cause cracking, toughness or higher leaching losses of the products if the seeds were heated to a high temperature (above 140°C) with low initial moisture content (Fasina et al., 1997).

Therefore, the moisture content of the seeds before micronization is critical to the quality of the end products. Tempering, the addition of moisture, is therefore an important step before micronization as it influences the distribution of the water inside the seeds (Arntfield et al., 1997).

## 2.4.4.1.2. Effects on Other Properties of Legumes

Micronization also affects other properties of legumes. The first application of infrared heating to legumes was to dry the seeds for storage (Zhao, 2000). Infrared drying was found to be a viable method for the destruction of microorganism, insects and fungal infections on legume seeds (Deshpande and Deshpande, 1991; Sarantinos and Blank, 1996). The gelatinization of starch and the denaturation of proteins can improve the nutrient availability of legumes. Kadam et al. (1987) reported significant improvements in biological values and net protein utilization values for diets containing winged beans treated with infrared heat compared to untreated or oven heated samples. The effects of several processing methods were compared in their experiments, including dryair heating with a hot air oven, infrared radiation with a gas burning infrared heater, and cooking in boiling water. The results indicated that the digestibility of proteins in infrared treated bean meals was greatly increased. Both the infrared treatment and cooking almost completely inactivated the trypsin inhibitors and the lectins. The highest reduction of tannins was obtained by infrared heating. Infrared heating may also affect the removal of oligosaccharides in legumes

(Sarantinos and Blank, 1996). Infrared heat treatment can also decrease the offflavours of legumes (McCurdy, 1992).

Therefore, micronization can improve legume quality. It can reduce spoilage problems and allow prolonged storage.

2.4.4.2. Potential of Micronization for Further Studies

Even though micronization has been shown to produce many beneficial changes, excessive heating also has been reported to result in some negative effects. For example, over heating may cause cracking, toughness or higher leaching losses of the products (Fasina et al., 1997). Excessive heat treatments cause loss of heat-labile nutrients such as lysine, cysine, methionine and thiamine (Kouzeh-Kanani et al., 1981). Overheated micronized soybean flour also developed rancid odours and off flavours (Kouzeh-Kanani et al., 1982).

Therefore, the micronization processing conditions should be carefully regulated to avoid undesirable quality changes. Micronization is still in its early stage of development, but sufficient information is available to indicate the potential that exists and that may be developed in future.

#### CHAPTER 3

# MATERIALS AND METHODS

## 3.1. Materials

## 3.1.1. Pea Samples

Pea samples were provided by Tom Warkentin of the Crop Development Center in Saskatoon and Bruce Brolley of Manitoba Agriculture, Food and Rural Initiatives (MAFRI). There were six varieties of marrowfat peas from three locations, twelve varieties of green peas from four locations, and fourteen varieties of yellow peas from four locations for the 2005 crop (Table 6). Based on the hydration results of these samples, processing was done using four varieties for each type of pea (Table 7). They were pooled for all Saskatchewan locations, still from the 2005 crop. In the attempt to repeat the experiments from the 2005 crop within the limits of what was available, three varieties of marrowfat and green peas, and four varieties of yellow peas from the 2006 crop from central Saskatchewan were examined (Table 8). All samples were stored at room temperature in plastic containers.

#### 3.1.2. Chemical and Reagents

The chemicals and reagents used in this study are listed in Table 9. 3.1.3. Instruments and Supplies

The instruments and supplies used in this study are listed in Table 10.

Type of pea	Locations	Sample weight [g]	Varieties
Marrowfat peas	Rosthern	50	WM-10, Rambo,
	Outlook	50	Midichi, 82-10, 35-1-
	Sutherland	50	5, 98-4
Green peas	Davidson	50	Nitouche, CDC
	Rosthern	50	Montero, Stratus,
	Indian Head	50	CDC Striker, Vortex,
			CDC Sage, Cooper,
	Western	2000	Camry, Nessie, CDC
	Manitoba	2000	Dundurn, SWA6154,
			Bluebird
Yellow peas	Davidson	50	Alfetta, CDC Mozart,
	Rosthern	. 50	Cutlass, Carneval,
	Indian Head	50	Eclipse, CDC
	Western	2000	Golden, CDC
	Manitoba		Bronco, CDC 653-8,
			SW Midas, SW
			Carousel, Tudor,
			CDC 728-8, CDC
			985-36, SW Marquee

Table 6. Pea Samples Grown in 2005 from Eastern and Central Saskatchewan and Western Manitoba

Varieties	Sample weight [kg]
Rambo	4
98-4	1.8
82-10	4.2
Midichi	4.2
Camry	5.39
Stratus	6.6
Cooper	6.54
Nitouche	6.03
SW Midas	5.925
Eclipse	5.495
Alfetta	5.62
CDC Mozart	7.4
	Rambo 98-4 82-10 Midichi Camry Stratus Cooper Nitouche SW Midas Eclipse Alfetta

Table 7. Pea Samples Grown in 2005 from Central Saskatchewan

Table 8. Pea Samples Grown in 2006 from Central Saskatchewan

Type of pea	Varieties	Sample weight [kg]
Marrowfat peas	Rambo	2.120
	98-4	2.120
	82-10	2.120
Green peas	Camry	4.200
	CDC Striker	4.205
	Cooper	4.200
Yellow peas	SW Midas	4.205
	Eclipse	4.200
	Alfetta	4.205
	CDC Mozart	4.205

Chemicals	Manufactures
ABTS	Sigma Chemical Co., St. Louis, MO
Acetic acid	Fisher Scientific Inc., Nepean, ON
Acetone	Fisher Scientific Inc., Nepean, ON
AG1-X8 Chloride anion exchange resin	Bio-Rad Laboratories, Richmond, CA
Ascorbic acid	Canada Colors and Chemicals Ltd.
DPPH	Sigma Chemical Co., St. Louis, MO
Diethyl ether	Sigma Chemical Co., St. Louis, MO
EDTA	Sigma Chemical Co., St. Louis, MO
Ethyl acetate	Sigma Chemical Co., St. Louis, MO
Ferric chloride	Fisher Scientific Inc., Nepean, ON
Feulic acid	Sigma Chemical Co., St. Louis, MO
Folin Ciocalteau reagent	Sigma Chemical Co., St. Louis, MO
Formic acid	Sigma Chemical Co., St. Louis, MO
Hydrochloride acid	Fisher Scientific Inc., Nepean, ON
Methanol	Fisher Scientific Inc., Nepean, ON
Phytic acid	Sigma Chemical Co., St. Louis, MO
Sodium carbonate	Sigma Chemical Co., St. Louis, MO
Sodium chloride	Fisher Scientific Inc., Nepean, ON
Sodium hydroxide	Fisher Scientific Inc., Nepean, ON
Sulphosalicylic acid	Sigma Chemical Co., St. Louis, MO
Trolox	Sigma Chemical Co., St. Louis, MO

Table 9. Chemicals and Reagents Used for Evaluation and Processing of Peas

Instruments and Supplies	Manufactures		
Accumet® pH meter 925	Fisher Scientific Inc., Nepean, ON		
Air convention oven	Blue M. Electric Company, IL, USA		
Aminex® HPX-87N column	Bio-Rad Laboratories, Hercules, CA.		
1-10,1-100,100-1000 pipetters	Fisher Scientific Inc., Nepean, ON		
0.45 μm syringe filter	Fisher Scientific Inc., Nepean, ON		
Centrifuge (RC5C)	Mandel Scientific Company Inc. GP. ON		
Digital water bath (SW22)	Hulabo Labortechnik GMBH, Seelbatch, BW,		
	Germany		
Durapore membrane Filters (0.45µm HV)	Millipore Ltd. Ireland		
HPLC	Waters Corporation, Milford, MA, USA		
Infrared thermometer	Cole-Parmer Instruments Co. Vernon Hills, IL,		
	USA		
Glass wool	Supelco, Bellefonte, PA		
Glass jar	Fisher Scientific Inc., Nepean, ON		
Grinder	Applica Consumer Products, Inc. Miramar,		
	FL,USA		
Micronizer	Research Inc. Mpls, MN, USA		
Nitrogen compressed	Praxair Canada Inc. Mississauga,ON		
PC-351 Hotplate stirrer	Corning Inc. Corning, New York		
Rotary vacuum	Fisher Scientific Inc., Nepean, ON		
Sonicator (Branson 3200)	Branson Cleaning Equipment Company,		
	Shelton, CT, USA		
Superheated steam system	Designed by Biosystems Engineering		
	Department, U of Manitoba, MB		
Table centrifuge	GLC-1, Sorval, Newton, CT, USA		
Ultraspec 2000 UV/visible spectrophotometer	Biochrom Ltd, Cambridge, England		
Wrist-action shaker	Appropriate Technical Resources, Laurel, MD		
Zwick Roell texture analyzer	Zwick Ltd. Kennesaw, GA, USA		

Table 10. Instruments and Supplies Used for Evaluation and Processing of Peas

## 3.2. Methods

# 3.2.1. Experimental Design

This study was divided into three stages. In the first stage, hydration properties including water absorption capacity and rate were determined for all varieties of pea samples and locations from 2005 as listed in Table 6. In the second stage, phytic acid, phenolic content as well as the hydration and antioxidant properties of both raw samples and processed samples were evaluated using the composite 2005 and 2006 crop samples in Table 7 and 8. In the third stage, selected varieties of peas based on the hydration, antinutritional factor and antioxidant data were processed using superheated stream and micronization. The effectiveness of the processing was based on a texture evaluation, with a commercially deep fried pea snack used as the target texture.

## 3.2.2. Hydration Determination

Hydration properties of pea samples evaluated included water absorption capacity, hydration rate, stone seed percentage, and moisture content. The moisture content was determined for both raw and processed samples.

# 3.2.2.1. Water Absorption

Water absorption or hydration capacity (HC) of pulses is defined as the amount of water that whole seeds absorb after soaking in excess water for 16 h at room temperature ( $22 \pm 2 \, ^{\circ}$ C), and is expressed as the amount of water absorbed per 100 seeds. The procedure (Appendix 1) followed the method for

determining hydration capacity and percentage of unhydrated seeds of pulses according to pulse lab at Grain Research Laboratory (GRL), Winnipeg, MB.

3.2.2.2. Stone Seed

Seeds that remain entirely or partially unswollen after 16 h soaking at room temperature ( $22 \pm 2$  °C) are defined as stone seeds or unhydrated seeds. The number of unhydrated seeds (US) is expressed as a percentage of the total number of seeds:

Stone seeds:  $\% = N_2 * 100 / N_1$ 

 $N_1$  = original number of seeds = 100

 $N_2$  = number of unhydrated seeds

The stone seeds produce a distinctive "ping" sound when dropped onto a stainless steel tray, following hydration, as descried in the method for stone seeds.

#### 3.2.2.3. Hydration Rate

The hydration rate was monitored by determining the change in mass of the seeds every 30 minutes while soaking in excess water for a total of 16 h at room temperature ( $22 \pm 2$  °C). Details of the method are provided in Appendix 2.

### 3.2.2.4. Moisture Content

The moisture determination followed the oven drying procedure (Appendix 3). The moisture content of raw whole pea sample was measured when they arrived. The final moisture content of processed peas was determined as soon as the samples cooled to room temperature after processing.

#### 3.2.3. Tempering

Tempering of peas involved the addition of a certain amount of tempering solvent (eg. distilled water) to certain amount of peas for specified times in order to reach a desired moisture content (tempering level). The amount of solvent required was calculated as described below (Arntfield et al., 1997). Mass of distilled water added

= Pea weight \*  $[\%H_2O$  (target) -  $\%H_2O$  (original)] /  $[100 - \%H_2O$  (target)]

where  $\%H_2O$  (target) is the tempering level required in the peas, and the  $\%H_2O$  (target) is the original moisture content of peas.

Before processing with SHS and Micronization, raw peas were tempered to 16% and 33% at either room temperature or 35 °C in a covered glass jar. The seeds and water mixture was shaken every 30 min in the first two hours, then left for 16 h at room temperature.

#### 3.2.4. Superheated Steam System (SHS)

The SHS system from Dr. Cenkowski's lab in the Biosystem Engineering Department at University of Manitoba was used to process pea samples. Both

untempered and tempered peas were processed. Different conditions were used to produce a range of pea samples. Temperatures of 135 °C and 140 °C for 15, 17, 18 and 20 min were used. For each trial, 15 g of samples were tested. After the SHS treatment, samples were left at room temperature to cool down. About 2 g of samples were used for the moisture content determination, and 8 seeds were selected randomly for texture analysis. Remaining samples were placed in sealed bags stored in the freezer for further analysis.

#### 3.2.5. Micronization

In this study, a small electrical infrared lamp was used to micronize the untreated peas and tempered peas. Fifteen grams of sample were placed in one layer on a hand made aluminium tray (12x5x2 cm) under the lamp. The distance between the lamp and tray was maintained at 85 mm. The peas were heated for 2 min 30 s, 2 min 45 s, 3 min and 3 min 15 s with continuous shaking of the tray by hand using tongs.

The sample temperature was determined using an infrared thermometer during micronization to avoid burning. The final temperature of the micronized peas was  $120 \pm 5$  °C. After micronization, samples were left exposed to cool down to room temperature. About 2 g of samples were used for the moisture content determination, and remaining samples were placed in sealed bags stored in the freezer for further analysis.

# 3.2.6. Texture Evaluation

The texture evaluation was performed with a Zwick Roell texture machine (Zwick Ltd, Kennesaw, GA, USA). The processed peas were cooked using SHS and Micronization separately prior to texture measurement. Peas were removed from the freezer at least 2 hours before texture testing to let them return to room temperature. Eight seeds from each batch were measured. The "TestXpert II" (Zwick Ltd, Kennesaw, GA, USA) software was used to program the test method and record the results of the texture testing. The tool separation at start position is the working distance and it was set to 7.8 mm (approximately 2 mm greater than diameter of the largest pea). The Warner-Bratzler Blade was used test the texture. The maximum force of both processed peas and commercial peas was recorded as a comparative parameter. Force deformation curves were obtained for all samples.

# 3.2.7. Phytic Acid Determination

The content of phytic acid in both ground raw peas and micronized peas was determined following the modified chromatographic procedure of Latta and Eskin (1980) (Appendix 4).

#### 3.2.8. Determination of Phenolic Acids

The content of individual phenolic acid, in ground raw peas was determined following an HPLC method using samples prepared using an organic

solvent extraction, base hydrolysis with ascorbic acid and EDTA, and sequential acid hydrolysis (Luthria and Pastor-Corrales, 2006). (Appendix 5).

#### 3.2.9. Total Phenolics Determination

The content of total phenolics in both ground raw peas and micronized peas was determined by using Folin-Ciocalteau colorimetric method (Singleton and Rossi, 1965) as modified by Gao et al. (2002) (Appendix 6).

# 3.2.10. Antioxidant Properties Determination

Antioxidant levels of ground raw peas and micronized peas were assessed by using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH•) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS•) methods.

# 3.2.10.1. DPPH• Method

The procedure followed used the DPPH radical cation decolourization assay: A woking solution of 0.0025 g/100 mL of DPPH (Formula weight = 394.32 g/mol) was used. A ground sample weight of 0.3 g of sample was added to 3 mL of methanol and stirred for 2 hours. The mixture was then centrifuged at 1610 xg for 10 min and 0.1 mL of supernatant was added to 3.9 mL of the DPPH working solution. The absorbance of the mixture was recorded at a wavelength of 515 nm at time 0 (just before adding sample) and again after 30 minutes. The % decolouration was calculated as follows.

% Discolouration = [1 – (abs t=30) / (abs t=0)] \* 100 abs: absorbance;

t: time

The antioxidant activity was calculated as trolox equivalents as follows:  $\mu$ mole of trolox equivalent /g = (x  $\mu$ mole/100  $\mu$ L \* 4 mL \* 30)/ sample mass where x  $\mu$ mole is obtained from the equation of standard % discolouration curve.

3.2.10.2. ABTS• Method

A modified procedure was followed by applying the ABTS radical cation decolourization assay: Antioxidants were extracted by weighing 0.2 g of the material and adding 10 mL of methanol. The sample was mixed for 2 h at room temperature and centrifuged for 10 min at 13000xg. The supernatant was decanted. A fresh (daily) trolox stock solution (10 mM) was prepared by dissolving 0.0625 g Trolox in 25 mL of 50% methanol. An ABTS solution (7 mM) was made up in distilled water. To 5 mL of this solution, 88  $\mu$ L of 140 mM K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> (made the day before and, kept in darkness for 12-16 h) was added. The ABTS solution was then added drop by drop to a 50% methanol solution to achieve an absorbance of 0.7 at 734 nm. This was the diluted ABTS-solution used for further analysis. A trolox calibration curve was prepared as shown in Table 11.

	Conc	Concentration (µmoles / 100 µL)						
	0	0.0125	0.025	0.0375	0.05	0.1		
Stock solution (mL)	0	0.25	0.5	0.75	1	1.25		
50% Methanol (mL)	10	9.75	9.5	9.25	9	8.75		

Table 11. Amounts of Stock Trolox Solution Added into the 50% Methanol Solution for Preparation of Standard Solutions

In test tubes wrapped in aluminum foil, 100  $\mu$ L of diluted Trolox stock solutions or 1 mL of sample extract was added. To this 9000  $\mu$ L of 50% methanol and 2.9 mL of ABTS solution were added and the test tubes were incubated at 30 °C for 6 min. The absorbance was measured at 734 nm. Results were reported in trolox equivalents per mL of extract. They were converted to  $\mu$ mole trolox equivalents / 100 g sample.

## CHAPTER 4

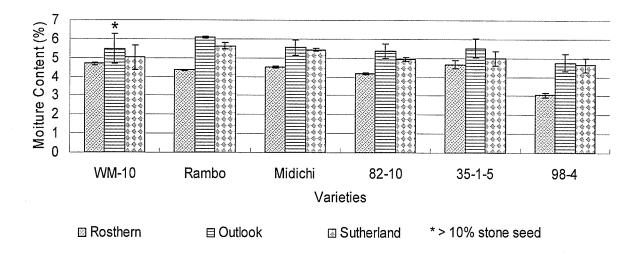
# RESULTS AND DISCUSSION

## 4.1. Determination of Hydration Properties of Raw Samples

The first stage of this project involved an assessment of the ability of the different varieties of peas to absorb water. Since only those varieties for which water can consistently be absorbed to an acceptable level can be used in the processing of whole seeds, the determination of hydration is important. Samples for further processing have been based on the hydration data. Hydration properties including hydration capacity and hydration rate were tested for all samples upon arrival. Initial moisture content and percent of stone seeds of all raw samples were also determined.

# 4.1.1. Initial Moisture Content of Samples

Three batches of peas were used in this study (Table 6, 7, and 8). The initial moisture content of all samples was measured as soon as they arrived. Figures 10, 11, and 12 show the initial moisture content of marrowfat, yellow and green peas based on the samples grown in Saskatchewan and Manitoba in 2005. The corresponding percent of stone seeds are shown in Tables 12, 13, and 14.



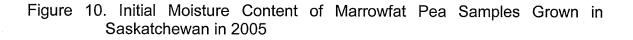


Table 12. Percent of Stone Seeds of Marrowfat Peas of Samples Grown in Saskatchewan in 2005 (%)

Varieties	Locations	Locations					
	Rosthern	Outlook	Sutherland				
WM-10	$1.5 \pm 0.7$	$14.0\pm8.5$	$9.0\pm2.8$				
Rambo	0	0	0				
Midichi	$0.5\pm0.7$	7.0 ± 1.4	$3.0\pm2.8$				
82-10	0	$6.0 \pm 2.8$	$2.0 \pm 1.4$				
35-1-5	$4.0 \pm 1.4$	$8.5\pm3.5$	$6.5\pm3.5$				
98-4	$2.5\pm0.7$	$7.5\pm0.7$	$5.5\pm3.5$				

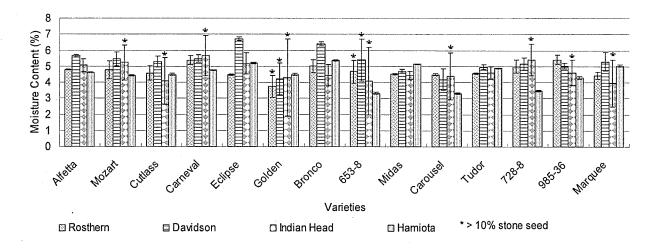


Figure 11. Initial Moisture Content of Yellow Pea Samples Grown in Saskatchewan and Manitoba in 2005

Table	13.	Percent	of	Stone	Seeds	of	Yellow	Pea	Samples	Grown	in
	Sa	askatchew	an	and Ma	nitoba in	200	)5 (%)				

Varieties	Locations		<u>ANG ANG ANG ANG ANG ANG ANG ANG ANG ANG </u>	
	Rosthern (SK)	Davidson (SK)	Indian Head	Hamiota
			(SK)	(MB)
Alfetta	$0.3\pm0.3$	$1.0\pm0.7$	$\textbf{6.0} \pm \textbf{1.4}$	18.6 ± 1.0
CDC Mozart	9.0 ± 1.4	$7.3\pm1.0$	$19.3\pm1.0$	$26.5\pm2.7$
Cutlass	7.7·± 1.6	$6\pm2.8$	$24.3 \pm 2.4$	$18.7\pm1.6$
Carneval	4.7 ± 1.7	3 ± 1.4	$20.3\pm6.7$	$15.4\pm0.9$
Eclipse	$0.7\pm0.5$	$1.3\pm1.0$	$9.3\pm3.8$	$13.2 \pm 1.0$
CDC Golden	10.7 ± 1.8	$18\pm2.8$	$35\pm7.1$	$40.1\pm3.7$
CDC Bronco	$7.0 \pm 2.1$	1.3 ± 1.0	$9.3\pm5.2$	$8.9\pm0.6$
CDC 653-8	$12.0\pm4.2$	$22.7\pm3.3$	$31.0 \pm 5.7$	$28.9 \pm 1.5$
SW Midas	$0.6 \pm 0.6$	$2\pm0.71$	$4.5\pm2.1$	5.9 ± 1.0
SW Carousel	$1.7 \pm 0.3$	$9.3\pm0.47$	$26 \pm 5.7$	$31.2 \pm 3.2$
Tudor	$0.3\pm0.3$	$2\pm1.4$	$6.0\pm2.8$	$\textbf{6.4} \pm \textbf{1.0}$
CDC 728-8	$\textbf{6.3} \pm \textbf{0.4}$	5.7 ± 1.2	$17.0\pm4.2$	$20.5\pm1.6$
CDC 985-36	$5.7\pm0.4$	2.7 ± 0.4	11.3 ± 5.2	8.7 ± 1.0
SW Marquee	$3.0\pm0.7$	$8.3\pm2.4$	$25.0\pm4.2$	19.8 ± 2.1

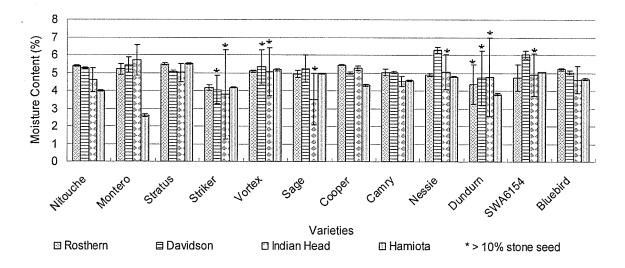


Figure 12. Initial Moisture Content of Green Pea Samples Grown in Saskatchewan and Manitoba in 2005

Table 14. Percent of Stone Seeds of Green Pea Samples Grown in Saskatchewan and Manitoba in 2005(%)

Varieties	Locations		•	
	Rosthern (SK)	Davidson (SK)	Indian Head	Hamiota
			(SK)	(MB)
Nitouche	0	$0.7\pm0.4$	7.7 ± 1.8	$15.2\pm1.9$
CDC Montero	5.3 ± 1.0	6.7 ± 1.2	$9.3\pm1.0$	$10.6\pm1.0$
Stratus	0	0.7 ± 0.4	$6.3\pm2.4$	$5.8\pm0.3$
CDC Striker	3.7 ± 1.8	$14.0 \pm 2.8$	$40.0\pm4.2$	$\textbf{42.2} \pm \textbf{5.7}$
Vortex	1 ± 1.4	$10.3 \pm 2.4$	18.7 ± 1.9	$19.8\pm2.3$
CDC Sage	$\textbf{4.3} \pm \textbf{0.4}$	8.0 ± 1.4	21.7 ± 1.8	$18.7\pm2.2$
Cooper	0	1.0 ± 1.4	$2.3\pm1.7$	$6.8 \pm 1.0$
Camry	$0.3\pm0.3$	1.0 ± 1.4	$5.0 \pm 1.4$	9.4 ± 1.0
Nessie	1 ± 1.4	3.0 ± 1.4	14.0 ± 1.4	$16.7 \pm 2.5$
CDC Dundurn	$23.7\pm3.3$	$29.3 \pm 2.4$	$\textbf{36.3} \pm \textbf{5.2}$	41.6 ± 4.9
SWA 6154	$7.3\pm2.4$	$\textbf{4.3} \pm \textbf{2.4}$	14.7 ± 4.7	$30.4 \pm 3.7$
Bluebird	1 ± 1.4	1.7 ± 1.8	$8.3\pm2.4$	7.9 ± 1.0

Results showed that the initial moisture content of peas varied with location, variety, and the percent of stone seeds. Greater standard deviations for initial moistures values were seen for samples with a higher level of stone seeds (Figs. 10,11,12). For marrowfat peas (Fig. 10, Table 12), peas grown in Outlook (SK) had highest initial moisture content for all the varieties; however, they also had the highest level of stone seeds. All varieties of peas grown in Sutherland (SK) had higher moisture content but lower percent stone seeds than those grown at Rosthern (SK). The one exception was the variety Rambo, which showed 0% stone seeds in all three locations with relatively high moisture content. WM-10 grown in Outlook showed the highest stone seed level (14%); levels of 9% were found in Sutherland, and 1.5% in Rosthern. Therefore, these two varieties (Rambo and WM-10) were selected for marrowfat peas to do the hydration rate determination in order to investigate these extremes.

For yellow peas (Fig. 11, Table 14), Rosthern and Davidson (SK) produced higher initial moisture content with much lower stone seed level than Indian Head (SK). Peas grown in Hamiota (MB) had very high level of stone seeds and lower moisture content in most varieties. Alfetta showed low percent stone seeds in the first three locations and CDC 653-10 showed more than 10% of stone seeds in these three locations. As a result, Alfetta and CDC 653-10 were chosen to do the hydration rate determination to compare the differences.

A similar trend was shown for green peas (Fig. 12, Table 15), where peas grown in Indian Head had a much high level of stone seeds although their moisture content were still comparable to peas grown in other locations. Hamiota

produced higher percent of stone seeds and not very high moisture content. Striker and CDC Dundurn were selected for the hydration rate determination because they had obvious differences in both moisture content and stone seed level.

Adbul-Kadir et al. (1990) reported that the increased hardness of seeds was due to low initial moisture content during growing. Other studies also reported that an infrared treatment might cause cracking, toughness or higher leaching losses of the products if the seeds were heated to a high temperature (above 140°C) with low initial moisture content (Fasina et al., 1997). In addition, initial moisture has to be considered for calculating the hydration capacity on dry basis. Therefore, determination of moisture content is crucial before processing. Higher initial moisture content of peas is usually desired for heat processing because there is less hardness and cracking products, while keeping in mind that the aim is to produce a dry product and moisture must be low enough to prevent mold growth.

# 4.1.2. Hydration Capacities of Samples Grown in Saskatchewan and Manitoba in 2005

Figures 13, 14, and 15 show the hydration capacities on a dry basis for marrowfat, yellow and green peas, respectively. Average values were obtained from duplicate analysis. Results showed that, in general, water absorption ability was highly related to the initial moisture content and percent of stone seeds. For marrowfat peas, the variety Rambo, which had high initial moisture and no stone seeds, had the highest hydration capacity values. Conversely, low hydration capacities were seen for samples such as WM-10, with a high level of stone seeds and low initial moisture content. As was the situation with the initial moisture content, greater standard deviations in hydration capacity were seen from samples with a higher level of stone seeds.

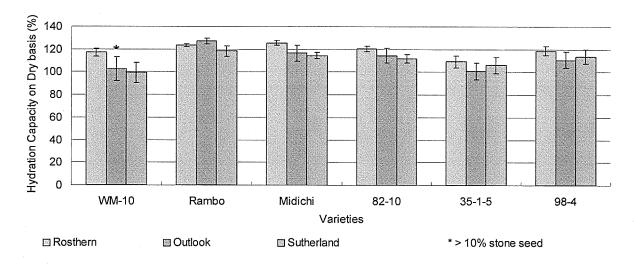
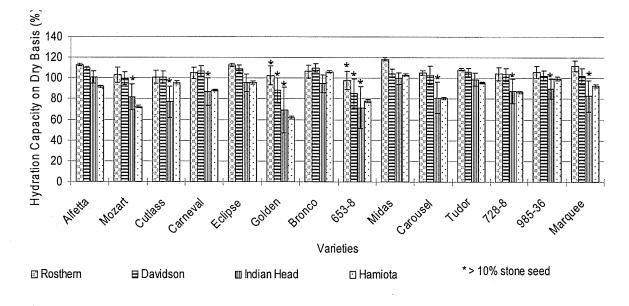
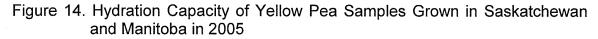
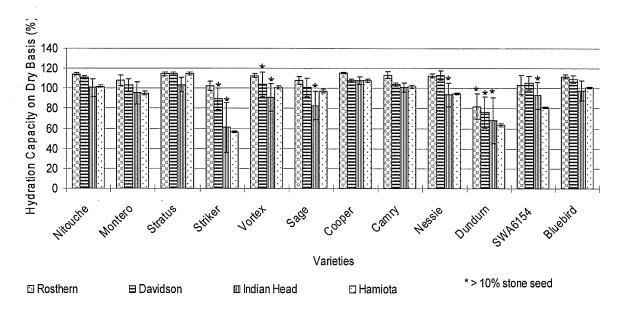
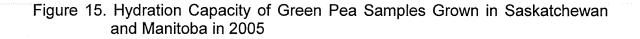


Figure 13. Hydration Capacity of Marrowfat Pea Samples Grown in Saskatchewan in 2005









Overall, hydration capacities ranged from 57% for CDC Striker (green) grown at Hamiota, MB to 127% for Rambo (marrowfat) grown at Outlook, SK. They were affected by both variety and location. Marrowfat peas tended to have higher hydration capacities than green or yellow peas. Peas grown in Eastern Saskatchewan (Indian Head) and Western Manitoba (Hamiota) tended to have lower hydration capacities than those grown in Central Saskatchewan (Rosthern, Davidson).

4.1.3. Hydration Curves of Samples Grown in Saskatchewan and Manitoba in 2005

Selected varieties of different types of peas were chosen to determine hydration rate according to the water absorption abilities and moisture content of samples. Varieties having the highest and lowest average levels of water absorption in each type of pea were selected. Sample mass was weighed every 30 minutes while soaking in excess water for 6.5 hours, and then left overnight; final weight was obtained after a total of 16 hours. Figures 16, 17, and 18 show the water uptake rates of selected varieties of marrowfat, yellow and green peas. Curves represent an average of duplicate analysis.

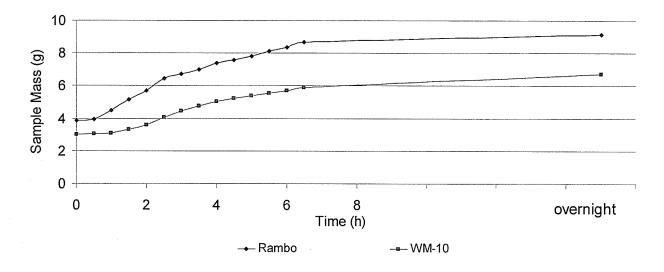


Figure 16. Hydration Curves of Selected Marrowfat Pea Samples Grown in Saskatchewan in 2005

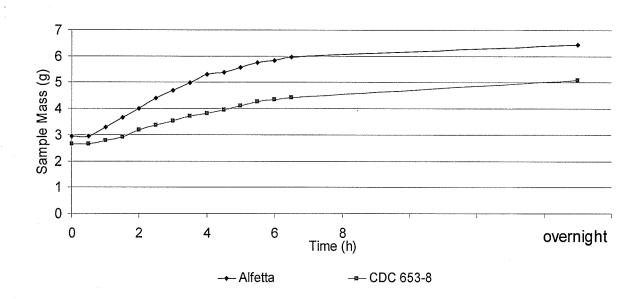


Figure 17. Hydration Curves of Selected Yellow Pea Samples Grown in Saskatchewan and Manitoba in 2005

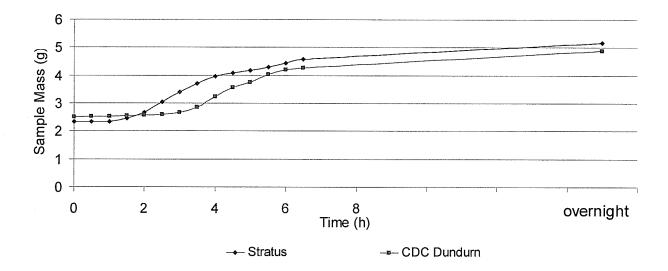


Figure 18. Hydration Curves of Selected Green Pea Samples Grown in Saskatchewan and Manitoba in 2005

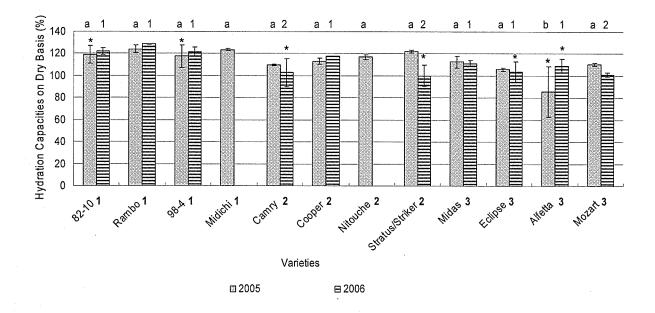
While the shape of hydration rate curves were similar for all peas tested; for marrowfat and yellow peas, difference in hydration capacity for the two extreme samples examined were seen throughout the hydration period. In addition, there was a delay of 30 min or more in the onset of water uptake for peas with lower hydration capacities. For example, CDC Dundurn (green pea) started absorbing water only after 2 hours. The greater level of water intake for the marrowfat peas that was seen in the hydration capacity data was also evident in the hydration curves, where the weight gain was in 3-5 g range compared to 2-3.5 g for yellow peas and 2-3 g for green peas.

Based on the results above, processing was done using four varieties for each type of pea that exhibited the best hydration properties (Table 7). For processing, samples were pooled for all Saskatchewan locations, still using the 2005 crop. In the attempt to repeat the experiments from the 2005 crop within the

limits of what was available, three varieties of marrowfat and green peas, four varieties of yellow peas from the 2006 crop from same locations in central Saskatchewan were examined (Table 8). Hydration properties for all these composite samples in Table 7 and 8 were also determined before processing.

## 4.1.4. Hydration Capacities of 2005 and 2006 Crop Samples

Figure 19 shows the hydration capacities of samples from 2005 and 2006 crop peas. Results shown are the mean values of duplicates. Hydration capacity ranged from 85.7% for Alfetta (6% of stone seeds) to 124.1% for Rambo in 2005, and from 100.1% for Striker (12.5% of stone seeds) to 128.7% for Rambo in 2006. Rambo had the highest water absorption ability in both years. As noted earlier, low hydration capacities are associated with high level of stone seeds and other factors; marrowfat peas tended to have higher hydration capacities than green and yellow peas in both years.



 $^{1}$  \* >5% stone seeds

<sup>2</sup> different letters indicate significant difference in 2005 samples (P<0.05)

- <sup>3</sup> different numbers indicate significant difference in 2006 samples (P<0.05)
- <sup>4</sup> 1 marrowfat peas
  - 2 green peas
  - 3 yellow peas

Figure 19. Hydration Capacities of Composite Peas Grown at Various Locations in Saskatchewan in 2005 and 2006

4.1.5. Hydration Curves of 2005 and 2006 Crop Samples

Hydration rate determination was tested for all samples grown in two years. Representative hydration curves from the 2005 and 2006 crop are shown in Figures 20 and 21 respectively. Varieties having the highest and average levels of water absorption in each type of pea for a particular year were selected. Although the curves are similar in shape, a delay of 30 ~ 60 min in onset of water uptake for peas with lower hydration capacities was again evident. Both Alfetta (2005) and Striker (2006) started taking in water only after one hour of soaking. Rambo performed consistently, absorbing water quickly and to the highest level of hydration capacity in both years. Midas and Cooper also showed consistent water absorption but to lower final moisture levels than Rambo.

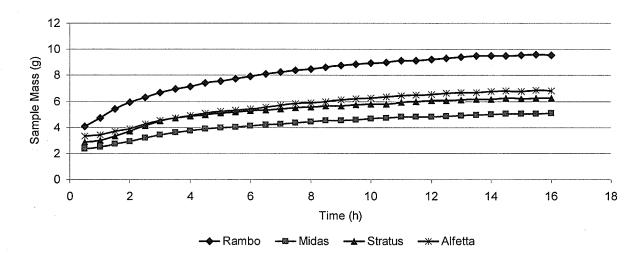


Figure 20. Hydration Curves of 2005 Crop Peas

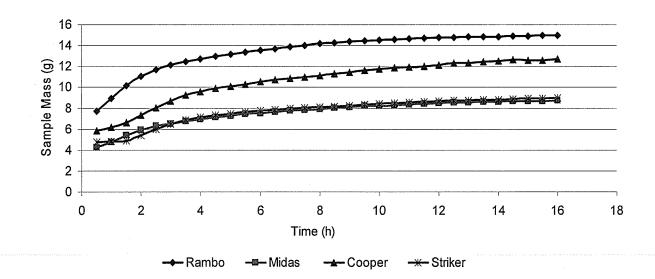


Figure 21. Hydration Curves of 2006 Crop Peas

# 4.2. Texture Evaluation of Pea Products under Different Processing Technologies

Based on the hydration data, two varieties of each type of pea were selected for processing into snacks with acceptable texture. Varieties (Midas, Mozart, Cooper, Stratus, Rambo and Midichi) with higher hydration capacities and lower levels of stone seeds were selected.

At first, whole raw peas without any pretreatment were processed, and then peas under different tempering conditions were processed. The texture evaluation was performed with a Zwick Roell texture machine (Zwick Ltd, Kennesaw, GA, USA). The average maximum forces based on eight replications were compared. Force deformation curves were obtained for all samples.

## 4.2.1. Texture Evaluation of Pea Products Produced with Superheated Steam

Different processing conditions (130 °C 10 min, 130 °C 15 min, 140 °C 10 min, and 140 °C 15 min) using superheated steam were explored for both untreated raw peas and tempered peas tempered to different levels (16%, 24%, and 33%). Tables 15, 16 and 17 show the effect of different processing conditions on maximum force obtained when compressing yellow, green and marrowfat pea products, respectively. The effects of the tempering level on maximum force were significantly different (P< 0.05) for all types of pea products. The different processing conditions also significantly influenced the maximum force to compress pea products (P< 0.05). For most samples, tempered peas

exhibited lower maximum force than the untreated raw peas. For all pea types, compression force was decreased by increasing the processing time at the same temperature, and also by increasing the temperature for a given processing time. This may because the degree of starch gelatinization increased with higher temperature and longer time, so the texture becomes more open and crispy. By increasing the tempering level from 16 to 24%, most of the compression force decreased; however, it is interesting to note that when tempered to 33% and heated to 140 °C, the force increased for yellow peas (Table 15) and green peas (Table 16). This may because at the higher moisture level, more energy was required to heat the seeds and as a result, they remained raw in the centre. Due to the structure and water movement for the marrowfat pea (Table 17) there was no hardening at the higher moisture content. The residual moisture content of pea products after SHS was decreased by about 30% from the initial moisture content, so that the products looked very dry after the processing. This is a characteristic of SHS, which dried the peas suddenly under the high pressures and temperatures used. However, if the starch of peas did not gelatinize enough, the seeds remain hard, resulting in the high compression forces.

Yellow and green peas with 24% tempering level and marrowfat peas with 33% tempering level processed using SHS at 140 °C for 15 min showed lowest compression force with values all lower than 100 N, for all three pea types, which are still not the ideal values. Other products all exhibited higher maximum forces, some even more than 300 N. All varieties used contained some stone seeds and it was very difficult to separate them before processing. This may have

influenced the results because the stone seeds appeared to become harder during processing and were easier to burn.

				Maximu	m force (N)				
	<u> </u>		idas ıg Level (%)			Mozart Tempering level (%)			
	untreated	16	24	33	untreated	16	24	33	
130 °C 10 min	274.7 ± 9.0 <sup>a1</sup>	297.7 ± 11.2ª1	267.7 ± 16.5 <sup>a1</sup>	213.2 ± 7.3 <sup>a2</sup>	294.2 ± 7.5 <sup>a1</sup>	$273.5 \pm \\ 8.4^{a1}$	244.7 ± 4.8 <sup>a3</sup>	219.0 ± 17.3 <sup>a2</sup>	
130 °C 15 min	250.2 ± 6.4 <sup>a1</sup>	180.1 ± 8.3 <sup>b2</sup>	131.6 ± 7.5 <sup>b3</sup>	114.7 ± 6.5 <sup>b4</sup>	252.6 ± 12.6 <sup>b1</sup>	181.4 ± 10.7 <sup>b2</sup>	132.4 ± 19.6 <sup>b3</sup> *	131.8 ± 17.0 <sup>b3</sup> *	
140 °C 10 min	216.1 ± 21.5 <sup>b1</sup>	175.5 ± 13.7°²	137.1 ± 17.3 <sup>b3*</sup>	167.2 ± 14.7 <sup>c2</sup>	202.4 ± 22.1 <sup>b1</sup> *	168.9 ± 19.1 <sup>b2</sup> *	129.8 ± 11.6 <sup>b3</sup>	175.4 ± 23.7 <sup>c2</sup> *	
140 °C 15 min	178.5 ± 5.0 <sup>c1</sup>	126.3 ± 10.2 <sup>d2</sup>	77.9 ± 6.6 <sup>c3</sup>	121.0 ± 6.9 <sup>b2</sup>	179.1 ± 6.8 <sup>c1</sup>	131.7 ± 9.9 <sup>c2</sup>	92.4 ± 5.1 <sup>c3</sup>	132.3 ± 6.9 <sup>b2</sup>	

Table 15. Effects of Different SHS Processing Conditions on the Maximum Force of Untreated Raw and Tempered Yellow Pea Products (2005 crop)

<sup>1</sup> mean ± standard deviation
 <sup>2</sup> different letters in the same column indicate significant difference (P< 0.05)</li>
 <sup>3</sup> different numbers in the same row indicate significant difference (P< 0.05)</li>
 <sup>4</sup> \* stone seed present
 <sup>5</sup> numbers in bold indicate the lowest values in the corresponding conditions

Table 16. Effects of Different SHS Processing Conditions on the Maximum Force of Untreated Raw and Tempered Green Pea Products (2005 crop)

				Maximu	m force (N)						
			oper Ig Level (%)			Stratus Tempering level (%)					
	untreated	16	24	33	untreated	16	24	.33			
130 °C 10 min	316.1 ± 27.5 <sup>a1</sup>	297.7 ± 11.2ª1	270.8 ± 11.5 <sup>a1</sup>	208.5 ± 7.4 <sup>a2</sup>	310.8 ± 29.8 <sup>a1</sup>	293.8± 6.5 <sup>a1</sup>	267.8 ± 16.5 <sup>a1</sup>	219.7 ± 18.6ª2			
130 °C 15 min	262.6 ± 12.1 <sup>b1</sup>	181.3 ± 8.0 <sup>52</sup>	138.3 ± 7.5 <sup>b3</sup>	114.3 ± 7.0 <sup>b4</sup>	267.8 ± 16.5 <sup>b1</sup>	186.4 ± 10.8 <sup>b2</sup>	131.6 ± 7.5 <sup>b3</sup> *	107.0 ± 4.5 <sup>b4</sup>			
140 °C 10 min	208.2 ± 7.8 <sup>c1</sup>	191.5 ± 37.0 <sup>b1</sup>	163.8 ± 17.2 <sup>c2</sup> *	183.7 ± 11.7 <sup>c1</sup>	219.7 ± 18.6 <sup>c1</sup>	182.4 ± 16.7 <sup>ь1</sup>	137.0 ± 17.3 <sup>b3</sup> *	187.6 ± 14.3 <sup>c1</sup>			
140 °C 15 min	166.5 ± 5.9 <sup>d2</sup>	133.2 ± 12.5 <sup>c2</sup>	95.0 ± 9.2 <sup>d4</sup>	$127.3 \pm 6.4^{b3}$	186.4 ± 10.8 <sup>d1</sup>	130.9 ± 8.7 <sup>c3</sup>	95.0 ± 10.3 <sup>c4</sup>	$140.6 \pm 5.8^{d3}$			

<sup>1</sup> mean ± standard deviation
 <sup>2</sup> different letters in the same column indicate significant difference (P< 0.05)</li>
 <sup>3</sup> different numbers in the same row indicate significant difference (P< 0.05)</li>
 <sup>4</sup> \* stone seed present
 <sup>5</sup> numbers in bold indicate the lowest values in the corresponding conditions

Table 17. Effects of Different SHS Processing Conditions on the Maximum Force of Untreated Raw and Tempered Marrowfat Pea Products (2005 crop)

				Maxi	mum force (N	I)			
		Ram empering			Midichi Tempering level (%)				
	untreated	16	24	33	untreated	16	24	33	
130 °C	301.3 ±	288.6 ±	264.8 ±	201.5 ±	295.1 ±	287.6 ±	265.0 ±	213.8 ±	
10 min	14.1 <sup>a1</sup>	3.6 <sup>a1</sup>	11.3 <sup>a2</sup>	9.9 <sup>a3</sup>	11.7 <sup>a1</sup>	9.5 <sup>a1</sup>	14.2 <sup>a2</sup>	8.1 <sup>a3</sup>	
130 °C	258.6 ±	169.8 ±	138.2 ±	105.0 ±	260.4 ±	176.7 ±	132.8 ±	110.3 ±	
15 min	15.9 <sup>b1</sup>	8.5 <sup>b2</sup>	7.5 <sup>b3</sup>	7.0 <sup>b4</sup>	15.0 <sup>b1</sup>	13.2 <sup>b2</sup>	8.7 <sup>b3</sup>	2.1 <sup>b4</sup>	
140 °C	211.7 ±	172.8 ±	159.8 ±	103.1 ±	219.7 ±	240.1 ±	137.1 ±	109.2 ±	
10 min	8.7 <sup>c2</sup>	12.9 <sup>b3</sup>	10.6 <sup>c4</sup>	10.4 <sup>b5</sup> *	18.6 <sup>c2</sup>	84.1 <sup>c1</sup> *	17.4 <sup>b4</sup>	4.2 <sup>b5</sup>	
140 °C	157.7 ±	133.2 ±	110.6 ±	64.0 ±	176.2 ±	137.7 ±	120.9 ±	71.1 ±	
15 min	11.8 <sup>d1</sup>	12.4 <sup>c2</sup>	8.2 <sup>d3</sup>	4.6 <sup>c4</sup>	10.3 <sup>d1</sup>	20.7 <sup>d2</sup> *	9.1 <sup>b3</sup>	6.4 <sup>c4</sup> *	

<sup>1</sup> mean ± standard deviation
 <sup>2</sup> different letters in the same column indicate significant difference (P< 0.05)</li>
 <sup>3</sup> different numbers in the same row indicate significant difference (P< 0.05)</li>
 <sup>4</sup> \* stone seed present
 <sup>5</sup> numbers in bold indicate the lowest values in the corresponding conditions

4.2.2. Texture Evaluation of Pea Products produced with Micronization

Different micronizing times were explored for both untreated raw peas and peas tempered to different levels. The effects of different micronization conditions on maximum force of these peas are shown in Table 18, 19 and 20 for yellow, green and marrowfat peas, respectively. Since the infrared heat was intensive, untreated raw peas burned when they were micronized for longer times (>2min 45sec). Tempering improved the texture of peas. The maximum force values were much lower than those seen for the SHS processed products. As was the case with SHS, both different tempering levels and different processing times significantly influenced the compression force of pea products (P<0.05). In addition, all varieties had stone seeds which were hard to separate. As this phenomenon affected the results, it is highly recommended that further research should focus on methods for separating stone seeds before processing. Since this weak point of pea products represented a potential hazard during consumption, a sensory test was not conducted in this study.

Unlike the SHS processed products, yellow and green pea with 16% tempering level and marrowfat peas (24%) showed the lowest maximum force. The optimum processing time to give the lowest compression force varied with pea type. Yellow peas required micronization for 2 min 45 s; green peas required 3 min and marrowfat required 3 min 15 s. The lowest maximum forces were seen in the green pea variety Stratus (18.4 N) and yellow pea variety Midas (18.8 N), which were both lower than values obtained for the commercial peas tested in the study (32.2 N). The moisture content of micronized peas was reduced by

about 15% from the initial moisture content so that the residual moisture level was higher than the SHS processed products. In addition, higher residual moisture level was obtained with higher tempering levels. This may be related to better moisture equilibrium within the seed and insufficient energy to get this moisture to the surface and remove it or case-hardening of the seed surface, which also lessened water evaporation during micronization.

				Maximu	m force (N)				
	Т		das g Level [%]		Mozart Tempering level [%]				
	untreated	16	24	. 33	untreated	16	24	33	
2 min 15 s	35.4 ± 6.3 <sup>a3</sup>	46.1 ± 4.0 <sup>a4</sup>	59.1 ± 5.4 <sup>a2</sup>	77.1 ± 5.0 <sup>a1</sup>	36.4 ± 4.6 <sup>a3</sup>	52.6 ± 8.2 <sup>a2</sup>	65.7 ± 10.0 <sup>a1</sup> *	79.7 <del>:</del> 2.9 <sup>a1</sup>	
2 min 30 s	30.2 ± 6.3 <sup>a3</sup>	30.3 ± 2.6 <sup>b3</sup>	39.8 ± 13.0 <sup>b2</sup> *	$54.9 \pm 4.5^{b4}$	28.5 ± 13.2 <sup>b3</sup> *	32.1 ± 2.3 <sup>b3</sup>	39.1 ± 1.4 <sup>b2</sup>	65.9 ± 6.7 <sup>b1,</sup>	
2 min 45 s	В	18.8 ± 1.7 <sup>c3</sup>	32.0 ± 2.5 <sup>b2</sup>	41.8 ± 1.7 <sup>c1</sup>	В	21.5 ± 1.7 <sup>c3</sup>	34.4 ± 3.1 <sup>b2</sup>	44.0 - 3.5 <sup>c1</sup>	
3 min	В	22.4 ± 2.2 <sup>c2</sup>	30.1 ± 11.2 <sup>b1</sup> *	32.6 ± 2.4 <sup>d1</sup>	В	34.0 ± 16.5 <sup>b1</sup> *	25.9 ± 3.5 <sup>c2</sup>	28.2 - 2.0 <sup>d2</sup>	

Table 18. Effects of Different Micronization Conditions on the Maximum Force of Untreated Raw and Tempered Yellow Pea Products (2005 crop)

<sup>1</sup> mean ± standard deviation
<sup>2</sup> different letters in the same column indicate significant difference (P< 0.05)</li>
<sup>3</sup> different numbers in the same row indicate significant difference (P< 0.05)</li>
<sup>4</sup> \* stone seed present
<sup>5</sup> B burned at this condition
<sup>6</sup> numbers in bold indicate the lowest values in the corresponding conditions

Table 19. Effects of Different Micronization (	Conditions on the Maximum	Force of Untreated Raw and Terr	pered Green
Pea Products (2005 crop)			

			Ν	laximum	force (N)			
	Te	Coop empering		Stratus Tempering level [%]				
	untreated	16	24	33	untreated	16	24	33
2 min 15 s	$40.2 \pm 9.8^{a3}$	50.5 ± 10.9 <sup>a2</sup> *	57.2 ± 5.4 <sup>a2</sup>	74.0 ± 4.8 <sup>a1</sup>	38.5 ± 7.5 <sup>a3</sup>	48.5 ± 2.0 <sup>a2</sup>	55.6 ± 4.5 <sup>a2</sup>	75.8 ± 2.4 <sup>a1</sup>
2 min 30 s	$32.2 \pm 8.5^{b3}$	30.5 ± 2.2 <sup>b3</sup>	36.2 ± 2.9 <sup>b3</sup>	52.1 ± 2.8 <sup>b4</sup>	31.0 ± 5.8 <sup>a3</sup>	30.9 ± 1.1 <sup>b3</sup>	43.2 ± 11.4 <sup>b2</sup> *	68.8 ± 6.3 <sup>b1</sup>
2 min 45 s	В	24.5 ± 1.5 <sup>b3</sup>	31.8 ± 2.5 <sup>b2</sup>	38.1 ± 2.2 <sup>c2</sup>	В	21.3 ± 1.0 <sup>c3</sup>	$36.0 \pm 2.8^{c2}$	46.8 ± 3.3 <sup>c1</sup>
3 min	В	19.4 ± 1.1 <sup>c2</sup>	23.0 ± 1.9 <sup>c2</sup>	31.2 ± 1.3 <sup>c1</sup>	В	18.4 ± 1.3 <sup>c2</sup>	23.7 ± 2.0 <sup>d2</sup>	32.9 ± 2.6 <sup>d1</sup>

<sup>1</sup> mean ± standard deviation
<sup>2</sup> different letters in the same column indicate significant difference (P< 0.05)</li>
<sup>3</sup> different numbers in the same row indicate significant difference (P< 0.05)</li>
<sup>4</sup> \* stone seed present
<sup>5</sup> B burned at this condition
<sup>6</sup> numbers in bold indicate the lowest values in the corresponding conditions

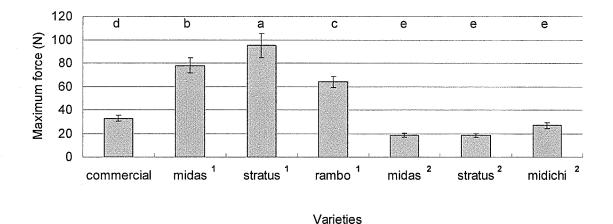
Table 20. Effects of Different Micronization Conditions on the Maximum Force of Untreated Raw and Tempered Marrowfat Pea Products (2005 crop)

			1	Maximum	force (N)			
	Те	Ram	ibo Level [%]		Te	Midio		
	untreated	16	24	33	untreated	16	24	33
2 min 15 s	50.1 ± 8.7 <sup>a3</sup>	68.8 ± 3.1 <sup>a2</sup>	74.8 ± 3.6 <sup>a4</sup>	82.4 ± 4.3 <sup>a1</sup>	55.4 ± 7.5 <sup>a3</sup>	60.9 ± 4.4 <sup>a2</sup>	68.2± 8.9 <sup>a2</sup> *	80.8 ± 20.0 <sup>a1</sup>
2 min 30 s	46.6 ± 2.4 <sup>a2</sup>	53.5 ± 5.0 <sup>b2</sup>	60.7 ± .3.4 <sup>b1</sup>	54.9 ± 4.5 <sup>b2</sup>	50.1 ± 9.0 <sup>a2</sup>	52.6 ± 2.9 <sup>b2</sup>	54.7 ± 3.6 <sup>b2</sup>	67.0 ± 4.5 <sup>b1</sup>
2 min 45 s	В	40.4 ± 2.3 <sup>c2</sup>	47.6 ± 14.6 <sup>c2</sup> *	43.0 ± 2.3 <sup>c2</sup>	В	39.3 ± 2.6 <sup>c2</sup>	41.9 ± 2.6 <sup>c2</sup>	55.2 ± 3.4 <sup>c1</sup>
3 min	В	32.5 ± 2.3 <sup>d1</sup>	28.9 ± 1.8 <sup>c1</sup>	34.6 ± 3.1 <sup>d1</sup>	В	31.3 ± 2.6 <sup>c1</sup>	26.9 ± 2.5 <sup>d1</sup>	32.1 ± 3.0 <sup>d1</sup>

<sup>1</sup> mean ± standard deviation
<sup>2</sup> different letters in the same column indicate significant difference (P< 0.05)</li>
<sup>3</sup> different numbers in the same row indicate significant difference (P< 0.05)</li>
<sup>4</sup> \* stone seed present
<sup>5</sup> B burned at this condition
<sup>6</sup> numbers in bold indicate the lowest values in the corresponding conditions

### 4.2.3. Comparison of Texture between Commercial and Processed Peas

Texture evaluation was also determined for commercial peas which were deep fried in oil. The maximum force of these commercial peas was used as a reference parameter. Figure 22 shows the comparison of maximum force between commercial and pea products from SHS and Micronization. Pea products from processed with SHS had extremely significant higher maximum force than the commercial peas (32.8 N) (P<0.0001). The highest value (95.0 N) was seen in Stratus green peas. However, the micronized peas had significant lower compression forces than the commercial peas (P<0.05). It was very interesting to note that Stratus showed the lowest maximum force (18.4 N) in all micronized pea products although the three micronized samples were not significantly different. Even though marrowfat peas also showed low maximum force, peas began to pop after a certain time during the micronization. The texture of marrowfat pea products was not as crispy as yellow and green peas, this may due to the large and square size of marrowfat peas. It was difficult to keep every seed heated evenly because of the method used to shake the samples. Since marrowfat peas absorbed moisture better and had higher starch contents, they were expected to produce a useable new pea snack food.



1 samples treated with SHS

2 Micronized samples

Figure 22. Comparison of Maximum Force between Commercial and Processed Peas

In addition, force deformation curves were obtained for each replicate. Figure 23, 24 and 25 are the examples of force deformation curves of commercial, micronized, and superheated peas, respectively. Micronized peas showed a similar shape of curve to the commercial peas, which have a few small peaks before it reached the maximum force. These may be because the texture of the products was crispy, not only the seed coat but the inside of seeds. The presence of air pockets and cell walls within the seed caused a series of breaks which were responsible for all the small peaks. However, the force generated within the superheated peas which at specific conditions during compression increased linearly without any peaks prior to reaching the maximum force. This is because there was no open structure so that the texture was very hard and not crispy, so it did not break until it reached the maximum force.

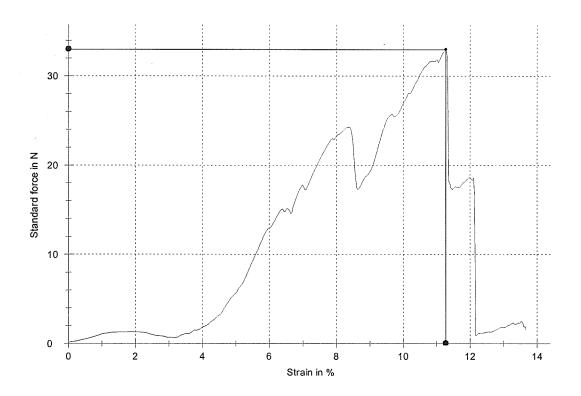


Figure 23. Typical Force Deformation Curve of Commercial Pea

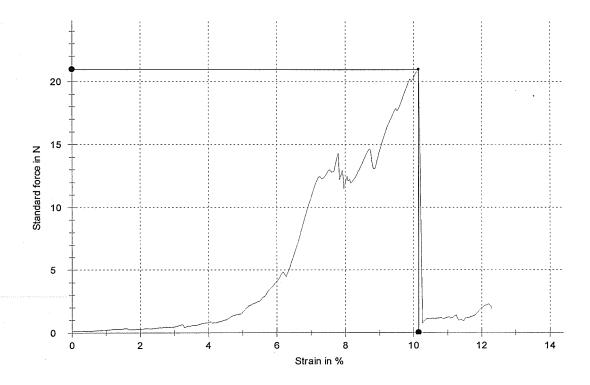


Figure 24. Typical Force Deformation Curve of Micronized Pea

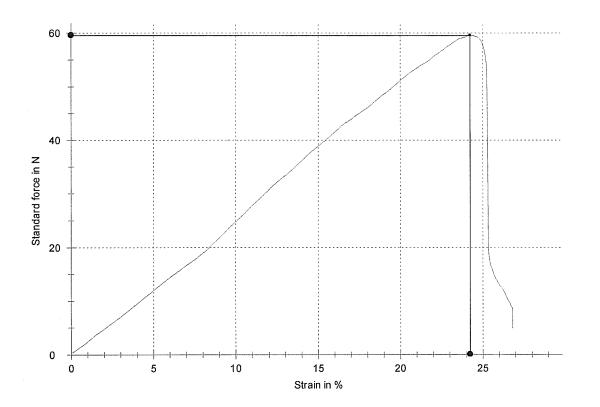


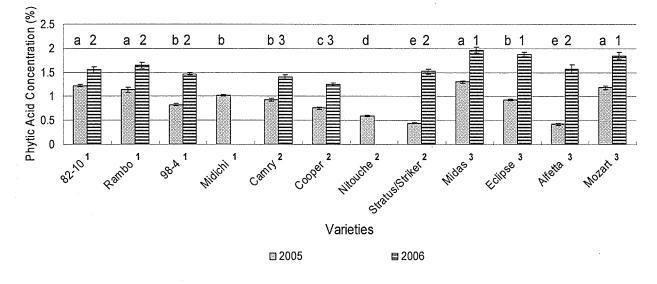
Figure 25. Typical Force Deformation Curve of SHS Pea

To summarize, micronization is a more effective technique than SHS that at specific conditions used to produce whole pea snack food. Green peas with 16% tempering level for 3 min and yellow peas with 16% tempering level for 2 min 45 s produced a preferred texture for the snack. However, due to the existence of stone seeds, there needs to be developed an effective method for separating the stone seeds prior to processing. Once this drawback has been solved, micronization will be a very practical processing technique to produce whole pea snacks.

### 4.3. Phytic Acid Levels in Raw and Micronized Peas

## 4.3.1. Phytic Acid Content of Raw Peas from 2005 and 2006

Figures 26 shows the phytic acid content of raw peas from 2005 and 2006 for composite samples from various locations in Saskatchewan. Overall, Midas showed the highest phytic acid content in both years. Comparing the results of the same varieties across years, the content of phytic acid in 2005 peas tended to be lower than in 2006. The unpaired t test was applied by using Graphpad Instat3 program (t=5.338) to compare the average value between two years. T value was 5.338 (p<0.0001) and indicated that 2005 peas contain significantly lower level of the phytic acid than 2006 samples. The average content of phytic acid in 2005 (0.97%) increased to 1.62% in 2006. Marzo, et al. (1997) reported the amount of phytic acid in dry peas varies with varieties, growing conditions and irrigation conditions. Climatic differences existed between 2005 and 2006 in Saskatchewan. The climate in 2005 was characterized with heavy rains in summer and normal precipitation and slightly below normal temperature. It was much drier in 2006, with temperatures and heat units close to normal. Therefore, wet climate and lower temperature may cause the low level of phytic acid. These yearly climatic differences also contributed to the difference in total phenolic acid content and antioxidant properties of peas which will be discussed later.

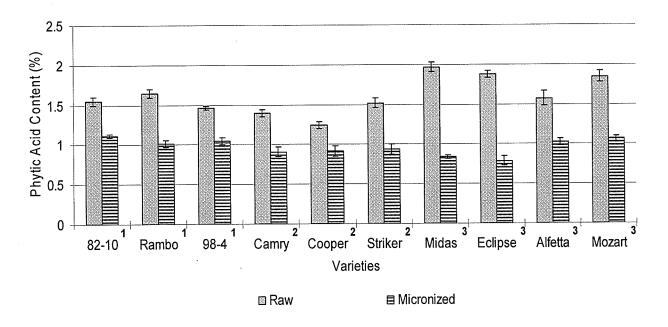


<sup>1</sup> different letters indicate significant difference in 2005 samples (P<0.05)</li>
 <sup>2</sup> different numbers indicate significant difference in 2006 samples (P<0.05)</li>
 <sup>3</sup> 1 marrowfat peas
 2 green peas
 3 yellow peas

Figure 26. Phytic Acid Content of Raw 2005 and 2006 Peas

### 4.3.2. Effects of Micronization on Phytic Acid of Peas

The effects of micronization on the phytic acid content of peas are shown in Figure 27. For the 2006 samples, the average percentage of phytic acid of raw peas was decreased by approximately 40% following micronization. A paired t test was applied by using Graphpad Instat3 program. The t value was 7.379 (p<0.0001) indicating that micronization significantly decreased the phytic acid level. Since phytic acid is seen as an antinutritional factor in legumes (O'Dell, 1979), micronization can be used to decrease the phytic acid content. Urbano, et al. (2000) reported several methods for decreasing the inhibitory effect of phytic acid on mineral absorption including soaking, germination, fermentation, and cooking. Micronization may be a new method to decrease the inhibitory effects that need to be further studied. As there was soaking prior to micronization step, phytase activity during this step may also had contributed to lower phytic acid levels.



1 marrowfat peas 2 green peas

3 yellow peas

Figure 27. Effects of Micronization on Phytic Acid Content of Peas

#### 4.4. Phenolic levels in Raw and Micronized Peas

## 4.4.1. Determination of Phenolic Acid Contents of Raw Samples

In this study, peas from the 2005 crop year were used to determine the level of phenolic acids. Three fractions were produced during the determination of phenolics: a methanol extract, representing free phenolics, and phenolics released following base and then acid hydrolyses. Phenolics were quantified at two different wavelengths (270 and 325 nm) as not all of the phenolic acids absorbed at 270 nm. Levels of phenolic acids for the 2005 crop representing the total of all three extracts are summarized in Table 21. Overall, the most prominent phenolic acids tended to be synaptic acid and ferulic acid regardless of variety. The p- and m-coumaric acids were also relatively high but were not detected in all samples. Levels of individual phenolic acids were lower in the marrowfat peas than the yellow or green peas. While Eclipse and Nitouche had the highest levels of synaptic acid, ferulic acid, the highest levels of gallic acid, procatechuic acid and hydroxybenzoic acid were found in the variety Mozart.

Varieties	Phenoli	c Acid Co	ntent (mg/	/100g wh	oleme	eal)						<u>.</u>
	GA	PCA	HBA	GTA *	VA	CA *	SYA	pCA *	SA *	FA *	mCA *	oCA
82-10 <sup>1</sup>	0.014	0.068	0.065			0.057		0.172	0.124	0.094	0.021	
Rambo <sup>1</sup>	0.034	0.021	0.048					0.109	0.052	0.079		0.008
Midichi <sup>1</sup>		0.01	0.025						0.059	0.086	0.023	
98-4-1 <sup>1</sup>	0.021	0.012	0.024						0.063	0.088		
Midas <sup>2</sup>	0.024	0.031	0.038			0.045		0.130	0.198	0.432	0.101	
Eclipse <sup>2</sup>	0.029	0.023	0.062			0.040		0.134	0.215	0.636	0.11	
Alfetta <sup>2</sup>	0.034	0.047	0.056				0.01		0.155	0.450	0.118	
Mozart <sup>2</sup>	0.048	0.048	0.059	0.04				0.103	0.137	0.443	0.091	
Camry <sup>3</sup>	0.025	0.029	0.059					0.140	0.168	0.329	0.096	
Nitouche <sup>3</sup>	0.027	0.018	0.053						0.216	0.669	0.097	
Stratus <sup>3</sup>	0.022	0.035	0.056			0.047	0.01	0.146	0.186	0.437	0.113	
Cooper <sup>3</sup>	0.031	0.020	0.056					0.143	0.145	0.339	0.079	

Table 21. Phenolic Content of Peas at 270 and 325 nm

GA: Gallic acid; PCA: Procatechuic acid; HBA: Hydroxybenzoic acid; GTA: Gentistic acid; VA: Vanillic acid; CA: Caffeic acid; SYA: Syrinigic acid; pCA:p-Coumaric acid; SA: Synapic acid; FA: Ferulic acid; mCA: m-Coumaric acid; and oCA: o-Coumaric acid

\* Phenolic acids quantified at 325nm

Rest of acids quantified at 270nm

<sup>1</sup> Marrowfat pea

<sup>2</sup> Yellow pea

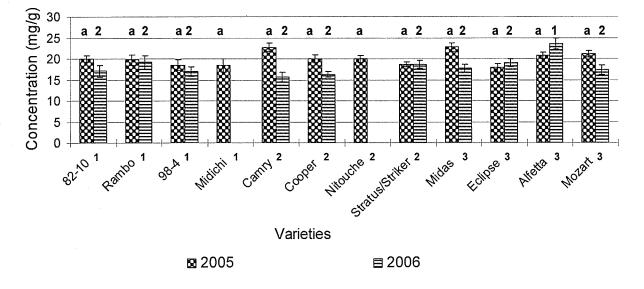
<sup>3</sup> Green pea

#### 4.4.2. Determination of Total Phenolics of Raw and Micronized Peas

As the total phenolics were more valuable parameter for examining the effects of processing, further work was done using the assay for total phenolics. Peas from the 2005 and 2006 crop years and micronized peas were examined. As discussed earlier, the climatic differences influenced the phytic acid concentration in the two years of the study. Differences in total phenolics in these two years were also seen and are discussed in this section as well as the effect of micronization on total phenolics.

## 4.4.2.1. Total Phenolics of Raw Peas from both Years

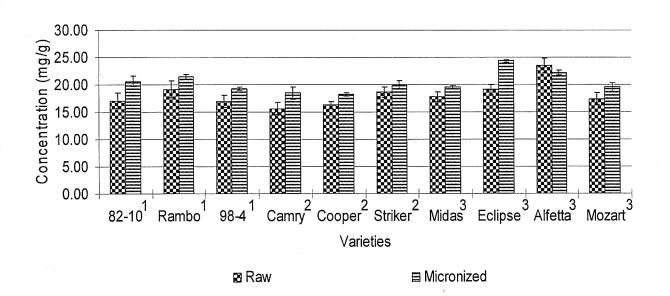
Figures 28 shows the total phenolics content of raw peas from 2005 and 2006. Yellow peas were seen to have higher average amounts of total phenolics than marrowfat and green peas in both years. Comparing the results of same varieties across years, the content of total phenolics in 2005 peas tended to be higher than in 2006. The average content of total phenolics in 2005 was 20.383mg/g, and decreased to 18.105mg/g in 2006. The unpaired t test showed t value was 0.0304 (p=0.0304), so indicated that 2005 peas contain significantly higher level of the total phenolics than 2006 peas. This significant difference could also result from the different climate and temperature in the two successive years. Therefore, the amount of total phenolics in dry peas not only varies with variety, but also environmental conditions.



- <sup>1</sup> different letters indicate significant difference in 2005 samples (P<0.05)</li>
   <sup>2</sup> different numbers indicate significant difference in 2006 samples (P<0.05)</li>
   <sup>3</sup> 1 marrowfat peas
  - 2 green peas
  - 3 vellow peas
- Figure 28. Phenolic Acid Content of Raw 2005 and 2006 Peas

## 4.4.2.2. Effects of Micronization on Total Phenolics of Peas

Due to the limited availability of sample from the 2005 crop year, the study of the effects of micronization on total phenolics in peas was mainly done using 2006 samples (preliminary testing was done with materials from 2005). The results are shown in Figure 29. The average amount of total phenolics of raw peas increased by 12.2% after micronization. The paired t test showed the t value was 4.149 (p=0.0025) and the statistical analysis indicated that micronization significantly increased the total amount of phenolics. Since phenolics have been proven to have antioxidant activity in many studies, it is important to investigate the effects of treatments and processings on these compounds. The results are in agreement with Alonso, et al. (1998), who analysed polyphenols in peas and found that their concentrations varied with processing. Based on these results, micronization appears to be a novel processing technique that increases the total phenolics in peas. However, more research needs to be done to explain the mechanism responsible for the increases.



- 1 marrowfat peas
- 2 green peas
- 3 yellow peas

Figure 29. Effects of Micronization on Total Phenolics

#### 4.5. Antioxidant Properties Determination of Raw and Micronized Peas

Antioxidant levels of raw peas and micronized peas were assessed by using the DPPH• and ABTS• methods. To compare the results of raw and micronized peas, 2006 samples were used.

4.5.1. Results of Raw Samples using DPPH• and ABTS• Method

Table 22 shows the antioxidant activity of the raw samples (Table 7 and 8) in two successive years using the DPPH• method. The marrowfat variety 98-4 exhibited the highest antioxidant activity among all varieties in 2005 samples, yet Cooper showed the highest values in 2006. Turkey-Kramer Multiple Comparisons Test was applied by using Graphpad Instat3 program to compare the average level of antioxidant activity of all varieties in 2005 sample. One-way ANOVA statistical analysis indicated significant differences among all varieties (p=0.0003). However, the average level of yellow peas was not significantly different from green peas (p>0.05), while the average level of marrowfat peas was significantly higher than both yellow (p<0.01) and green (p<0.001) peas. The same test was also employed for 2006 samples; the one-way ANOVA statistical analysis indicated that varieties had extremely significant differences in antioxidant levels (p<0.0001). Significant difference were also seen among the three types of peas. Comparing the results across years, 2005 peas seemed to have higher antioxidant activities than 2006 peas. Except for the varieties Cooper and Camry, all pea varieties had lower level of antioxidant activity in 2006.

	Varieties	2005 Peas	2006 Peas
Marrowfat	Rambo	$81.9 \pm 5.6$ <sup>b</sup>	70.8 ± 1.7 <sup>2</sup>
	98-4	114.2 $\pm$ 4.5 <sup>a</sup>	$58.8 \pm 1.6 \ ^{\textbf{4}}$
	82-10	$73.4\pm2.5$ <sup>c</sup>	$52.6\pm5.9^{\text{ 4}}$
	Midichi	$66.1\pm4.3$ <sup>d</sup>	NA
Yellow	Midas	$72.8\pm7.2$ <sup>b</sup>	$64.0 \pm 4.3$ <sup>3</sup>
	Eclipse	$62.4\pm4.6$ <sup>d</sup>	42.1 $\pm$ 3.7 $^{\texttt{5}}$
	Mozart	$66.3\pm4.8$ <sup>d</sup>	$47.4\pm3.3~^{5}$
	Alfetta	$78.9\pm3.4$ <sup>b</sup>	$62.5\pm6.9~^{3}$
Green	Cooper	72.0 ± 5.1 °	$83.2\pm4.0~^{1}$
	Nitouche	$63.6\pm4.4$ <sup>d</sup>	NA
	Stratus	$60.7\pm2.4$ <sup>d</sup>	NA
	Camry	$63.2\pm1.8$ <sup>d</sup>	$76.6\pm3.8~^{\textbf{2}}$
	Striker	NA	$73.3\pm2.9~^{\textbf{2}}$

Table 22. Scavenging Capacity of Raw Samples Using DPPH• Method (µmole Trolox equivalent/100g sample)

NA: Variety not applicable in that year

 $^1$  mean  $\pm$  standard deviation  $^2$  different letters in the same column indicate significant difference (P=0.0003)  $^3$  different numbers in the same column indicate significant difference (P< 0.0001)

Table 23 shows the antioxidant activity levels of raw pea samples in two successive years using the ABTS • method. Comparing the results of both years to those from the DPPH• results, the ABTS• method resulted in more radical scavenging activity of antioxidants than was obtained using DPPH• method. Prakash (2001) reported that the various methods used to measure antioxidant activity of food products can give varying results depending on the specificity of the free radical being used as a reactant. She also mentioned that antioxidants in food may be water soluble, fat soluble, insoluble, or bound to cell walls, hence they react at different rates. In addition, different antioxidant compounds have different sensitivity in terms of their ability to react with different radicals. Although there was a big difference between the results using the two methods, 98-4 and Cooper again exhibited the highest antioxidant activity in 2005 and 2006 respectively. Comparing the results across years, 2005 peas had higher level of antioxidant activities than 2006 peas in all varieties which is similar to the DPPH• results. This also agreed with the total phenolics results discussed earlier which is not surprising as the total phenolics have been related to antioxidant properties (Cai et al., 2004; Zheng and Wang, 2001). These year to year variations supports the previous observation that the climatic differences do influence pea quality including hydration, antinutritional factors and antioxidant activities. Since it was wetter with lower temperatures in 2005, it would appear that these are the conditions that increase these quality characteristics in pea products.

	Varieties	2005 Peas	2006 Peas
Marrowfat	Rambo	147.8 ± 2.5 <sup>c</sup>	117.2 ± 2.6 <sup>5</sup>
	98-4	$200.5\pm0.5~^{\textbf{a}}$	144.4 ± 9.3 <sup>2</sup>
	82-10	175.7 ± 2.8 <sup>b</sup>	127.4 ± 1.4 <sup>4</sup>
	Midichi	143.0 ± 5.2 <sup>c</sup>	NA
Yellow	Midas	$149.8\pm5.4~^{\textbf{c}}$	134.8 ± 5.7 <sup>3</sup>
	Eclipse	158.0 ± 5.2 <sup>c</sup>	124.1 ± 1.7 <sup>4</sup>
	Mozart	147.1 ± 1.0 <sup>c</sup>	113.3 $\pm$ 4.1 <sup>5</sup>
	Alfetta	142.3 ± 4.2 <sup>c</sup>	$122.0 \pm 4.6$ <sup>4</sup>
Green	Cooper	178.4 ± 0.0 <sup>b</sup>	158.9 ± 4.1 <sup>1</sup>
	Nitouche	199.3 ± 8.1 <sup>a</sup>	NA
	Stratus	175.3 ± 8.5 <sup>b</sup>	NA
	Camry	155.7 ± 3.3 <sup>c</sup>	132.3 ± 9.6 <sup>3</sup>
	Striker	NA	$132.8 \pm 0.7$ <sup>1</sup>

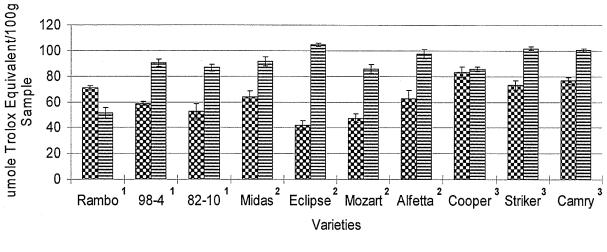
Table 23. Scavenging Capacity of Raw Samples Using ABTS. Method (µmole Trolox equivalent/100 g sample)

NA: Variety not applicable in that year

 $^1$  mean  $\pm$  standard deviation  $^2$  different letters in the same column indicate significant difference (P< 0.05)  $^3$  different numbers in the same column indicate significant difference (P< 0.0001)

## 4.5.2. Effects of Micronization on Antioxidant Properties of Peas

The effects of micronization on antioxidant properties were investigated using 2006 samples only and the results are shown in Figures 30 and 31 for the DPPH• and ABTS• methods, respectively. A paired t test was used to evaluate the average results of all varieties from each method, and the statistical analysis indicated that micronization significantly increased the antioxidant activities of peas based on the DPPH• (t=3.844, p=0.0039) method, but not with the ABTS• method (t=2.214, p=0.0541). Except for Rambo, 98-4, and Striker, the effects of micronization on antioxidant activity in peas were similar for the two methods. The different results from the different methods may be due to the antioxidant compounds responding differently to the radicals as discussed earlier.

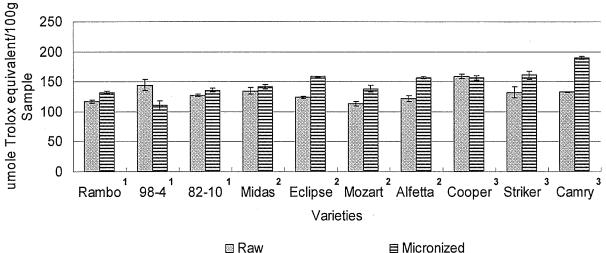


Raw

Micronized

- 1 marrowfat peas
- 2 yellow peas
- 3 green peas

Figure 30. Effects of Micronization on DPPH• Free Radical Scavenging Capacity of 2006 Samples



🖾 Raw

1 marrowfat peas

2 yellow peas

3 green peas

Figure 31. Effects of Micronization on ABTS• Free Radical Scavenging Capacity of 2006 Samples

#### CHAPTER 5

#### CONCLUSIONS AND RECOMMENDATIONS

Hydration properties are very important to the processing of peas. Varieties of peas suitable for processing can absorb water consistently to an acceptable level (>110 % on dry basis) with an appropriate initial moisture content (>5 %) and low percent of stone seeds (<5 %). The initial moisture content of peas varies with variety, location, and percent of stone seeds. Water absorption ability was highly related to the initial moisture content and percent of stone seeds. Low hydration capacities (< 100 % on dry basis) were seen for samples with a high level of stone seeds (>5 %) and were associated with a low level of initial moisture content (<5 %). Marrowfat peas tended to have higher hydration capacities than green or yellow peas. Hydration rate curves were similar for all peas tested; however, there was a delay of 30 min or more in the onset of water uptake for peas with lower hydration capacities.

Different SHS processing conditions (130 °C 10min, 130 °C 15min, 140 °C 10min, 140 °C 15min) were explored for both untreated raw peas and peas tempered to different levels. Results showed that both the processing condition and tempering levels had significant effects on the maximum force during compression testing of processed peas (P<0.05). Yellow and green peas with a 24% tempering level and marrowfat peas with a 33% tempering level processed using SHS at 140 °C for 15 min showed lowest maximum compression force with values lower than 100 N, for all three pea types; other products all exhibited

higher maximum forces, some even more than 300 N. The difficulty in separating the stone seeds before processing influenced the results greatly due to their hardness.

Different micronization processing conditions (2min15s, 2min30s, 2min45s, 3min, 3min15s) were explored for both untreated raw peas and peas tempered to different levels. As was the case with SHS, both different tempering levels and different processing times significantly influenced the maximum compression force of the pea products (P<0.05). Unlike the SHS products, yellow and green pea with 16% tempering level and marrowfat peas (24%) showed the lowest maximum force. The lowest maximum force were seen in green pea of the variety Stratus (18.4 N) and yellow pea of the variety Midas (18.8 N), which are lower than values obtained for the commercial peas tested in the study (32.2 N).

Comparing the texture to commercial peas, pea products from SHS processed at the specific conditions used in this study had significantly higher maximum force than the commercial peas (32.8 N) (P<0.0001). However, the micronized peas (2min45s for yellow peas, 3min for green peas, and 3min 15s for marrowfat peas) had significantly lower compression forces than the commercial peas (P<0.05). It was very interesting to note that Stratus showed the lowest maximum force (18.4 N) among all micronized pea products although the three micronized samples were not significantly different. Therefore, micronization is a more effective technique than SHS to produce whole pea snack food. Green peas with 16% tempering level micronized for 3 min and

yellow peas with 16% tempering micronized level for 2 min 45 s produced preferred textures for the snack.

Micronization significantly decreased the phytic acid level and increased the total phenolics levels (P<0.001). Since phytic acid is seen as an antinutritional factor in legumes and total phenolics have been proven to have antioxidant activity, micronization was seen as a beneficial process. In addition, micronization significantly increased the antioxidant activities of peas based on the DPPH• method, but not with the ABTS• method. This difference between the different methods may due to the antioxidant compounds responding differently to the radicals. Considering the effects of micronization on total phenolics level, it can be said that micronization could improve the antioxidant activities of peas.

As the existence of stone seeds interfered with processing, it is highly recommended that further research should focus on a method for separating stone seeds before processing. Once this drawback has been solved, micronization will be a very practical processing technique to produce whole pea snacks. Since marrowfat peas absorbed moisture better and had higher starch contents (Christensen and Mustafa, 1999), they were expected to produce an acceptable new pea snack food. They did not perform as well in terms of texture as green peas in this study, possibly because the large and square size of marrowfat peas. It was difficult to keep every seed heated evenly because of the method used to shake the samples. Therefore, future studies should investigate modifying the processing of marrowfat peas to ensure even heat distribution.

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**Appendix 1.** Procedure for Hydration Capacity Determination

- 1. One hundred seeds were randomly removed from a pea sample and placed in a weighing dish.
- 2. They were visually inspected and seeds with cracked or broken seed coats, disease or other damage were discarded and replaced with sound seeds.
- 3. The weight of the 100 sound seeds was recorded.
- 4. The weighed seeds were placed into a labelled glass jar.
- 5. Distilled water was added at a ratio of 1:3 seeds: water and soaked for 16 hours.
- 6. After 16 hours, the water was drained by pouring the contents through a strainer and gently shaking off excess water. The contents were returned to the containers.
- The soaked seeds were placed into a stainless steel tray lined with a paper towel.
- 8. The seeds were gently blotted with a paper towel to remove excess surface water.
- 9. The soaked seed were weighed and the weights were recorded.

Calculations:

1. HC (Hydration Capacity) on as-is basis:

 $\% = [(W_2 - W_1)/W_1] \times 100$ 

2. HC' (Hydration Capacity) on a dry basis:

% = { $[100 \times (W_2 - W_1)] / [W_1 \times (100 - MC)]$ } × 100

# 3. MC (Moisture Content):

 $\% = [(W_3 - W_4)/W_3] \times 100$ 

Where:

W<sub>1</sub> = weight of seeds before soaking

W<sub>2</sub> = weight of seeds after soaking

MC = moisture content

W<sub>3</sub> = weight of seeds before drying

 $W_4$  = weight of seeds after drying

**Appendix 2.** Procedure for Hydration Rate Determination

- 1. Ten whole peas were weighed and then soaked in a beaker with 100 mL distilled water.
- 2. Timing began when the 100 mL water was added.
- 3. Every 30 min for a total of 16 hours, the timer was stopped and all peas removed with a scapula.
- 4. All peas were quickly blotted dry on a paper tower and weighed (until a constant weight was established).
- 5. Once the peas were returned to the beaker with water, timing resumed.
- 6. The increase in weight as a function of time was plotted.

### **Appendix 3.** Oven Drying Procedure for Moisture Determination

- 1. Aluminum dishes were pre-dried for 1 hour at 130 °C.
- 2. After cooling in a desiccator for 10 min, the dishes were weighed using an analytical balance.
- 3. Two grams of sample were accurately weighed into each dish.
- 4. The whole pea samples were dried at 130 °C for 2 hours.
- 5. The samples were cooled in a desiccator for 30 min and reweighed.
- 6. Calculated %moisture = (g lost) \* 100/ (g of original sample weight)
- 7. Duplicate analysis was performed for each sample.

Appendix 4. Procedure for Phytic Acid Determination (Latta and Eskin, 1980)

1. Sample extraction

The ground sample (0.5 g) was weighed into a 25 mL flask and placed on a magnetic stirrer. While stirring 10 mL of 2.4% HCl was added and mixed for 1 h. A portion of the sample was centrifuge at 10,000xg in 50 mL tubes for 10 min. 5-10 mL of clear supernatant was removed and stored in a vial in the fridge.

2. Column chromatography

A glass column (0.7 cm \* 27 cm) packed with some glass wool and 0.5g of 200 – 400 mesh AG1-X8 chloride anion exchange resin (Blorad) was used. The column was prepared with 15 mL of 5% HCl and then rinsed with 20 mL deionised water. The sample was diluted (1 mL to 25 mL) in a volumetric flask. 10 mL of diluted sample was pipetted onto column. After the sample passed through the column, 15 mL of 0.1 M NaCl was added. The eluant discarded. A 25 mL volumetric flask was placed under the column. 15 mL of 0.7 M NaCl was added to the column and the eluant was collected. The collected sample was diluted to 25 mL with distilled water, mixed and poured into large test tubes.

3. Preparation of standards and wade reagent

Phytic acid standards were prepared to contain 5, 10, 20, 30, 40 and 50  $\mu$ g/mL as shown below. They were stored in fridge and brought to room temperature prior to use.

 $50 \mu g/mL = 12.5 mL$  of SS in 50 mL volumetric flask

 $\mu$ g/mL = 10 mL of SS in 50 mL volumetric flask  $\mu$ g/mL = 7.5 mL of SS in 50 mL volumetric flask  $\mu$ g/mL = 5 mL of SS in 50 mL volumetric flask  $\mu$ g/mL = 2.5 mL of SS in 50 mL volumetric flask  $\mu$ g/mL = 1.25 mL of SS in 50 mL volumetric flask

4. Colorimetric test

Three mL of blank (distilled water), standards, and samples were pipetted into 15 mL glass tubes. Exactly 1 mL of Wade Reagent was added and the tube was covered with parafilm and mixed using a vortex mixer. The supernatant was pur into a cuvet and the absorbance read at 500 nm on a colorimeter using water to zero the instrument. Absorbance readings for samples and standards were subtracted from the blank reading to obtain the final reading. The final readings for the standard were plotted against the concentration to give a standard curve. Moisture determinations were run on all samples and the % phytic acid reported on dry matter basis.

5. Calculation

Concentration ( $\mu$ g/mL) was obtained form the equation based on the standard curve. The concentration as a % was calculated as shown below. Concentration as % =  $\mu$ g/mL ×10 mL/ (0.5×1000×1000) ×25×10×25

- **Appendix 5.** Procedure for Phenolic contents Determination using HPLC Method (Luthria and Pastor-Corrales, 2006).
  - Peas were ground to pass through a 0.825 mm sieve. Ground samples (0.5 g) were treated with methanol (7 mL) containing 10% acetic acid (85:15).
  - 2. The mixture was sonicated for 30 min and the volume of the extract was adjusted to 10 mL with distilled water.
  - A 1 mL aliquot of extract was filtered (through a 0.45 μm syringe filter) and analyzed for free phenolic acid content by HPLC.
  - 4. The remaining 9 mL of the extract were used for a sequential hydrolysis experiment with base followed by an acid. 10 mL of distilled water and 5 mL of 10 M NaOH containing 2% ascorbic acid and 13.4 mM (0.0134 M). EDTA was added to the extract. The mixture was flushed with nitrogen and stirred overnight (16 h) at ambient temperature.
  - 5. The pH of the extract was adjusted to 2 by drop wise addition of 6 N HCl.
  - 6. The liberated phenolic acids were extracted with diethyl ether-ethyl acetate (3x15 mL) by votexing and centrifuging (10000 xg for 10 min). The DE/EA extract was divided half and 7.5 mL diethyl ether-ethyl acetate were added to each portion.
  - 7. The combined diethyl ether-ethyl acetate layer was evaporated to dryness under rotary vacuum.
  - 8. The residue was re-dissolved 3x in 1.5 mL methanol: water (75:25% v/v) each time. The final volume was made up to 5 mL in a volumetric flask.

(Samples were then filtered through a 0.45  $\mu$ m syringe filter). These samples were analysed with HPLC.

- The aqueous part from the base hydrolysis fraction was further treated with
   2.5 mL of concentrated (12 N) HCl, flushed with nitrogen, and incubated in a water bath at 85 °C for 30 min.
- 10. The liberated phenolic acids were extracted with diethyl ether-ethyl acetate (3x15 mL) by votexing and centrifuging 10000 xg for 8 min). The DE/EA layers (supernatants) were combined. Note: Due to the size of the centrifuge tubes the aqueous portion was divided in half and 7.5 mL diethyl ether-ethyl acetate was added to each portion.
- 11. The combined diethyl ether-ethyl acetate layers were evaporated to dryness under rotary vacuum
- 12. The residue was re-dissolved 3 times in 1.5 mL methanol: water (75:25% v/v) each time. The final volume of the solubilised material was made up to 5 mL in a volumetric flask. Samples were then filtered through a 0.45  $\mu$ m syringe filter. The sample was then analysed by HPLC. Using mobile Phase A = 0.1% formic acid and mobile Phase B = 100% methanol.

**Appendix 6.** Procedure for Total Phenolics Determination (Singleton and Rossi, 1965) as modified by Ago, et al. (2002)

1. Reagents:

The following reagents were used:

Acidified methanol (HCI/methanol/water, 1:80:10, v/v)

Folin-Ciocalteau reagent (diluted 10 fold in distilled water just before use)

Sodium carbonate solution (60 g/L)

Ferulic acid

2. Preparation of Ferulic Standards

A stock solution was prepared by dissolving 200  $\mu$ g Ferulic acid per mL acidified methanol. Standards were prepared by diluting the stock solution according to the following scheme:

Cond.(µg/mL)	200	180	160	140	120	100	80	60	40	20	0
F.A (mL)	10	9	8	7	6	5	4	3	2	1	0
A.M (mL)	0	1	2	3	4	5	6	7	8	9	10

F.A : Ferulic acid

A.M : Acidified methanol

3. Sample Preparation

Ground samples (200 mg) were extracted with acidified methanol (4 mL) at room temperature for 2 hours on a wrist-action shaker. The mixture was centrifuged at 402xg for 10 min on a GLC -1 Sorval table centrifuge.

## 4. Measurement

The standards and samples were treated in preparation for spectrophotometric determination as follows:

The sample extract supernatant (0.2 mL) was added to 1.5 mL of freshly diluted (10-fold) Folin Ciocalteu reagent and mixed using a vortex mixer. The mixture was allowed to equilibrate for 5 min then 1.5 mL of sodium carbonate solution (60 g/L) was added and mixed using a vortex mixer. The mixture was incubated at room temperature for 90 min and then the absorbance was measured at 725 nm using acidified methanol as the blank.

Note:

a. All absorbance test replicate samples were run in duplicate.

b. For samples and standards, absorbance measured at exactly 90 min