

USE OF PEA (*Pisum sativum* L.) FLOURS AND FRACTIONS AS FOOD
INGREDIENTS: EFFECT ON TEXTURE, SENSORY RESULTS, ANTIOXIDANT
ACTIVITY AND TOTAL PHENOLIC CONTENT

by

Heather Maskus

A thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Food Science

University of Manitoba

Winnipeg, Manitoba

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Content**

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Abstract

The purpose of the research was to formulate food products such as tortillas and extruded, expanded snack food products using pea flour and pea fractions. Tortillas were made from 5 different formulations of pea flour, pea hull and wheat flour. Each was evaluated for diameter, thickness and rollability characteristics as well as colour, cohesiveness and firmness. Through optimization by response surface methodology for cohesiveness and firmness of tortillas, it was found that 27% pea flour and 5% pea hull from the Eclipse yellow pea variety and 26% pea flour and 4% pea hull from the Cooper green pea variety could be incorporated in tortillas without compromising texture based on results from the 2006 crop year. Through sensory testing, it was found that these optimized formulations scored a seven of nine on a nine point hedonic scale, similar results for the control wheat flour tortillas.

Pea flour, pea hull and pea starch were also used to make an expanded, extruded snack food product. By testing the shear strength, bulk density and expansion ratio of the final products and comparing the attributes to existing products on the market, it was apparent that a texturally acceptable product was created. Pea flour tended to have a positive effect on the texture of the extrudate (up to 50% pea flour addition which was tested). Processing temperature also had a significant effect on the expansion ratio and bulk density of the final product but did not affect shear strength. Of three samples tested in sensory evaluation, the sample which most closely represented ideal product characteristics was a 50% whole yellow pea flour sample without added hull and extruded at a final barrel temperature of 120°C as opposed to a 50% whole yellow pea

flour without added hull processed at 135°C or a 50% whole yellow pea flour with 10% added hull processed at 135°C.

The antioxidant activity of tortillas and extrudates made using pea flour and pea fractions was tested using the ABTS, DPPH and ORAC methods while total phenolic content was measured using the Folin-Ciocalteu method. A reduction in antioxidant activity and total phenolic content occurred in processed pea flour, pea hull and wheat flour tortillas. For extrudates, a reduction occurred in antioxidant activity and the total phenolic content when using the ABTS and Folin-Ciocalteu methods respectively while the ORAC method indicated that the antioxidant activity increased with processing. These differences may have occurred due to the reaction of different antioxidants with different antioxidative capacity between each method. Temperature of processing likely had an impact on the results for antioxidant activity and total phenolic content. By incorporating pea flour and pea fractions in tortillas and extruded, expanded snack foods, pea producers will benefit from the use of peas in a value added food product while consumers will benefit from having a product using a nutritionally superior ingredient.

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LIST OF ABBREVIATIONS

ABTS	2,2'-AZINOBIS-(3-ETHYLBENZOTHAZOLINE-6-SULFONIC ACID)
AOA	ANTIOXIDANT ACTIVITY
AUC	AREA UNDER THE CURVE
BD	BULK DENSITY
BU	BRABENDER UNITS
CDC	CROP DEVELOPMENT CENTER
CIGI	CANADIAN INTERNATIONAL GRAIN INSTITUTE
CMC	CARBOXY-METHYL CELLULOSE
CRC	CEREAL RESEARCH CENTER
CWRS	CANADA WESTERN RED SPRING
DNA	DEOXYRIBONUCLEIC ACID
DPPH	2,2-DIPHENYL-1-PICRYLHYDRAZYL
ER	EXPANSION RATIO
FAB	FARINOGRAPH ABSORPTION
FAE	FERULIC ACID EQUIVALENTS
GAE	GALLIC ACID EQUIVALENTS
JAR	JUST ABOUT RIGHT
ORAC	OXYGEN RADICAL ABSORBANCE CAPACITY
ROS	REACTIVE OXYGEN SPECIES
SEM	SCANNING ELECTRON MICROSCOPY
TE	TROLOX EQUIVALENTS
TPC	TOTAL PHENOLIC CONTENT
USDA	UNITED STATES DEPARTMENT OF AGRICULTURE

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CHAPTER 1: INTRODUCTION

Canadian production of dry field peas (*Pisum sativum* L.) is responsible for 50% of the exported pea market (Agriculture and Agri-Food Canada, 2006). In the export market, Canadian peas are used equally for both human food and animal feed, those which remain in Canada are mostly used for animal feed with only about 10% of domestic peas used for food (Agriculture and Agri-Food Canada, 2005). More value may be added to this crop by increasing its use in domestic foods. The objective of this research was to formulate food products using pea flour and pea fractions as ingredients. Specifically, different concentrations of pea flour and pea fibre blended with wheat flour to make tortillas and pea flour, pea fibre and pea starch in different concentrations were used to make an expanded snack food product. The quality of the final products was assessed using texture analysis as well as through sensory analysis. Antioxidant activity and total phenolic content of the final products were determined and compared to the values for the blends used to make the products.

Use of peas in food products not only increases the value of the crop at the farm level but it will also provide products which are superior as compared to similar products which do not contain pea components. Peas are high in fibre and contain both insoluble and soluble fibre. Peas are high in protein, and contain the essential amino acid lysine, which is limited in cereal crops. Peas are also low in fat. On the market, there are a limited number of products which contain pea or pea fractions apart from soups. Some research efforts have used pea fractions as ingredients in the past. Some examples of products include pea protein gels, pasta-like products and the use of pea fibre in meat products.

The hypotheses for this experiment are as follows:

- As the concentration of pea flour in tortillas increases, it will interrupt the gluten network in the wheat based product and cause failures in the texture of tortillas at a critical point.
- The addition of pea fibre in tortillas will increase the absorption of water by the tortilla blend.
- The antioxidant activity of the composite pea flour tortillas will be higher for samples with additional pea fibre due to an increased concentration of antioxidant activity in the hull component of the peas.
- As the concentration of pea flour increases in extruded snack foods, the expansion of the products will decrease in comparison to control samples
- The increased level of pea fibre added to extrusion formulations will limit the air cell size in extruded snack foods.

CHAPTER 2: REVIEW OF LITERATURE

2.1. Canadian pea production and utilization

Of the production of dry field peas (*Pisum sativum* L.), Canada contributes 25% of the world supply and 50% of the exported pea market (Agriculture and Agri-Food Canada, 2006). Saskatchewan is the main grower of Canadian peas and accounts for 78% of national production (Agriculture and Agri-Food Canada, 2006). Ninety percent of the peas that remain in Canada are used for livestock feed (Agriculture and Agri-Food Canada, 2005) while Canadian peas on the export market are used equally for both animal feed and human food (Agriculture and Agri-Food Canada, 2005).

Several dry pea types are grown in Canada which include yellow, green, small yellow, maple and marrowfat (Agriculture and Agri-Food Canada, 2005). Cultivars may vary in size, shape, colour, leaf structure, maturity, yield, mildew resistance and cooking quality (Saskatchewan Pulse Growers' Association, 2005). Primary processing, which involves cleaning and sorting, makes up the majority of the pea processing industry, however, secondary processing, including splitting, canning, drying, milling or other processing applications (Agriculture and Agri-Food Canada, 2005) to make the commodity ready for consumer consumption, will continue to grow at a faster rate.

2.2. Composition of peas

The pea seed is composed of three parts, the seed coat, the cotyledon and the embryonic axis, representing respectively 10%, 89% and 1% of the whole seed depending on the variety (Dueñas et al., 2004). Peas are known for their beneficial nutritional profile, which is discussed in greater detail below.

2.2.1. Fat

Peas typically contain approximately 1.2% fat. The majority of this is made of polyunsaturated fatty acids representing 0.495% being mostly 18:2 and 18:3 (0.411g/100g and 0.084g/100g respectively). Monounsaturated fats are responsible for 0.242% of the whole seed, mostly being 18:1 while saturated fats make up 0.161% and is mostly 16:0 (0.125g/100g). (USDA, 2008)

2.2.2. Protein

Peas are composed of approximately 25% protein. The major amino acids present include glutamic acid, aspartic acid, arginine, lysine and leucine in concentrations of 4.196%, 2.896%, 2.188%, 1.772% and 1.760% respectively (USDA, 2008).

2.2.3. Fibre

According to the USDA Nutrient Data Laboratory (2008), peas contain approximately 26% total dietary fibre. Dalgetty and Baik (2003) found that insoluble dietary fibre in pea seed and pea flour was 11.3% and 5.3% respectively, while soluble dietary fibre was found to be 8.7% for both the pea seed and pea flour.

2.2.4 Carbohydrates

Carbohydrates are present in peas at a concentration of approximately 60% according to the USDA (2008). The most abundant carbohydrate in peas is starch, accounting for 43% of the whole seed with branched amylopectin (linear chains of

(1→4) α -D-glucose residues bonded with (1→6) α -linkages) making up 50-70% of the starch. The remainder of the starch is amylose, consisting of α -(1→4) linked with D-glucopyranosyl accounting for 33-49% (Ratnayake et al. 2002). Pea starch granules are generally oval shaped and 2-40 μ m in size (Ratnayake et al. 2002). It is possible to isolate pea starch using two methods; pin milling and wet milling. Pin milling, commercially the most common method, involves extensive particle size reduction followed by air classification. Wet milling, alternatively, utilizes a 0.02% sodium hydroxide alkaline treatment and repeated filtration through polypropylene screens (Ratnayake et al. 2002)

The swelling power and solubility of legume starches varies greatly with regard to. Gelatinization of pea starch begins at the hilum where the crystalline structure is disrupted, moving next to the central portion of the granule where B polymorphs are arranged causing swelling in the central part of the granule (Ratnayake et al. 2002). Gelatinization temperatures at onset, peak and final gelatinization stages of smooth pea starch are 55-61°C, 60-67.5°C and 75-80°C respectively (Ratnayake et al. 2002). These properties of pea starch contribute to the functionality of peas as a food ingredient.

2.2.5. Minerals

The ash value of field peas is typically 2.65%, representing the major minerals, potassium, phosphorous, magnesium, sodium, calcium and iron (981, 366, 115, 15, 55 and 4.43 mg/100g pea respectively) (USDA, 2008).

2.2.6. Antioxidants

The determination of antioxidant activity is important because it relates to the scavenging of reactive oxygen species (ROS) which are responsible for the oxidative degeneration of tissues such as proteins, lipids and DNA (Wu et al., 2004). Some common antioxidant compounds include include *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, *p*-coumaric acid, ferulic acid and apigenin-8C-glucoside (Dueñas et al., 2004, Lopez-Amores et al., 2006 and Troszyńska et al., 2002). Depending on the pea variety as well as environmental conditions, concentrations of these compounds may differ. For instance, protocatechuic acid was found in concentrations of 2.77 µg/g and 19.82 µg/g in the cotyledon of ZP-849 and Fidelia varieties respectively while the pea hull of these varieties contained 50.15 µg/g and 76.99 µg/g for ZP-849 and Fidelia variety peas respectively (Dueñas et al., 2004).

The seed coat is generally rich in antioxidants, likely to ward off oxidative stresses such as those from ultra violet light and heat (Troszyńska et al., 2006). There are many methods that may be used to measure antioxidant activity and four will be discussed, including oxygen radical absorbance capacity (ORAC), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) as well as a method to determine total phenolic content (TPC). Although the methods essentially measure antioxidant activity (AOA) of the samples (with the exception of the total phenolic method), the use of different radicals as well as extraction solvents, preparation methods and experimental conditions can cause variations between the results of the methods.

Although some data exist on the antioxidant and total phenolic potential of peas, information is lacking on the effects of processing antioxidants in products utilizing peas as an ingredient.

2.2.6.1. DPPH

The DPPH method is based around the method established by Brand-Williams et al. (1995). Antioxidants are permitted to react with the free radical, DPPH. As the free radical is reduced, the DPPH chromagen loses its absorption at 515nm as the odd nitrogen electron is reduced by the reception of a hydrogen atom from the antioxidant present. The loss of absorption can then be related to the ability of the compound being tested to retain the absorption of the free radical as it is protected from oxidation.

A good antioxidant is one which is capable of quickly donating the H^+ atom causing a rapid decrease in the absorbance of DPPH chromagen. With the exception of being in the presence of some Lewis bases, solvent types and oxygen, DPPH is a relatively stable free radical (Molyneux, 2004).

The unpaired electron is delocalized over the entire DPPH molecule, causing a deep violet colour and preventing dimerization, which would normally occur in the case of other free radicals. With reduction of DPPH to $DPPH^+$, the violet colour is lost and yellow colour remains due to the presence of the remaining picryl group (Ozcelik et al., 2003). In the reaction, the DPPH molecule is intended to represent free radicals formed whose activity is to be hindered by the antioxidant.

DPPH can be used in aqueous and non polar solvents in order to analyze both hydrophilic and lipophilic antioxidants. Lipophilic antioxidants can be determined

without the use of solubilizing agents such as β -cyclodextrin which is used in the ORAC method. β -Cyclodextrin has been shown to have strong interference in $\text{HO}^\bullet$ scavenging capacity estimation (Cheng et al., 2006).

Pea varieties, when analyzed for their antioxidant activity using the DPPH method ranged from 0.03-2.75 μmol trolox equivalents (TE)/g and 0.01-1.28 μmol TE/g for yellow and green peas respectively (Xu and Chang, 2007). Different extraction solvents played a significant role in the antioxidant analysis, 70% methanol and 80% acetone yielded the highest AOA values for yellow and green peas respectively (Xu and Chang, 2007). Xu et al., (2007) similarly found that the AOA of yellow and green peas using the DPPH method was 1.95 $\mu\text{molTE/g}$ and 1.53 $\mu\text{molTE/g}$ respectively with no significant difference between the types of pea.

2.2.6.2 ABTS

Re et al. (1999) used 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) or ABTS to produce a blue green chromophore through a reaction of ABTS with potassium persulfate. Absorption maximum is measured at 645, 734 or 815 nm through the addition of an antioxidant to the pre-formed radical cation a reduction of ABTS occurs causing a loss of blue green colour. Therefore the degree to which the decolourization of the ABTS^+ free radical colour is inhibited may be determined as a function of concentration and time. This value is then related to the reactivity of the antioxidant capacity of Trolox which is used as a standard under the same conditions. AOA values for peas using the ABTS method is lacking in literature.

2.2.6.3 ORAC

ORAC is a high throughput method for the determination of AOA. During the radical-antioxidant reaction, fluorescent intensity is measured. A fluorescent probe, fluorescein, is used as the free radical because it has a specific end point and as it is oxidized, will lose its fluorescence. This measures the peroxyl radical scavenging capacities of specific antioxidants. The difference between the area under the curve (AUC) for the reference sample and the test sample is taken and the measurement is recorded in ORAC units ($\mu\text{mol TE/g}$). (Cheng et al., 2006)

The data compiled by the USDA ORAC database (2007) indicates AOA for dry peas. This source indicated that the hydrophilic and total ORAC values for “peas, split mature seeds raw” and “peas, yellow, mature seeds, raw” have a hydrophilic as well as total ORAC values of 524 $\mu\text{molTE/100g}$ and 741 $\mu\text{molTE/100g}$ respectively. Higher values for “peas, yellow, mature seeds, raw” are likely due to the presence of the seed coat, which has higher AOA as indicated previously.

2.2.6.4 Total Phenolic Content

Both the seed coat and the cotyledon of peas are rich in phenolics. Generally, a high concentration of phenolics is associated with a high AOA (Dueñas et al., 2004). Phenolic AOA is generally dependant on their structure, polyphenols, for instance tend to have greater antioxidant capacity than monophenols (Troszyńska et al., 2006).

Yellow and green peas demonstrated total phenolic concentrations as indicated by Xu and Chang (2007) of 1.4 mg gallic acid equivalents (GAE)/g when extracted with 70% methanol. The USDA (2007) obtained similar results and found that peas

contained approximately 80mg GAE/100g depending on variety. The structure of phenolics greatly affects their antioxidant capacity, flavonoids tend to have greater AOA when compared to non-flavonoids (Lopez-Amores et al., 2006).

However, AOA cannot be directly correlated to the TPC of the sample considering that different phenolic compounds exhibit differences in their AOA as explained previously. Also, many factors tend to alter free radical scavenging ability of an antioxidant, including interactions with other endogenous compounds, the nature of the free radical and the substrate requiring protection by the antioxidant (Del Pozo-Insfran et al., 2006).

2.2.6.5 Effect of processing on antioxidant activity and total phenolic content

Depending on the material evaluated, both increases and decreases in AOA and TPC have been observed following thermal processing. Muffins analyzed by Li et al. (2007) using purple wheat bran exhibited a decrease in total phenolics from the raw material to the baked state. Specifically, methanol extracts of the purple wheat bran, heat treated purple wheat bran, the purple wheat bran muffin and wheat bran muffin resulted in a TPC of 3.34, 3.68, 0.26 and 0.35 mg ferulic acid equivalents (FAE)/g respectively. It is likely that these reductions were the result of diluting the raw flour with other ingredients as well as due to the binding or damage of aromatic structures of the phenolics when subjected to high temperature processing as was observed by Granito et al. (2007) when assessing the cause of the reduction on TPC of beans from 1917mg/100g to 854mg/100g following thermal processing.

AOA also changed following thermal processing. It was found that after processing of beans, AOA was reduced from 51.71 g/100g to 18.86 g/100g (Granito et al., 2007). However, Wu et al. (2004) concluded that the effect of processing on AOA was dependent on the vegetable studied and that the most thermally resistant foods tended to include those which contained active polyphenolic flavonoids rather than vitamins. Randhir et al. (2007) also supported this notion; stating that where increases in AOA occurred, there was likely a synergistic effect of other phytochemicals and changes in the structures of thermally altered phenolics.

2.3. Canadian consumption of dry peas

Canadian consumption of field peas in Canada in 2007 was 1.22kg/person (Statistics Canada, 2007). This amount has increased slightly since 1981, where consumption was 0.94kg/person, but the value has remained stable for the past 7 years (Statistics Canada, 2007). Some of the barriers to increased consumption of field peas are due to the inconvenience to cooking peas as well as antinutritional components which are present in peas, such as phytic acid.

2.3.1. Antinutritional factors

Phytic acid is a natural antinutritional substance found in peas and other pulses. It is the hexaphosphoric ester of cyclohexane (inositol hexaphosphoric acid) (Febles et al., 2002). Capable of complexing with minerals (divalent cations) and proteins, it is undesirable due to its ability to limit absorption of these components in the body. However, phytic acid has also been recognized for its abilities as an antioxidant,

capable of stopping free radical generation in the body which has been linked to degenerative diseases, coronary heart disease and cancer (Febles et al., 2002). In raw pea seeds, phytic acid has been found in concentrations of 1.19-1.33 g/100g (Alonso et al., 1998) and is characteristically found in the cotyledon of seeds (Alonso et al., 1998).

Alonso et al. (2000) found that extrusion significantly reduced phytic acid, condensed tannins and polyphenols.

2.3.2. Cooking time

A major barrier to the consumption of peas in North America involves their long cooking times and the need to pre-soak peas before use. In order to overcome this obstacle, milling of dry field peas into flour for convenient incorporation of the pea flour in commonly consumed products, such as tortillas or extruded, expanded snack foods is recommended. In order to mill peas into flour, they first must be cleaned, tempered, pitted, split, and separated into their hull and cotyledon fractions. However, development of suitable product formulations is necessary in order to incorporate a functional amount of pea flour in products. Rasper (1976) found that when ground legumes were used in bread, the gas retention of the dough was inadequate. Pea fibre has also been used in breads. Dalgetty and Baik (2006) incorporated pea hulls in bread at levels of 3, 5 and 7%, and the results indicated that levels of 5% could be utilized without altering bread texture. This study will investigate the feasibility of incorporating pea flour, pea hull and pea starch in extruded, expanded snack foods as well as the use of pea flour and hull in composite blends in wheat flour tortillas. This approach is based on the fact that tortillas are unleavened bread and will be able to

incorporate a greater percent of pea considering that the tortilla will not be as greatly affected by the lack of ability of peas to retain air cells in the tortilla.

2.4. Tortillas

A flour tortilla is defined as a round, chemically leavened flat bread which is produced from gluten structured dough (Serna-Saldivar et al., 2004)

2.4.1. Consumption data of tortillas

Over a span of four years, 2000 to 2004, the growth rate of tortilla sales in the United States has been 57%. Being the second highest selling product in the packaged bread category, United States wholesale prices for tortillas (corn and wheat) in 2004 were greater than four billion dollars (Serna-Saldivar et al., 2004). In Canada, tortilla sales have contributed to increasing consumption of cereals by 23% from the early 1990's to the late 1990's (Statistics Canada, 2007). Wheat tortillas were preferred to corn tortillas at a ratio of 2:1 (wheat:corn) by the average American consumer and are consumed in an amount of 4.5 billion pounds of wheat flour tortillas per year (Serna-Saldivar et al., 2004).

2.4.2. Factors affecting tortilla quality

Desirable wheat flour tortillas are characterized by a soft, flexible crumb structure which does not crack when folded. It should be light coloured, opaque and well puffed (Pascut et al., 2004). In order to achieve these properties, interactions among many different factors must be balanced. These will be further discussed.

2.4.2.1. Farinograph absorption/Water addition

In tortilla formulation, the amount of water added to the flour to make dough is directly related to the farinograph absorption (FAB) of the flour. In a study by Srinivasan et al. (2000), flour with an FAB of 62.6 for 500 Brabender Units (BU) was made into tortillas using 3 levels of water 50.5% (low), 52.5% (normal) and 54.0% (high). It was observed that with decreased water levels, the tortilla properties of diameter and thickness were unchanged; however, rollability suffered and was inferior to the control. Thicker, smaller diameter tortillas resulted when an increased level of water was used. These tortillas also had similar or better rollability as compared to the control due likely to the plasticizing effect of wheat protein by the water to improve fluid like properties (Srinivasan et al., 2000). When using triticale composite flour blends, Serna-Saldivar et al. (2004) found that the composite flour reduced the optimum water absorption of the flour. Because the use of composite flour of pea will significantly alter the absorption of water, it is necessary to test different FAB values for the tortillas (Srinivasan et al., 1999)

2.4.2.2. Mixing Time

Mixing time of dough also has an effect on the quality of tortillas. Under (3 min) and over mixing (11 min) of dough resulted in tortillas with lower hardness and resilience as compared to control dough tortillas (7 min) (Serna-Saldivar et al., 2004). Where tortillas made from undermixed doughs had smaller diameter and shorter shelf life compared with the control, overmixed doughs yielded thinner tortillas, larger in diameter with a shorter shelf life. (Serna-Saldivar et al. 2004) Again, the use of a

composite flour will affect mix times, as mixing is a function of protein development. Serna-Saldivar et al. (2004) observed that triticale composite flours resulted in a shorter mix time than for wheat alone.

2.4.2.3. Fibre

Both soluble and insoluble fibres in tortilla/pea composite flour appear to affect the final quality of tortillas but by different mechanisms. Using 8% soluble fibre, resulting tortillas were characterized by a dense, pasty crumb (Seetharaman et al., 1997). This is a result of insufficient gluten development as well as extensive starch gelatinization. Other effects of using increased levels of soluble fibre (carboxy-methyl cellulose (CMC) in this case) included longer mixing times and also, the dough was not as manageable for the dough mixing/tortilla forming equipment (Seetharaman et al., 1997). Using scanning electron microscopy (SEM), it was observed that at 8% soluble fibre, a smooth film consisting of CMC and protein covered the starch granules (Seetharaman et al., 1997). This resulted in long, wide gaps and indicated poor gluten development due to a lack of interconnected strands within the protein matrix (Seetharaman et al., 1997). Fully gelatinized starch granules were present in the tortilla following baking. Starch gelatinization was also affected by soluble fibre levels in tortillas. Due to greater moisture retention at the surface when soluble fibre was present, the starch was near total gelatinization at the surface where, in control tortillas, starch on the surface retained its birefringence. This was due to a low availability of water as well as the heat and environmental conditions to which the tortillas were subjected (Seetharaman et al., 1997).

Insoluble fibre also had an effect on tortillas. In this case, dough containing 8% insoluble fibre was observed to have a thin film of protein matrix enveloping starch and fibre particles (Seetharaman et al., 1997). Overall, it appears as though the gluten structure, associated with additional insoluble fibre had a stronger gluten structure, however, the gluten network was physically disrupted by insoluble fibre particles causing the collapse of air bubbles and decreasing shelf stability (Seetharaman et al., 1997). Air bubbles and channels present were smaller, contributing to the dense crumb of the tortilla. Larger diameters, higher moisture contents and shorter shelf life were characteristic of tortillas containing insoluble fibre (Seetharaman et al., 1997).

2.4.2.4. Protein

Wheat protein, in particular, gluten, is developed during mixing. Gluten forms as an elastic, thin, continuous film covering starch granules which holds them in place (McDonough et al., 1996). This creates a cohesive network within the dough. When tortilla dough is hot pressed, surfaces are dehydrated and the gluten/starch matrix shrinks causing the formation of a semi-continuous surface (McDonough et al., 1996). This allows the tortilla to puff and expand when steam and leaving gases are formed. Although not air tight, the starch/protein network captures heated air and is capable of retaining moisture for a brief period following baking (McDonough et al., 1996).

Generally, hard red winter wheat flour is used for the commercial production of flour (Pascut et al., 2004). Typically, this is bread quality flour with moderate to strong protein quality and dough strength. When using weak protein flours for tortilla production, wheat proteins with different functionality may help to improve the texture

of the end product (Pascut et al., 2004). By using flour with a higher protein quality, or through the addition of vital wheat gluten, the eventual breaking of tortillas due to staling may be delayed (Pascut et al., 2004).

When gluten was used to enhance tortillas, the product was thicker, smaller in diameter and had better rollability during storage as compared to control tortillas (Srinivasan et al., 1999). Following fractionation and reconstitution of wheat proteins from wheat flour, it was found that gluten is mainly responsible for the variation that different cultivars of wheat contribute to baking quality (Uthayakumaran and Lukow, 2005).

2.4.3. Determining tortilla quality

The evaluation of textural properties of tortillas is important to determine the acceptability of the final product. Instrumental methods may be used initial to determine an optimized formulation for the product. Final acceptability may be determined with a sensory test.

2.4.3.1. Rollability

Rollability of tortillas is a good indication of the textural quality of tortillas. Rolling of tortillas around a cylindrical dowel will give an indication of its ability to be rolled as when it is used. The response of the tortilla can be assessed on a 6 point scale (1-no signs of cracking, 2-edge cracking only, 3-edge cracking and/or cracking in the center, 4-cracking and breaking on one side, 5-cracking and breaking on both sides (clean break) but still rollable, 6-unrollable).

Srinivasan et al. (2000) determined rollability of wheat flour tortillas varying in mixing times, and temperature of dough mixing during shelf life storage on a 5 point scale (1 being unrollable to 5 being no signs of cracking or breaking). It was found that all tortillas initially had a perfect score of 5 and that after 15 days of storage, the dough mixed at a lower temperature (30°C) was the first of the samples to become unrollable.

2.4.3.2. Physical Characteristics

In the human eye, colour is perceived three dimensionally from the response of red, blue and green receptors. When quantifying the colour of food, the CIELAB L*, a* and b* values are used on a scale to measure lightness (L*), red (+a*), green, (-a*), yellow (-b*) and blue (+b*). In order to relate these values more closely to human perception, a* and b* values may be converted to hue and chroma values (Berrios et al., 2004).

Colour of tortillas based on the L*, a* and b* values can be used to determine the uniformity of the samples and is useful in characterizing the attributes of the tortillas especially after reformulation, changes in processing or throughout shelf life studies. Diameter and thickness of tortillas may also be used to characterize the final product. As indicated earlier, limited water availability, particularly when novel ingredients are included, can result in smaller diameter, thicker tortillas (Srinivasan et al., 2000)

2.4.3.3. Texture

The texture of a food influences how a food is processed and handled. It also plays a major role in food habits and is a determining factor with regard to shelf life and consumer acceptance. Instrumental methods are convenient to use to test texture considering that the test can be done under controlled conditions and with more strictly defined parameters (Stable Micro Systems, 2000).

In a compression test, each sample used must be identical in terms of size and shape in order to obtain repeatable measurements. In a penetration test, the probe is generally smaller and is more sensitive to deviations in the product structure, however, using a larger probe, the larger the surface area being tested and therefore, the lower the sensitivity of the probe to the sample surface (Stable Micro Systems, 2000).

2.4.3.3.1. Cohesiveness (area under the curve)

Cohesiveness is evaluated as the positive AUC (force (g)*time(s)) and is commonly a parameter used to describe meat, fish and poultry but has also been used to describe baked products such as tortillas, pancakes or pizza crust (Stable Micro Systems, 2000). A larger AUC indicates greater work to perform the test and therefore greater energy to complete the task (Stable Micro Systems, 2000). Serna-Saldivar et al. (2004), measured tortilla extensibility using the strips of tortillas (35×75mm). Grips held the tortilla strips 30mm apart and the strip was extended at 1.0mm/sec to determine the work during extension of wheat flour and triticale flour tortillas. It was found that as triticale was added to the flour, work required during extensibility was reduced from 1,561 g×mm for 100% wheat to 441 g×mm for 100% triticale tortillas.

2.4.3.3.2. Firmness (peak force)

Hardness or firmness of a sample may be assessed through the use of a penetration/puncture test. The probe is made to penetrate the sample and the force (g) required to reach a determined depth or time under defined conditions is measured. The greater the force to break the sample, the more resistant the material and therefore, the firmer the sample (Stable Micro Systems, 2000). Using a triticale blend, it was found that as the level of triticale increased from 100% wheat to 100% triticale, the force to rupture tortillas decreased from 54.3g to 11.2g respectively (Serna-Saldivar et al., 2004).

Uthayakumaran and Lukow (2005) determined that for wheat tortillas made from different cultivars which varied in their glutenin-to-gliadin ratio as well as their protein content, force to break in a penetration test as measured by a TA.XT2 texture analyzer ranged from 914.0g to 1571.9g.

2.4.3.4. Sensory Evaluation

Although instrumental texture tests indicate quantitative values of defined parameters, they are not indicative of the level of consumer acceptance of a final product. There are several reasons to correlate instrumental texture measurements with sensory evaluation; 1) quality control, 2) consumer response predictions, 3) determining sensory sensations perceived by experimental outcome 4) to develop instrumental methods to simulate sensory responses (Stable Micro Systems, 2000).

By observing reaction to food traits based on sight, smell, taste, touch and hearing, sensory evaluation is a scientific method capable of evoking, measuring, analyzing and interpreting those consumer responses to determine sentiment about a

final product (Stone and Sidel, 2004). A hedonic scale is a method that can be readily applied to consumer testing.

2.4.3.4.1. Consumer acceptance nine point hedonic scale

In the 1940's, the nine point hedonic scale was first used by the Food Research Division in Quartermaster Food and Container Institute of Chicago (Stone and Sidel, 2004). The basis of the hedonic scale is to determine consumer preference or acceptance through a continuous scale of like and dislike statements. Although panellists tend to avoid selecting extremes of the scale, the method has proven to be reliable, with reproducibility of responses among different groups of people who form the sensory panel (Stone and Sidel, 2004). The tests are also easily understood from the panellist perspective, with little instruction necessary to complete the evaluation (Stone and Sidel, 2004).

The performance of specific attributes of a product such as appearance, flavour and texture can also be measured in a sensory evaluation and compared to a control sample through liking ratings and preference (Maskowitz et al., 2006). Results may be further examined by correlating consumer responses to other forms of analysis such as physical or chemical analyses or other, more descriptive, sensory analyses (Maskowitz et al., 2006).

A substitution of 50% triticale for wheat in tortillas was found to be acceptable without affecting texture, colour, flavour and overall acceptability (Serna-Saldivar et al., 2004).

2.5. Extrusion

Extrusion processing is a commonly used method of making foods, in particular snack foods. It is a thermal process which combines shear forces to create a change in the structure of food materials (Thakur and Saxena, 2000). Gelatinization of starch and the denaturation of protein are some of the changes that extrusion may induce (Suknark et al., 1997, Wang et al., 1999). The amino acid lysine, which is present in peas, may also undergo Maillard browning during extrusion cooking of a pea blend (Alonso et al., 2000, Athar et al., 2006, Wang et al., 1999). Expanded extrusion processing has been done with peas and is under the patent application process (Berrios et al., 2008). Extrusion of split and whole dry field peas were puffed using the addition of sodium bicarbonate and high amylase corn starch using eight barrel sections with a temperature profile of no heat added, 60°C, 80°C, 100°C, 100°C, 120°C, 140°C and 160°C for each of the respective zones. Screw speed was set at 500 rpm with a feed rate of 25 kg/h and a die diameter outlet of 3.5mm (Berrios et al., 2008).

Brittle foams produced from starch are commonly used to make snack foods using extrusion processing. By metering the feed material through a temperature and pressure gradient and eventually through a die, water becomes heated and flashes off upon exiting the apparatus as the pressure drops leaving the aerated structure. By altering parameters and blend formulations, bubble nucleation and vapour diffusion will change the characteristics of the foam structures (Hutchinson and Siodlak, 1987).

2.5.1. Consumption data of snack foods

Pepsi-Co (2003) reported that in 2002 \$1.6 billion dollars worth of snack foods such as potato chips, tortilla chips, pretzels, popcorn, nuts and extruded snacks were consumed by Canadians. Pepsi-Co (2007), in their 2007 annual report, indicated their leading brand of puffed, extruded snacks had worldwide retail sales of about 3 billion dollars.

2.5.2. Factors affecting extrusion

Many factors will affect the final product generated by extrusion. These include the ingredient blend (moisture, protein, starch and fibre) as well as parameters defined by extrusion conditions such as screw speed, screw configuration, feed rate and barrel temperature.

2.5.2.1. Moisture

Moisture content affects many aspects of extrusion. An increase in moisture content will cause a reduction in viscosity, causing a reduction in torque and specific mechanical energy input as well as reducing the temperature of the outgoing product. The expansion ratio (ER) and specific length also tend to be lower with a higher moisture content, which also directly relates to an increase in bulk density (BD). As Chinnaswamy and Hanna (1988) indicated, when decreasing moisture content from 30% to 14%, an increase in the ER for corn starch extrudates was observed (from 7.5 to 14.2). Moisture content lower than 14% resulted in a limited expansion ratio for corn starch. At 14% moisture, expansion was likely due to restricted material flow which

increased the time the material was exposed to extrusion temperatures to encourage gelatinization, and increased shear rate of the material in the barrel. Very high shear rates occurring at very low moisture content caused much greater increases in temperature and residence times which results in starch degradation and dextrinization and reduced expansion potential (Chinnaswamy and Hanna, 1988).

2.5.2.2. Protein

Typically, protein added to a starch based material during extrusion, interferes with the components necessary for expansion (Falcone and Phillips, 1988)

2.5.2.3. Starch

The two major components of starch are amylose and amylopectin. In terms of expanded extruded products, amylopectin has a positive effect, due likely to the branched nature of the polymer, while amylose has a more negative functionality in terms of expanded extrusion, due likely to the alignment of linear chains preventing the formation of bubble nucleation (Falcone and Phillips 1988).

Starch gelatinization determines the degree to which a product is capable of expanding and is dependent on temperature, shear rate and moisture content of the feed material. Degradation of starch will occur if the temperature of extrusion is too high and consequently ER will be reduced (Chinnaswamy and Hanna, 1988).

2.5.2.4. Fibre

As the concentration of bran is increased, the average air cell size decreases while the number of air cells increases yielding a denser extrudate structure. It was found by Jin et al. (1995) that with the incorporation of 30% fibre in extrudates, cell walls tended to be thicker than at 10% fibre addition. Overall, bran used in expansion extrusion limits the formation of large air cells due to its nature of interference in the starch network. This causes the air cell walls to be weaker and incapable of capturing steam during the flashing off process before the extrudate is able to harden to a brittle foam structure (Jin et al., 1995).

2.5.2.5. Screw Speed

Starch has been extruded using a moisture content of 14% (db) at screw speeds of 75 to 190 rpm and feed rates ranging from 15 to 170 g/min with a barrel temperature of 140°C (Jin et al., 1995). The optimized parameters for expansion were found to be 150 rpm with a 60g/min feed rate. At high screw speeds, it is thought that there was a lower level of starch gelatinization due to shorter residence time thus reducing expansion (Jin et al., 1995). At lower screw speeds, it is thought that molecular degradation of starch occurred due to an increase in residence time, rendering the starch incapable of expansion (Jin et al., 1995). Thinner cell walls as well as larger air cell sizes were observed at lower screw speeds (Jin et al., 1995). Mechanical damage to food molecules, which are less cohesive than gelatinized, undamaged starch, also increased with higher screw speeds due to an increase in shear rate (Jin et al., 1995).

With regard to extrudate expansion, screw speed was not a significant factor, however it significantly changed the internal structure of the extrudate (Jin et al., 1995).

2.5.2.7. Screw Configuration

Screws are divided into three sections; feed, transition (compression) and metering. Each section is composed of many different screw segments. In the transition section, mixing of the ingredients occurs and the feed is worked into continuous dough from its original loose, flour like state direction of the discharge in the presence of heat provided and produced by frictional energy. The metering section hosts a rapid increase in temperature of the material due to an increased shear rate. In order to further improve mixing, kneading disks are used, which increase the conversion of mechanical energy to heat. Therefore, by changing the configuration of each section by altering the screw segments present, it is possible to achieve a much different end product.

2.5.2.7. Feed Rate

A lower bulk density is achieved by increasing the feed rate because this will increase the ER as well due to increased viscosity and shear rate in the extruder barrel. However, too great a feed rate will overwhelm the apparatus and cause a backup in the extruder barrel.

2.5.2.8. Temperature

A decrease in product temperature is achieved by decreasing barrel temperature. This will lead to decreased dough temperature and higher dough viscosity which results in a higher torque and die pressure.

In a study done by Chinnaswamy and Hanna (1988), barrel temperature was increased from 110°C to 140°C. Initially, ER increased from 11.5 to 13.2, however, temperature greater than 140°C resulted in a decreased expansion ratio to 10.2. The temperature used for extrusion will depend on the material being extruded. Corn grits and starch were found to have the best extrusion properties at a temperature of 170-200°C.

2.5.3. Determining extrudate quality

Texture is a major determinant of overall quality for snack foods. The microstructure of food is a result of both physical and chemical factors and defines the texture of the product (Jin et al., 1995). Texture can be characterized by shear strength, ER and BD.

2.5.3.1. Shear Strength

Shear strength is measured as the shear force required to break a product relative to its cross sectional area and is typically indirectly related to the expansion volume. Therefore the greater the expansion volume, the lower the shear strength (Chinnaswamy and Hanna, 1988). The strength of extrudates relates to the cell wall thickness of air pockets, thicker walls require more force to break (Jin et al., 1995).

2.5.3.2. Bulk Density

Unlike expansion ratio, bulk density accounts for expansion of the product in all directions (Falcone and Phillips, 1988) and is measured as mass of product residing in a specified unit of volume (Obatolu et al., 2006).

2.5.3.3. Expansion ratio

ER accounts for expansion of the extrudate in a perpendicular direction and is measured as the cross sectional area of the extrudate divided by the cross sectional area of the die hole (Obatolu et al., 2006). Expansion of material depends on processing material used as well as the processing conditions. The expansion of a product relates to the texture of the product, for instance, crispness (Chinnaswamy and Hanna, 1988). Berrios et al. (2008) were able to obtain expansion ratios of legumes ranging from 1.38 for garbonzo beans to 24.15 for split pea formulated with a leavening agent and Hylon V corn starch.

2.5.4. Sensory Evaluation Just About Right Scale

A scale method is used in the Just-About-Right (JAR) test to analyze the desirability of specified characteristics of the product. The test provides directional information with regard to optimization or reformulation of the product through a combination of intensity testing and hedonic scaling. The JAR test assumes panellist familiarity of characteristics for the product. Centering bias is another problem which may occur with this test when multiple samples of varying intensity are tested. The tendency of the panellist is to place the sample with intermediate intensity in the “Just

Right” position. It is important to prevent the compounding of intensity results with hedonic scores and therefore, an alternative method to JAR should be used to for directional reformulation data (Lawless and Heymann, 1998).

The consumer texture profile technique as described by Szczesniak et al. (1975) modifies the JAR test by separating the ideal characteristics from the actual sample intensity characteristics. The scales and attributes tested are identical. Approximately 30 respondents appear to be adequate to generate reliable results. The method remains useful for determining directional information on the reformulation of the product however, it may be impossible, physically and technologically, to provide consumers with their ideal product (Szczesniak et al., 1975). This method was initially used to evaluate the textures of puddings and cereals but may be readily applied to other foods requiring texture analysis.

Using a lentil base, it was determined through sensory evaluation that samples with an ER of 8.75 to 10.24 were most optimal for a continued hedonic sensory analysis (Berrios et al., 2008).

CHAPTER 3: PHYSICAL CHARACTERISTICS AND SENSORY EVALUATION OF COMPOSITE PEA AND WHEAT FLOUR TORTILLAS

3.1 Abstract

Composite pea and wheat flour tortillas were made using 5 different combinations of pea flour, pea hull and wheat flour. Tortillas were evaluated for their physical characteristics including diameter, thickness, rollability, colour (a^* , b^* , L^* and greenness), cohesiveness and firmness. Using response surface methodology, it was found that an optimized formulation to maximize pea flour and pea hull inclusion based on the cohesiveness and firmness values obtained through TA.XT2 texture analysis was 27% pea flour and 5% pea hull for the yellow pea (Eclipse) and 26% flour and 4% hull for the green pea (Cooper). Verification of the optimized formulation through sensory evaluation of the appearance, flavour, texture, overall acceptability and intent to purchase indicated that pea flour tortilla formulations were similar to the scores obtained for the control tortilla and were generally around a score of 7 or like moderately on a 9 point hedonic scale. These results indicate the potential for the inclusion pea flour in a tortilla product without affecting the quality of tortilla beyond consumer acceptability thereby increasing the consumption of peas in North America by their inclusion in a popular product such as tortillas.

3.2 Introduction

Dry field peas (*Pisum sativum* L.) are of considerable value to the Canadian economy. However, the consumption of this crop is low in North America. Canadians consume approximately 1.22kg of peas per person in a year, an amount that has

remained relatively stagnant over the past 7 years (Statistics Canada, 2007), despite a protein content of about 25% and a fibre level of another 15% (USDA, 2008). Peas also contain many minerals including potassium, phosphorous, magnesium, calcium and iron. Total ash content of peas is generally 2.6% (USDA, 2008). The consumption plateau of peas may be accounted for by a lack of preparation convenience, especially the need to pre-soak. This may be resolved by milling peas into flour and using it to replace a portion of wheat flour in commonly consumed products.

Tortillas, on the other hand, are increasing in popularity among North Americans and have been characterized the fastest growing sector of the baking industry (Ames et al., 2003). There has been increased use of tortillas in the United States (Holt et al., 1992) with growth from 1997 to 2000 increasing from two to four billion dollars (Srinivasan et al., 2000 and Serna-Saldivar et al., 2004). Potentially, the use of a wheat/pea composite flour blend to make tortillas could increase the consumption of peas in North America and provide a nutritional advantage to consumers as compared to a wheat tortilla counterpart. However, in doing this, tortillas must be capable of maintaining their properties which are necessary for high quality products.

By definition, tortillas are flatbread, chemically leavened and structurally based on gluten dough (Serna-Saldivar et al., 2004). Commercially, they are made using hard red winter wheat to produce moderately strong dough (Pascut et al., 2004). In terms of quality, tortillas are characterized by a soft, non-sticky, pliable texture which folds easily without cracking or breaking (Ames et al., 2003, Pascut et al., 2004 and Bejosano et al., 2005). Ideally, they are also puffed in appearance, opaque and light coloured

(Pascut et al., 2004 and Bejosano et al., 2005). The puffed nature of a tortilla is dependent on the ability of the gluten network to retain steam generated by heat and leavening agents (McDonough et al., 1996). Tortilla texture is directly indicative of quality and can be assessed through both instrumental methods as well as sensory evaluation (Stable Micro Systems, 2000).

Assessment of tortilla texture may be done using instrumental methods such as a penetration test where the force necessary to puncture the tortilla in a given time is used as an index of hardness (Stable Micro Systems, 2000) and work to break the tortilla as a function of force and time may be used to indicate the cohesiveness of the tortilla (Stable Micro Systems, 2000). This method uses both shear and compressive forces generated through penetration to characterize tortillas (Stable Micro Systems, 2000). A subjective, rollability test may also be used to characterize tortillas. This method has been used on either a 5 or 6 point scale as demonstrated by Srinivasan et al. (2000) and Bejosano et al., (2005).

Tortilla quality will change greatly with the use of inclusions such as pea flour. The effect of adding pea flour to tortillas has not been previously studied. However, other additives have been used in tortillas. These include the replacement of 24% cowpea flour or 46% peanut flour for wheat flour in flour tortillas (Holt et al., 1992), wheat flour tortillas made incorporating fractions of soluble or insoluble dietary fibre (up to 8%) (Seetharaman et al., 1997), maize and bean tortillas (Mora-Avilés et al., 2007), wheat and triticale tortilla blends (Serna-Saldivar et al., 2004), nixtamalized waxy barley tortillas (Ames et al., 2003) as well as 70% maize and 30% soybean tortillas with added sugar and salt (Obatolu et al., 2007). These inclusions affect the

diameter, thickness, rollability, diameter, colour, objective and subjective texture measurements of tortillas.

Sensory analysis may be used to assess the acceptability of the final product using a nine point hedonic scale. The basis of the hedonic scale is to determine consumer preference or acceptance through a continuous scale of like and dislike statements. Although panellists tend to avoid selecting extremes of the scale, the method has proven itself to be reliable with reproducibility or responses among different groups of people who form the sensory panel (Stone and Sidel, 2004). The tests are also easily understood from the panellist perspective with little instruction necessary to complete (Stone and Sidel, 2004). Response surface methodology is a useful tool to optimize wheat pea composite tortillas to manage with sensory evaluation (Holt et al., 1992).

The objective of this experiment was to develop a tortilla made from a blend of wheat and pea flour with acceptable textural properties. This is to be done through mechanical testing of tortillas made of various pea/wheat composite blends, response surface methodology to determine an optimal blend and a sensory analysis to verify the optimized tortilla formulation.

3.3 Materials and Methods

3.3.1 Pea composite flours

Tom Warkentin of the Crop Development Center (CDC) in Saskatoon, Saskatchewan, Canada supplied four varieties of whole yellow peas; Alfetta, Eclipse, SW Midas and CDC Mozart, as well as two varieties of whole green peas; Cooper and

Camry. Peas were harvested from the 2005 and 2006 crop year at 3 growing locations: Davidson, Indian Head and Rosthern Saskatchewan. The climatic data for these growing years and regions may be seen in Appendix A. Using the Canadian International Grain Institute (CIGI) special crops method, peas were split, dehulled and milled to flour. Specifically, peas were weighed and analyzed for moisture content using a Seedburo® moisture meter (Seedburo Equipment Company, automatic moisture meter model 1200A, 1022 west Jackson Blvd, Chicago Ill). Pitting of peas using a gap with of 5/8" was done to initiate cracks in the seed coat using a pitting machine (SK Engineering and Allied Works Bahraich -271801- India). Peas were then tempered for 5 h to 14% moisture content. Heat at 70°C with occasional stirring was applied for 20 min to the peas using a heater (SK Engineering and Allied Works Bahraich – 271801- India). Overnight cooling of the peas in cooling towers (SK Engineering and Allied Works Bahraich -271801-India) was done following heating.

The next day, peas were dehulled and split after moisture content was measured. A sheller (SK Engineering and Allied Works Bahraich -271801- India) dehulled the peas. To separate the hull from the cotyledon, aspiration was used (SK Engineering and Allied Works Bahraich -271801- India). A hammer mill (Jacobson Inc, Minneapolis MN) with a screen size of 1.5/64" was used to mill the split pea seeds to flour while a coffee grinder (Black and Decker CBG100W, Towson, Maryland) was utilized to grind the hull fraction to powder of particle size less than 850 µm.

Canada Western Red Spring (CWRS) straight grade wheat flour (Laura variety) was provided by CIGI which was used as the base for the tortillas. The flour was characterized by CIGI, having a protein content of 13.26% (combustion nitrogen

analysis), a moisture content of 13.41% and wet and dry gluten at 34.4% and 11.2% respectively.

Combinations of pea flour, pea hull and wheat flour were used to compose five different composite flours which included 15% pea flour with 0% hull, 15% pea flour with 5% hull, 25% pea flour with 1.5% hull, 35% pea flour with 0% hull and finally 35% pea flour with 5% hull. Flours were made on an as is basis, mixed, and stored at 4°C in Ziploc® bags until used.

3.3.2 Tortilla procedure

Composite flour moisture content was assessed using the AACC Moisture Air-Oven Method (44-15A) (AACC, 1999)

AACC method 54-21 (small bowl) (AACC, 1982) was used to determine the Farinograph absorption of the composite flour samples.

Tortillas were made using the method described by Ambalamaatil et al. (2006) by mixing 100g flour (14% moisture basis) with 1.5g baking powder (Magic commercial brand, Kraft Foods, Toronto, ON), 1.5g sodium chloride (Fisher Scientific, Ottawa Ontario) and 9g of vegetable shortening (Crisco, commercial brand, Orrville, Ohio) in a 200g mixer (National MFG. Co., Lincoln, Nebraska) for 2 min. Water addition was dependent on the FAB of the composite flours to reach 500 FU less 10mL. Water addition was tested for each tortilla blend at FAB-8mL, FAB-10mL, FAB-12mL, it was found that FAB-10mL was optimal for the composite flour blends. After distilled water was added, the tortillas were further mixed for a total of 7, 6.2 and 3 minutes for compositions of 15%, 25% and 35% respectively. Mixing times were tested for

different formulations of tortilla dough. Three doughs of each formulation were mixed and the peak mixing time was observed for each. The average peak mixing time was used to make tortillas. After forming dough into 35g balls, each was placed in a plastic container and covered with a damp cloth to rise for 5 minutes at room temperature. A lubricated Doughpro press pre-heated to 93°C at a thickness level between “thick” and “thin” (Proprocess Corporation, Perris, California) was used to press the balls into tortillas for 8s. Using a spatula, the tortillas were transferred to a 220°C frying pan. They were cooked for 30s on one side, flipped and cooked for 40s on the second side, flipped again and cooked on the initial side for an additional 10s. Before tortillas were placed in a polyethylene bag and left to cool overnight at 25°C, they were cooled on a wire rack for 1 min.

3.3.3 Diameter, thickness and rollability

Diameter of tortillas was evaluated using a ruler. Three measurements from each tortilla were taken and the values were averaged. Three thickness measurements using a vernier calliper were taken and averaged from 3 measurements from each tortilla. Rollability was measured three times for each tortilla formulation on a 6 point scale, 1 no indication of cracking, 2 edge cracking only, 3 edge cracking and/or cracking in the center, 4 cracking and breaking on one side, 5 cracking and breaking on both sides but still rollable, 6 unrollable after being rolled around a one centimetre diameter wooden dowel. An average rollability score was recorded.

3.3.4 Colour

Colour was measured spectrophotometrically using a Minolta Spectrophotometer (CM- 525i, Japan) The Minolta was calibrated against a white calibration plate and L*, a* and b* readings were taken (observer: 2 C, Illuminate 1: C and illuminate 2). Three readings were taken on each side of the tortilla in a random area while avoiding darker bubbles if possible. Each reading by the spectrophotometer given was an average of 3 readings.

3.3.5 Rheology

Instrumental testing of tortillas cooled to room temperature took place 24 h after they were made using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Scarsdale, NY/Stable Micro Systems, Godalming, Surrey, UK). Tortillas were positioned blister side down on a frame and punctured with a cylindrical probe (TA 108, 18 mm diameter). The cohesiveness of the tortillas, measured as AUC (g×s) as well as the firmness of tortillas measured as peak force (g) was used to characterize tortilla texture properties. The TA.XT2 settings for the test used are given in Table 1.

Table 1: Test parameters for tortillas using TA.XT2 texture analyzer

Parameter	Setting
Test mode	Compression
Pre-test speed	1.0 mm/s
Test speed	1.0 mm/s
Post-test speed	10.0 mm/s
Distance	40.0 mm
Trigger force	1.0 g

3.3.6 Response Surface Methodology

Response surface methodology was used to optimize the tortilla blends using the Design Expert Software (Version 7.1, Minneapolis, Minnesota). The level of pea flour was set to a maximum between 15% and 35% and was given the greatest importance level (+++++). Pea hull was set to be maximized in optimization between 0% and 5% with a moderate importance level (+++). Response variables peak force and area were also set to a maximum. Peak force maximum in the range of 426-895g and was given an importance level of moderate importance (+++) while the range for area was set to 1516.33 and 5192.18 with the same importance level as peak force (+++). Optimization criteria may be found in Table 2. The ranges given for peak force and area were the range obtained for the values for the control wheat flour tortillas.

Table 2: Optimization criteria of pea flour and pea hull in pea and wheat composite flour tortillas using Design Expert software

Variable	Range	Weight	Importance
Pea flour	15-35%	1	+++++
Pea hull	0-5%	1	+++
Peak force	426-895g	1	+++
Area	1516.33- 5192.18g×s	1	+++

3.3.7 Sensory evaluation

Formulations for green and yellow peas were determined through optimization results. Green pea optimization revealed a concentration of 26% Cooper pea flour and 5% Cooper ground pea hull while Eclipse yellow peas were used at a level of 27% pea flour combined with 5% ground pea hull. The varieties were selected based on acreage of yellow and green pea varieties (which may be found in Appendix B), those with greatest acreage were selected as the varieties to be used for sensory evaluation.

Tortillas for sensory evaluation were made following the same method as described previously for tortillas. However, after tortillas were made they were frozen at -40°C for two weeks until the sensory analysis.

A nine point hedonic scale was used to assess the attributes of three tortillas, yellow pea blend, green pea blend and wheat flour control in terms of consumer acceptability. Seventy-two un-trained panellists were recruited from the University of Manitoba Faculty of Agricultural and Food Sciences. The panellists were presented with one quarter of a tortilla in a random order and coded with a random three digit number, which they were asked to assess in a random order. Panellists were given water to rinse their palate between tasting of different samples. Characteristics of the tortillas that they were asked to evaluate included appearance, flavour, texture, overall opinion and intent to purchase. Volunteers were given a small snack as compensation for their time. Examples of the sensory forms as well as the ethics approval forms may be found in the Appendix E.

3.3.8 Statistical Analysis

Tortilla characteristics were considered to be significantly different when $p < 0.05$. This analysis was done using Statistical Analysis Software (SAS, version 9.1) using a Tukey test. Three replications of each tortilla were made and were tested in triplicate for diameter and thickness ($n=9$ for each tortilla formulation), rollability of 3 tortillas was tested for each formulation ($n=3$), $n=18$ for colour measurements per tortilla formulation and $n=6$ for cohesiveness and firmness values for each tortilla formulation.

3.4 Results and Discussion

3.4.1 Diameter, thickness and rollability

As shown in Table 3, the addition of pea flour to wheat flour caused a significant increase ($p < 0.05$) in the diameter (from about 16.7cm to 17.3cm for control and treatment respectively) of the tortilla but did not have as great an affect on the thickness of the tortilla since the control (1.06mm) was significantly thinner than only the 2005 crop year tortillas (1.26mm) but not the 2006 crop year tortillas (1.06mm) (Crop year growing conditions found in the Appendix A). In terms of rollability, control tortillas had a significantly lower score (1.00 more desirable) than the wheat and pea composite flour tortillas (2.85 and 2.16 for 2005 and 2006 crop year respectively) (Table 3).

When comparing the effect of crop year of peas, diameter was found to be unaffected by crop year, where both thickness and rollability of the 2005 crop year were significantly larger than the 2006 crop year (Table 3).

Table 3: Physical characteristics of tortillas by crop year

Characteristic	Control	2005	2006
a*	-0.64 ± 0.98 ^{▶a}	-0.39 ± 2.69 ^{▲a}	-0.20 ± 3.49 ^{▲a}
b*	28.74 ± 2.84 ^{▶ab}	28.41 ± 5.78 ^{▲b}	30.14 ± 6.00 ^{▲a}
L*	80.86 ± 1.66 ^{▶a}	79.36 ± 3.34 ^{▲b}	79.04 ± 3.31 ^{▲b}
Greenness (-b*/a*)	35.68 ± 46.78 ^{▶a}	-13.42 ± 24.21 ^{▲c}	-0.98 ± 68.93 ^{▲b}
Diameter (cm)	16.71 ± 0.37 ^{◀b}	17.26 ± 0.37 ^{▼a}	17.32 ± 0.33 ^{▼a}
Thickness (mm)	1.06 ± 0.24 ^{◀b}	1.26 ± 0.18 ^{▼a}	1.06 ± 0.17 ^{▼b}
Rollability (1-6)	1.00 ± 0.00 ^{◻c}	2.85 ± 1.85 ^{◻b}	2.16 ± 1.42 ^{◻a}
Cohesiveness (g×s)	8468.23 ± 1892.31 ^{▲a}	3626.91 ± 1109.12 ^{♦b}	3435.83 ± 1275.60 ^{♦b}
Firmness (g)	910.88 ± 106.29 ^{▲a}	758.44 ± 143.74 ^{♦ab}	685.17 ± 154.49 ^{♦b}

Results shown as average ± standard deviation, significant difference for each characteristic between crop years is indicated by a different letter in the same row (p<0.05)

▲n=540 (6 varieties × 5 formulations × 3 samples × 6 replications)

▶n=108 (18 samples × 6 replications)

▼n=270 (6 varieties × 5 formulations × 3 samples × 3 replications)

◀n=54 (18 samples × 3 replications)

◻n=90 (6 varieties × 5 formulations × 3 replications)

◻n=15 (5 samples × 3 replications)

♦n=180 (6 varieties × 5 formulations × 3 samples × 2 replications)

▲n= 30 (15×2 replications)

The effects of crop year were slight in terms of tortilla diameter, thickness and rollability as seen in Table 3. Diameter was unaffected by differences in crop year; however, the thickness of the 2005 tortillas was greater than that of the 2006 tortillas. The thickness may be correlated to the hardness of the tortilla edge, which tended to be much harder than for the control tortillas, causing a hard edge which curled slightly. This also correlated with the results from the rollability test which indicated that rollability scores of the 2005 of the crop year were higher and less desirable than for the 2006 crop year. Crop year differences may be explained by differences in temperature

and rainfall for the seasons. Peas from both the 2005 and 2006 years were obtained from the same three growing locations; Indian Head, Davidson and Rosthern Saskatchewan. Climate data from Environment Canada (2006) indicates that generally, the 2005 crop year had cooler temperatures than the 2006 crop year and the total rainfall of the 2005 crop year had a greater rainfall during the pea growing season than in 2006 (climate data found in Appendix A). These climate differences could potentially alter the protein content of the peas or other properties which may alter the functionality of peas as it is used as flour.

Some varietal differences were found between diameter, thickness and rollability of the peas (Table 4). Cooper (17.46cm) had a significantly larger diameter than all varieties with the exception of Camry while the control sample had a significantly smaller diameter (16.71cm) compared to the treatments. With regard to thickness, the control (1.06mm) and Midas (1.14mm) were significantly thinner than Eclipse (1.23mm). For rollability, the control tortilla (1.00) was significantly lower than only the Eclipse variety tortillas (2.63) when looking at the effect of pea flour concentration using data from all cultivars.

Table 4: Physical characteristics of tortillas according to variety

Characteristic	Control	Yellow Pea varieties				Green Pea varieties	
		Eclipse	Alfetta	Midas	Mozart	Cooper	Camry
a*	-0.64 ±	1.40 ±	1.67 ±	1.49 ±	1.37 ±	-3.74 ±	-3.95 ±
	0.98 ^{▼b}	0.49 ^{▶a}	0.54 ^{▶a}	0.60 ^{▶a}	0.50 ^{▶b}	3.01 ^{▶c}	3.22 ^{▶c}
b*	28.74 ±	28.49 ±	28.97 ±	29.88 ±	28.89 ±	29.77 ±	29.65 ±
	2.84 ^{▼a}	5.90 ^{▶a}	5.95 ^{▶a}	6.47 ^{▶a}	6.12 ^{▶a}	5.64 ^{▶a}	5.54 ^{▶a}
L*	80.86 ±	80.86 ±	80.61 ±	80.47 ±	80.86 ±	76.36 ±	76.04 ±
	1.66 ^{▼a}	1.70 ^{▶a}	1.72 ^{▶a}	1.73 ^{▶a}	1.79 ^{▶a}	3.66 ^{▶b}	3.72 ^{▶b}
Greenness (-b*/a*)	35.68 ±	-23.52 ±	-19.11 ±	-23.96 ±	-23.59 ±	20.28 ±	26.72 ±
	46.78 ^{▼a}	14.64 ^{▶b}	8.14 ^{▶b}	13.37 ^{▶b}	10.53 ^{▶c}	27.2 ^{▶a}	11.06 ^{▶a}
Diameter (cm)	16.71 ±	17.26 ±	17.23 ±	17.17 ±	17.23 ±	17.46 ±	17.38 ±
	0.37 ^{◀c}	0.33 ^{▲bcd}	0.30 ^{▲cd}	0.40 ^{▲cd}	0.32 ^{▲bcd}	0.35 ^{▲a}	0.33 ^{▲abc}
Thickness (mm)	1.06 ±	1.23 ±	1.16 ±	1.14 ±	1.15 ±	1.14 ±	1.14 ±
	0.23 ^{◀b}	0.21 ^{▲a}	0.17 ^{▲ab}	0.19 ^{▲b}	0.19 ^{▲ab}	0.23 ^{▲ab}	0.21 ^{▲ab}
Rollability (1-6)	1.00 ±	2.63 ±	2.42 ±	2.54 ±	2.43 ±	2.47 ±	2.53 ±
	0.00 ^{▣b}	1.72 ^{▣a}	1.69 ^{▣ab}	1.61 ^{▣ab}	1.65 ^{▣ab}	1.71 ^{▣ab}	1.79 ^{▣ab}
Cohesiveness (g×s)	8468.23 ±	3479.35 ±	3350.58 ±	3687.47 ±	3587.17 ±	3586.02 ±	3497.62 ±
	1892.31 ^{▲a}	1084.29 ^{♦b}	1028.60 ^{♦b}	1253.41 ^{♦b}	1195.97 ^{♦b}	1300.85 ^{♦b}	1505.32 ^{♦b}
Firmness (g)	910.88 ±	719.07 ±	698.35 ±	744.69 ±	736.99 ±	717.20 ±	714.52 ±
	106.29 ^{▲a}	158.571 ^{♦a}	139.26 ^{♦a}	157.33 ^{♦a}	155.36 ^{♦a}	167.33 ^{♦a}	171.62 ^{♦a}

Results listed as average ± standard deviation. Significant difference is indicated by different letters in the same row (p<0.05)

▶ n=270 (5 formulations × 3 tortilla replications × 3 measurements × 3 readings/measurement × 2 crop years)

▼ n= 162 (1 formulation × 18 tortilla replications × 3 measurements × 3 readings/measurement)

▲ n=90 (5 formulations × 3 tortilla replications × 3 measurements × 2 crop years)

◀ n= 54 (1 formulation × 18 tortilla replications × 3 measurements)

▣ n=30 (5 formulations × 3 tortilla replications × 2 crop years)

▢ n= 18 (1 formulation × 18 replications)

♦ n=60 (5 formulations × 3 tortilla replications × 2 trials × 2 crop years), * n= 36 (18 tortillas × 2 trials)

Slight variety differences were also found for the diameter, thickness and rollability characteristics of tortillas. The green pea variety tortillas, in particular Cooper, tended to have a larger diameter than the yellow pea varieties. These differences could in part be due to the particle size of the pea flour milled from these peas, which would be a direct result of the composition, starch or protein, of the peas. Further work needs to be done on the effect of particle size on the properties of tortillas blended with pea and wheat flour. However, the addition of any variety caused a significant increase in diameter in comparison to the control, likely a result of a reduction in gluten which has been linked to the characteristic texture of tortillas (McDonough et al., 1996). In terms of thickness, slight differences were found between varieties, Eclipse resulted in the thickest tortillas. This again, may in part be due to the hard outer edge of treatment tortillas which had a much less soft, pliable outer edge as compared to the control due to a lack of gluten to provide the elastic properties of tortillas. In terms of rollability, it was found that the thickest tortilla, Eclipse variety, had the highest rollability scores, indicating rolling problems. These scores likely reflected the dry, hard outer edge of the tortilla due to a lack of gluten to provide the elastic characteristic and water retention of tortillas.

The control tortilla had a significantly smaller ($p < 0.05$) diameter than pea flour tortillas as seen in Table 5. With each increasing increment of percent pea flour added, the tortillas became significantly smaller in diameter. For thickness, the 25% and 35% formulations were found to be significantly thicker than the control and the 15% pea flour tortillas and rollability for the 35% pea flour

formulations were significantly greater (less desirable) than the other treatments as well as the control (Table 5).

Table 5: Diameter, thickness and rollability characteristics of pea and wheat composite flour tortillas by formulation

Category		Diameter	Thickness	Rollability
% Flour	15	17.51 ± 0.32 ^{▶a}	1.12 ± 0.23 ^{▶b}	1.25 ± 0.37 ^{♦b}
	25	17.23 ± 0.25 ^{▼b}	1.20 ± 0.20 ^{▼a}	1.65 ± 0.64 ^{♦b}
	35	17.10 ± 0.31 ^{▶c}	1.18 ± 0.16 ^{▶a}	4.18 ± 1.38 ^{♦a}
	Control	16.71 ± 0.37 ^{▲d}	1.06 ± 0.23 ^{▲b}	1.00 ± 0.00 ^{♦b}
% Hull	0	17.34 ± 0.35 ^{▶a}	1.15 ± 0.20 ^{▶a}	2.57 ± 1.70 ^{♦a}
	1.5	17.23 ± 0.25 ^{▼b}	1.20 ± 0.20 ^{▼a}	1.65 ± 0.64 ^{♦b}
	5	17.27 ± 0.39 ^{▶ab}	1.15 ± 0.19 ^{▶a}	2.86 ± 1.83 ^{♦a}
	Control	16.71 ± 0.37 ^{▲c}	1.06 ± 0.23 ^{▲b}	1.00 ± 0.00 ^{♦b}
Type	Yellow	17.22 ± 0.34 ^{◀b}	1.17 ± 0.19 ^{◀a}	2.50 ± 1.65 ^{▼a}
	Green	17.42 ± 0.34 ^{◻a}	1.14 ± 0.22 ^{◻a}	2.50 ± 1.74 ^{Δa}
	Control	16.71 ± 0.37 ^{▲c}	1.06 ± 0.23 ^{▲b}	1.00 ± 0.00 ^{♦b}

Results are given as the average ± standard deviation. Significant difference of samples followed by different numbers within the same section (% flour, % hull or type) within a column (p<0.05)

▶n= 216 (2 formulations × 6 varieties × 2 crop years × 3 trials × 3 replications)

▼n= 108 (1 formulation × 6 varieties × 2 crop years × 3 trials × 3 replications)

▲n=54 (1 formulation × 18 tortillas × 3 replications)

◀n= 360 (4 varieties 5 formulations 3 tortilla replications 3 measurements 2 crop years)

◻n= 180 (2 varieties 5 formulations 3 tortilla replications 3 measurements 2 crop years)

♦n = 72 (2 formulations × 6 varieties × 2 crop years × 3 trials)

♦n = 36 (1 formulation × 6 varieties × 2 crop years × 3 trials)

♦n=18 (1 formulation 18 tortilla replications)

▼n= 120 (4 varieties 5 formulations 3 tortilla replications 2 crop years)

Δn= 60 (2 varieties 5 formulations 3 tortilla replications 2 crop years)

The addition of pea hull did not have as much of an effect on diameter, thickness and rollability of tortillas as seen in Table 5. The control again, had a significantly smaller diameter than treatments and tortillas with no added hull (but treated with added pea flour) had a significantly larger diameter than tortillas with 1.5% added hull. Control tortillas also were significantly thinner ($p < 0.05$) than tortillas with added hull. Tortilla rollability of the control and those with 1.5% added hull were significantly lower than samples containing 0% and 5% hull using data from all varieties and concentrations.

Formulation of tortillas according to percent of pea flour added had a definite affect on the diameter, thickness and rollability of tortillas. Likely, the increasing increments, in addition to dilution of gluten normally present in wheat flour tortillas, also interfered with the gluten network present resulting in a decreasing diameter as percent flour was increased (unlike the control tortilla which had the smallest diameter). Likely there is a balance between the reduction in gluten, the elasticity of the tortilla and the structure of the gluten network present. Considering these reactions, it is seen that the control tortilla, with the greatest content of gluten, has the greatest extensibility resulting from a complete gluten network, although it has a smaller diameter, without force applied, it has extensibility potential when the tortilla is stretched during compression a result of the extent of the gluten network present. As the amount of pea flour is increased in the formulation, the diameter decreases, however, not for the same reasons of why the control tortilla has a smaller diameter but because of a lack of a gluten network, resulting in poor extensibility of the tortilla as verified by the lack of the extensibility/cohesiveness of the tortillas subjected to a compression/penetration test. The lack of extensibility is

likely due to the interference of pea flour with the gluten network. The thickness of the treatment tortillas became greater as the concentration of pea flour was increased in tortilla formulation with the lowest value for the control. These properties were reflected in the rollability scores of the tortillas of which the 35% flour tortillas had the least desirable rollability scores. Rolling thick, dry, hard tortillas lacking gluten caused interferences with the little gluten structure which was present and resulted in measurable cracking. The effect of hull addition on the other hand had less of an affect on the outcome of the diameter, thickness and rollability of tortillas partially because the hull content which was added was in lower quantities compared to the pea flour.

The effect of pea type (green or yellow) can also be seen in Table 5. For diameter, it was found that green pea tortillas were significantly larger ($p < 0.05$) than yellow pea tortillas which were significantly larger ($p < 0.05$) than the control. No significant difference was found between the green and yellow pea tortillas in terms of thickness, but, the control tortillas were significantly thinner ($p < 0.05$) and had a significantly lower ($p < 0.05$) rollability score than both of them.

Previous research which dealt with the use of inclusions in tortillas found similar results. When insoluble fibre was added to tortilla formulations, the resulting characteristics of the tortillas were larger diameters, higher moisture contents and shorter shelf life as indicated by Seetharaman et al. (1997). Gluten tended to have a more positive effect on tortilla properties as demonstrated by Srinivasan et al. (1999) who noted that a thicker tortilla with a smaller diameter resulted from tortillas formulated with additional gluten.

3.4.2 Colour

The addition of pea flour to wheat flour overall only had a significant effect on the L* and “greenness” values, where the control was found to be significantly greater in both cases (Table 3). In terms of crop year, no difference was found in a* and L* values, however, for b* and greenness values; the 2006 crop year tortillas had significantly higher values than the 2005 crop year tortillas.

Varietal differences were more pronounced in terms of colour as seen in Table 4. The a* values of yellow pea variety tortillas were significantly greater ($p < 0.05$) than the control which was significantly greater ($p < 0.05$) than green pea variety tortillas. However, for b* values, no significant differences were found between the varieties. Control and yellow pea varieties had a significantly greater ($p < 0.05$) L* value than green pea variety tortillas and, without surprise, the green pea variety tortillas had a significantly greater greenness value than the yellow pea variety tortillas. There were no differences due to variety within each of the pea types (green and yellow).

Colour data for tortilla formulation was separated into yellow and green pea types (Table 6). For yellow pea flour tortillas, the a* values for increased flour and hull content caused significant increases in positive values, indicating that the green level of tortillas increased by increasing yellow pea flour. Significant increases in b* values was the result of increased yellow pea addition to tortilla blends. An increase in hulls had less of an effect than added flour, 1.5% hull addition was significantly lower (less yellow, than the 0% and 5% hull addition). As pea flour was added to tortilla blends, the L* value was significantly reduced. Pea hull had a

significantly lower L^* value at 5% hull incorporation. In terms of greenness, 25% pea flour had a significantly greater value than 35% pea flour while hull incorporation resulted in a significantly lower greenness value for 0% while there was no significant difference between 1.5% and 5% hull addition (Table 6).

Green pea flour tortillas a^* values were significantly lower with the addition of increased flour content while for hull, the a^* values for 0% and 5% were significantly lower than 1.5% hull addition. For b^* values, as flour percentage was increased, b^* became significantly greater while 0% hull was significantly greater than 1.5% hull and 5% hull was significantly greater than 1.5%. L^* values were significantly lower with an increase in green pea flour while 0% and 1.5% hull were significantly greater than 5% hull for L^* values. Greenness values significantly decreased (became more green) as green pea flour addition increased from 15% to 25%, however, no significant difference was found between the 25% and 35% green pea flour formulations. Hull changes did not significantly change the greenness values of green pea flour tortillas

Overall, with respect to colour differences, crop year differences between 2005 and 2006 may be a result of climate differences. L^* (Lightness), b^* (blue and yellow) and greenness values were greater for the 2006 crop year which had a hotter, drier climate and may have caused a bleaching of the peas. Variety differences in colour were correlated to the pea type used. Green peas resulted in lower values for a^* values while yellow peas resulted in higher values for b^* colour.

Table 6: Colour data for tortilla formulations by type of pea (green or yellow)

Flour	Yellow Pea Types				Green Pea Types			
	a*	b*	L*	greenness	a*	b*	L*	greenness
Control	-0.64± 0.98 ^{ad}	28.74± 2.84 ^{ab}	80.86± 1.66 ^{ab}	35.68± 46.78 ^{aa}	-0.64± 0.98 ^{aa}	28.74± 2.84 ^{ab}	80.86± 1.66 ^{aa}	35.68± 46.78 ^{ab}
15%	1.25± 0.47 ^{ac}	23.58± 1.79 ^{ad}	82.02± 1.21 ^{aa}	-22.74± 14.07 ^{abc}	-0.89± 0.48 ^{aa}	24.57± 1.58 ^{ad}	79.69± 1.25 ^{ab}	48.26± 122.78 ^{aa}
25%	1.44± 0.34 ^{ab}	26.05± 1.95 ^{bc}	81.42± 0.63 ^{bc}	-19.07± 5.47 ^{ab}	-2.57± 0.51 ^{ab}	27.35± 1.96 ^{bc}	77.59± 0.72 ^{bc}	11.03± 2.20 ^{abc}
35%	1.73± 0.58 ^{aa}	36.03± 2.32 ^{aa}	79.02± 1.03 ^{ad}	-24.10± 12.01 ^{ac}	-7.44± 1.16 ^{ac}	36.04± 1.10 ^{aa}	72.01± 1.22 ^{ad}	4.98± 0.93 ^{cd}
Hull								
Control	-0.64± 0.98 ^{ad}	28.74± 2.84 ^{aa}	80.86± 1.66 ^{ab}	35.68± 46.78 ^{aa}	-0.64± 0.98 ^{aa}	28.74± 2.84 ^{abc}	80.86± 1.66 ^{aa}	35.68± 46.78 ^{aa}
0%	1.22± 0.53 ^{ac}	29.34± 6.74 ^{aa}	81.32± 1.83 ^{aa}	-28.62± 16.11 ^{ac}	-4.36± 3.39 ^{ac}	29.87± 6.27 ^{ab}	76.72± 4.05 ^{ab}	15.92± 17.16 ^{aa}
1.5%	1.44± 0.34 ^{ab}	26.05± 1.95 ^{ab}	81.42± 0.63 ^{ba}	-19.07± 5.47 ^{ab}	-2.57± 0.51 ^{ab}	27.35± 1.96 ^{bc}	77.59± 0.72 ^{ab}	11.03± 2.20 ^{aa}
5%	1.76± 0.51 ^{aa}	30.28± 6.36 ^{aa}	79.72± 1.56 ^{ac}	-18.22± 5.41 ^{ab}	-3.96± 3.41 ^{ac}	30.74± 5.76 ^{aa}	74.98± 3.84 ^{ac}	37.31± 124.48 ^{aa}

^{*} n=108 (18 replications × 6 measurements)

[♦] n= 144 (2 varieties × 2 formulations × 3 replications × 6 measurements × 2 crop years)

[□] n= 72 (2 varieties × 1 formulation × 3 replications × 6 measurements × 2 crop years)

[⊠] n = 144 (4 varieties × 1 formulation × 3 replications × 6 measurements × 2 crop years)

[◀] n = 288 (4 varieties × 2 formulations × 3 replications × 6 measurements × 2 crop years)

3.4.3 Rheology

3.4.3.1 Cohesiveness

The addition of pea flour significantly reduced ($p<0.05$) the cohesiveness values for wheat flour tortillas from about 8500 to 3500 g×s (Table 2). There was no significant difference for cohesiveness between the 2005 and 2006 crop years (Table 3) or between the pea varieties (Table 4).

Formulation, in particular the percent of pea flour added, had a significant effect on the cohesiveness of tortillas as seen in Table 7. Each increase in percent pea flour added to wheat flour tortillas significantly reduced ($p<0.05$) the cohesiveness of the tortillas (about 8500, 4600, 2800 and 2300 g×s for the control, 15% pea flour, 25% pea flour and 35% pea flour respectively). Addition of hull and pea type did not have as great an effect on the cohesiveness of tortillas and no significant differences were found in the cohesiveness of treatment tortillas; however, the control sample tortillas again had a significantly greater cohesiveness value.

Tortilla texture is a function of gluten which provides the elastic properties of tortillas which are reduced when pea flour is used to replace wheat flour in tortilla formulations. This explains the decreasing cohesiveness values with added pea flour in tortilla formulations. As the concentrations of pea hull and flour were increased, the ability to roll the tortillas around a 1 cm diameter wooden dowel was decreased. This may partially be explained by the ability of fiber to hold water over the pea flour fraction and contribute to the structure of the tortilla. The effects of

increasing pea flour and pea hull were also reinforced by the rollability tests performed on the products.

3.4.3.2 Firmness

The addition of pea flour and fibre tended to decrease the firmness values of wheat flour tortillas. There was no significant difference in the firmness value based on crop year differences (Table 7) or between different pea varieties (Table 4).

As the concentration of percent pea flour used increased, tortilla firmness became significantly lower, the 15% and 25% pea flour had a significantly greater firmness value (about 840 and 790 g respectively) as compared to the 35% pea flour tortillas (approximately 570 g) (Table 7). The addition of hull was identical to that of pea flour with 0% and 1.5% addition having a significantly greater firmness value as compared to 5% pea hull addition to tortillas (Table 7). Pea type did not have a significant effect on the firmness values of pea flour tortillas (Table 7).

Firmness was not as greatly influenced by the addition of pea flour and hull as cohesiveness. Lower firmness values due to a lack of interconnected gluten network to hold tortilla structure to provide resistance against the puncture force explains the lower firmness values for increasing levels of pea flour in pea composite flour tortillas.

Table 7: Composite flour tortilla cohesiveness and firmness by pea flour (%), pea hull (%) and type (yellow, green or wheat flour control)

Composition		Cohesiveness (area g×s)	Firmness (peak force g)
% Pea Flour	Control	8468.23 ± 189.23 ^{▲a}	910.88 ± 106.29 ^{▲a}
	15	4598.00 ± 751.45 ^{▶b}	841.57 ± 82.83 ^{▶ab}
	25	3792.54 ± 465.24 ^{▼c}	791.11 ± 77.61 ^{▼b}
	35	2334.15 ± 489.78 ^{▶d}	567.38 ± 86.86 ^{▶c}
% Pea Hull	Control	8468.23 ± 1892.31 ^{▲a}	910.88 ± 106.29 ^{▲a}
	0%	3825.50 ± 1323.34 ^{▶b}	743.68 ± 150.28 ^{▶ab}
	1.5%	3792.54 ± 465.24 ^{▼b}	791.11 ± 77.61 ^{▼b}
	5%	3106.65 ± 1207.33 ^{▶b}	665.27 ± 166.85 ^{▶c}
Type	Control	8468.23 ± 1892.31 ^{▲a}	910.88 ± 106.29 ^{▲a}
	Yellow	3526.14 ± 1106.43 ^{▶b}	724.78 ± 147.95 ^{▶b}
	Green	3541.82 ± 1370.03 ^{◻b}	715.86 ± 164.97 ^{◻b}

Results are shown as the average ± standard deviation. Significant difference indicated by different letters in the same columns of the same composition criteria (flour, hull and type) when $p < 0.05$

▶ n= 108 (2 formulations × 6 varieties × 2 crop years × 3 trials × 2 replications)

▼ n= 54 (1 formulation × 6 varieties × 2 crop years × 3 trials × 2 replications)

▲ n=27 (1 formulation × 18 tortillas × 2 replications)

◻ n= 180 (4 varieties × 5 formulations × 3 tortilla replications × 2 measurements × 2 crop years)

◻ n= 120 (2 varieties × 5 formulations × 3 tortilla replications × 2 measurements × 2 crop years)

3.4.4 Response surface methodology and optimization

Results of response surface methodology for the optimization of wheat and pea composite flours tortillas from the 2006 crop year are shown in Table 8. The optimized level of pea flour and pea hull is relatively similar between varieties. Overall, the greatest desirability (58.9%) was demonstrated by the Midas variety for yellow peas and Camry (52.1%) for the green pea varieties. However, the differences between the amount of pea flour and pea hull which has been optimized for addition into wheat flour tortillas is slight. Essentially, incorporating 26-27% pea flour and 4-5% pea hull will yield a predicted firmness value and predicted cohesiveness value of about 620g and 3000 g×s respectively regardless of variety.

The results of optimization were verified when selected varieties were made for sensory evaluation. The varieties Eclipse and Cooper were selected for sensory evaluation based on their acreage values in 2006. The acreage of the pea varieties may be found in Appendix B.

Table 8: Response surface methodology optimization results of composite pea and wheat flour tortillas based on instrumental texture measurements cohesiveness and firmness values

Variety	% Pea Flour	% Pea Hull	Predicted Firmness (g)	Predicted Cohesiveness (g×s)	Desirability
Yellow Pea					
Mozart	26.1	3.9	651.024	3120.78	48.6%
Midas	27.1	5.0	629.940	2960.91	58.9%
Eclipse	26.7	4.6	617.856	2871.95	50.5%
Alfetta	25.8	5.0	629.654	2997.93	49.6%
Green Pea					
Camry	25.8	5.0	625.577	3106.10	52.1%
Cooper	26.2	4.0	610.904	2980.66	48.4%

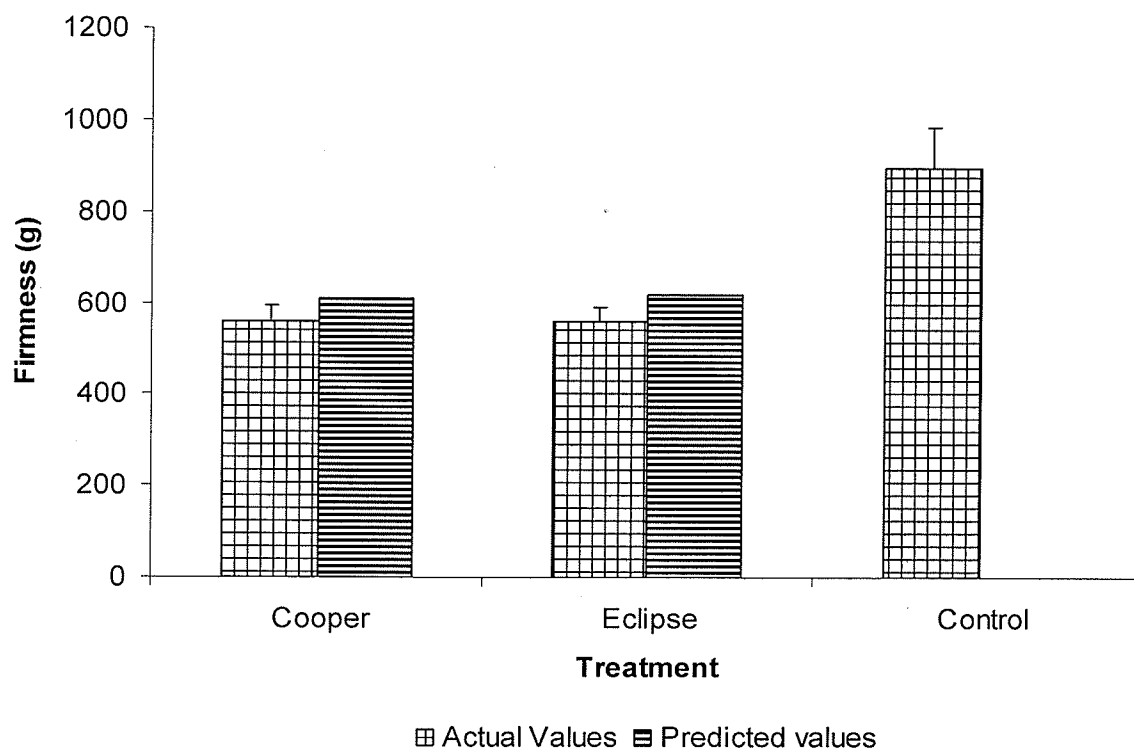


Figure 1: Actual and predicted values of firmness for composite Cooper green pea and composite Eclipse yellow pea and wheat flour tortillas used for sensory evaluation

Figure 1 illustrates the level of predictability of response surface methodology optimization to predict actual values of firmness for composite flour tortillas. As indicated, the predicted value was slightly higher than the actual firmness value measured using a TA.XT2 texture analyzer.

Figure 2 illustrates the level of predictability of response surface methodology optimization to predict actual values of cohesiveness for composite flour tortillas. As indicated, the predicted value was not significantly different from actual firmness value measured using a TA.XT2 texture analyzer.

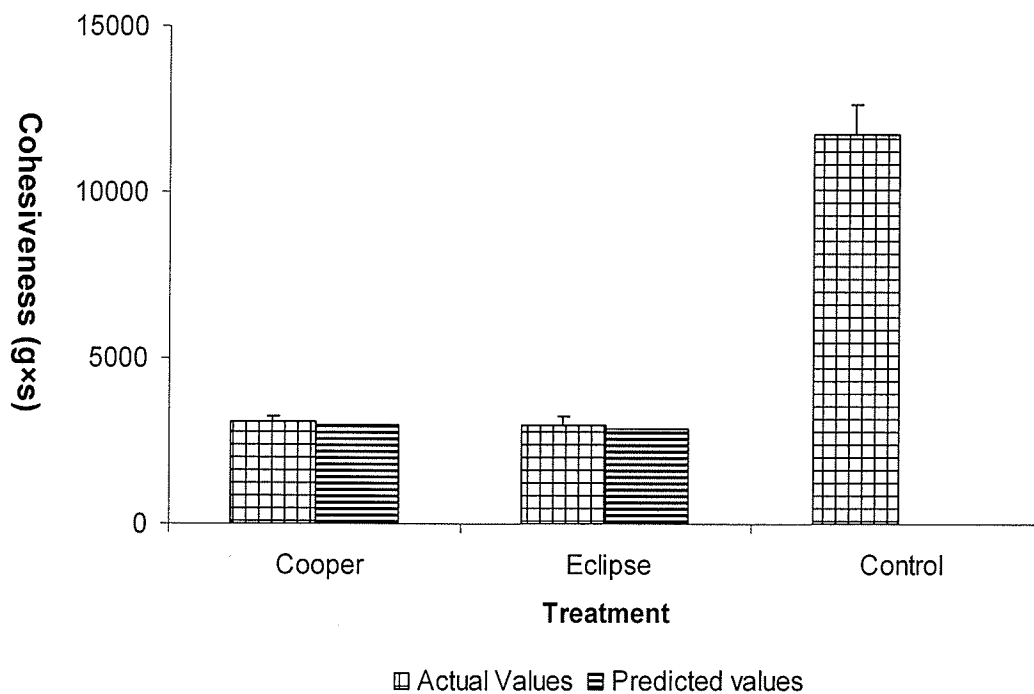


Figure 2: Actual and predicted values of cohesiveness (g×s) for composite Cooper green pea and composite Eclipse yellow pea and wheat flour tortillas used for sensory evaluation

The response surface methodology optimization results indicated the point of maximum content of pea flour and pea hull incorporated while minimizing the reduction in cohesiveness and firmness values of tortillas. While not part of the optimization, the results were reflected in the rollability values of tortillas, where problems arose at the 35% pea flour level.

3.4.5 Sensory evaluation

Results of sensory evaluation are shown in Figure 3. The only significant differences found were in terms of the appearance of the tortillas. Panellists deemed that the Eclipse yellow pea tortillas had a significantly more favourable appearance

as compared to the control tortillas. The Cooper green pea tortillas had a slightly lower acceptance score, but were not significantly different from the yellow pea tortillas or the control tortillas. The average sensory score of all attributes was approximately 7 or “like moderately”. The frequency of tortilla characteristic responses for sensory evaluation can be found in the Appendix C.

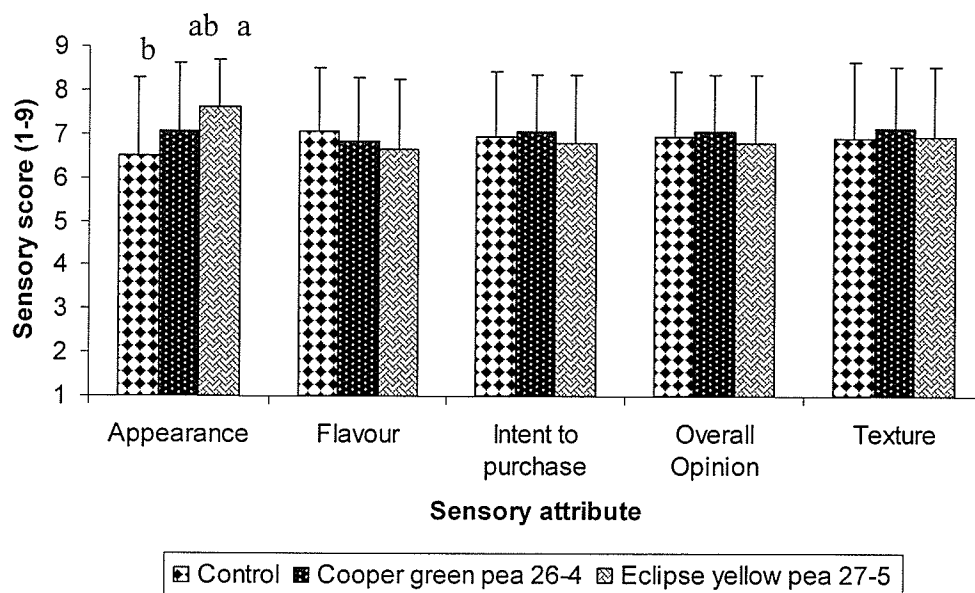


Figure 3: Sensory evaluation results of composite wheat and pea flour tortillas. Significant difference indicated by different letters in the same sensory attribute category.

The sensory results for composite pea flour tortillas had similar results to the control, however reasons for the moderately like score may be difference. The sensory scores of texture for pea flour tortillas were due to the lack of gluten for the texture results while the score of control tortilla texture resulted from an abundance of strength from the gluten quality and content resulting in control tortillas being too chewy. The variety Laura used in the control is known to be a relatively strong, hard red spring wheat. Flavour scores, of pea flour tortillas, although lower than the

control were not significantly different. The off flavours and astringent qualities of peas were likely reduced during processing. In terms of appearance, the control had a lower score than the pea flour tortillas. The control was characterized by pale appearance and blisters, whereas, the colour of both green and yellow tortillas was found to be attractive to the panellists.

3.5 Conclusions

From these results it appears to be possible to produce a pea and wheat composite flour tortilla with the aid of mechanical texture optimization followed by verification with sensory analysis (i.e. acceptability to consumers in terms of appearance, flavour, texture and overall acceptability). The formulations of pea flour, pea hull and wheat flour that can support texturally acceptable characteristics for tortillas were found to be at a level of 27% Eclipse yellow pea flour, 5% Eclipse yellow pea hull as well as a formulation containing 26% Cooper green pea flour, 4% Cooper green pea hull with the remainder of these formulations made up with wheat flour. The wheat flour used for the base of the tortilla formulations as well as for the control had an effect on the final characteristics of the final product. Different wheat flours could be explored to give the best properties for composite flour tortillas. However, future research regarding the shelf life of these tortillas as well as scale up testing of these formulations is necessary. As well, the functionality of specific particle sizes as well as protein content, starch characteristics should also be studied to see how differences in peas will affect the outcome of the final product.

CHAPTER 4: EXTRUSION PROCESSING AND SENSORY EVALUATION OF AN EXPANDED, PUFFED PEA SNACK PRODUCT

4.1 Abstract

Pea (*Pisum sativum*) based expanded snack foods were developed using formulations varying in pea flour, pea fibre and pea starch using extrusion processing. The product physical characteristics which included shear strength, bulk density and expansion index were characterized. It was found that the incorporation of pea fibre had the greatest effect on the texture of the final product where as the addition of pea flour only slightly affected the physical properties of the product. Temperature also had an effect on the physical properties bulk density and expansion ratio. However, temperature had no significant effect on the shear strength of the samples. Of three samples tested in sensory evaluation, the sample which most closely represented ideal product characteristics was a 50% whole yellow pea flour sample without added hull and extruded at a final barrel temperature of 135°C, indicating the potential to include pea flours and fractions as a snack food product.

4.2 Introduction

Pulse crops including peas, beans, lentils and chickpeas are of major economic importance to the Canadian economy. Saskatchewan is the major producer of Canadian dry field peas, accounting for 78% of national production (Agriculture and Agri-Food Canada, 2006). In terms of land use, this represents 2.9 million tonnes of peas produced in 2007. Canadian dry pea exports were valued at \$500 million in 2007 and 2008 (Agriculture and Agri-Food Canada, 2008). Of the

Canadian food use for Canadian peas, 10% (of the volume which is not exported) is consumed domestically. Secondary processing of peas following cleaning and sorting usually involves splitting, canning, drying or milling (Agriculture and Agri-Food Canada, 2005).

Extrusion processing of foods, in particularly snacks, is a very large market segment. A \$1.6 billion dollar market share was reported by Pepsi-Co (2007) in terms of Canadian consumption of snack foods including potato chips, tortilla chips, pretzels, popcorn, nuts and extruded snacks in 2002. Worldwide sales of the leading brand of Pepsi-Co puffed extruded corn snacks was approximately \$3 billion in 2007 as indicated in the Pepsi-Co annual report.

Extrusion processing is a high temperature, high pressure method to produce snack foods through the metering of feed material through temperature and pressure changes and eventually exiting through a die. Screws are divided into three sections: feed, transition (compression) and metering. Each section is composed of many different screw segments differing in their abilities to transport, mix and shear the dough. Depending on the configuration used, the outcome of the final product is changed. Water present in the system becomes heated and immediately dissipates when the pressure drops upon exiting the die, leaving an aerated, brittle foam structure. By altering the moisture, protein, starch and fibre in the ingredient blend as well alter parameters defined by extrusion conditions such as screw speed, screw configuration, feed rate and barrel temperature the viscosity, shear, component interactions/interferences, specific mechanical energy, torque, pressure, temperature, air cell size/frequency, mechanical damage to food components, bulk density and

expansion index will change. This results in differences in bubble nucleation and vapour diffusion which change the characteristics of the structures (Hutchinson and Siodlak, 1987, Chinnaswamy and Hanna, 1988, Falcone and Phillips, 1988, Jin et al., 1995)

Texture is a major determinant of overall quality for snack foods. The microstructure of food is a result of both physical and chemical factors and defines the texture of the product (Jin et al., 1995). Texture can be characterized by shear strength, expansion ratio and bulk density which are generally correlated to one another.

In order to verify instrumental texture analysis with consumer acceptability, sensory analysis must be completed. A Just-About-Right test is useful to gather directional information regarding a new product to guide further development by combining intensity and hedonic scaling by asking consumers opinions about how specified characteristics compare with what they consider to be ideal for each attribute (Lawless and Heymann, 1998). However, this may result in a confounding of the measurement and therefore it is recommended that a modified approach be taken to this method as suggested by Szczesniak et al. (1975). This involves the use of a consumer texture profile technique which separates ideal characteristics from the actual sample intensity characteristics.

Typically, extruded snack foods are characterized as being high in fat and low in nutritional value. However, the incorporation of nutritionally superior materials has been studied with respect to extruded snacks. These ingredients include whole and split peas (Berrios et al., 2008), garbonzo beans (chickpeas)

(Berrios et al., 2008), black bean flours (Berrios et al., 2008), cassava and pigeon pea flour (Rampersad et al., 2003), lentil flours and apple fibre (Berrios et al., 2008), yellow corn meal, soy fibre and cane sugar (Jin et al., 1995) and sorghum and cowpea flours (Falcone and Phillips, 1988).

There is now an opportunity to include more nutritious ingredients in snack foods. The rising costs of corn and wheat coupled with consumer demand for more healthful products has created the opportunity for ingredients such as peas to be used in food products. The nutritional profile in terms of protein content and fibre levels are generally superior as compared to corn which is typically used as the basis for puffed extruded snack food products. When comparing the glycemic index of corn meal to pea flour it was found that corn grits, green pea grits and yellow pea flour had a glycemic index relative to white bread of 100%, 70% and 70% respectively (Hardacre et al., 2006). A glycemic index ranging from 44 to 49 was found for three different varieties of peas (Chung et al., 2008) indicating that peas do not cause as large a spike in the blood glucose level following consumption as compared to white bread or corn grits. The objective of this experiment is to develop a pea based snack food using pea flour, pea fibre and pea starch to produce an acceptable product in terms of texture characteristics. Product texture acceptability is evaluated through a variation of the Just-About-Right sensory test.

4.3 Materials and methods

4.3.1 Formulations

Whole yellow pea flour (Eclipse variety), split green pea flour without hull and finely ground pea fibre (pea hull) was donated from Best Cooking Pulses of Portage la Prairie, Manitoba. A native, food grade pea starch made from yellow Canadian field peas was donated by Nutri-Pea Limited (Portage la Prairie, Manitoba) (Specification sheets may be found in Appendices F, G and H). Five different formulations with varying concentrations of pea flour, pea fibre and pea starch totalling 2.5kg were made for both yellow and green pea flours on an as is basis. The formulations included 30% pea flour-0% pea hull-70% pea starch (30-0), 30% pea flour-10% pea hull-60% pea starch (30-10), 40% pea flour-5% pea hull-55% pea starch (40-5), 50% pea flour-0% pea hull-50% pea starch (50-0) and 50% pea flour-10% pea hull-40% pea starch (50-10). Each formulation was run singularly with the mid point (40-5) processed in triplicate. Straight pea starch was run as the control.

Moisture content of formulations was determined using the AACC moisture air oven method 44-15A (AACC, 1999).

Final extruded samples made in this experiment were compared to commercial samples readily available in the market. Two commercial samples were used, Frito-Lay Canada Cheetos Puffs © (Cambridge, ONT) as well as Hawkins Cheezies Corn Snacks © (Belleville, ONT).

4.3.2 Extrusion

Blends were extruded with an APV co-rotating twin screw extruder (MPF19-25, 2.2kW motor, 19/25D, APV Baker Ltd. Peterborough England) and a circular die hole with a 4.5mm diameter using a high shear screw configuration. Prior to extrusion, blend feed and moisture injection rate were calibrated. Total moisture content of the blends was adjusted to 15% through the addition of water. Temperature of extrusion was set to 30°C, 70°C and 90°C for the first three of five barrel temperature zones respectively. The effect of temperature was also investigated; temperatures were tested by varying the final two barrel temperatures to 110°C, 120°C and 135°C. Screw speed was kept at a constant 240rpm. Following extrusion, the extrudates were dried in a convection oven at 135°C for 5 minutes, allowed to cool and placed in a polyethylene bag overnight. Texture was analyzed the following day. A table of extrusion conditions and screw configuration may be found in Appendices I and J.

4.3.3 Texture analysis

Texture analysis was done with a Zwick Roell texture analyzer (BDO-FB005TN, Zwick GmbH & Co. KG Germany) using a shear three point bend test in the compression mode. Extrudates were cut into 4cm lengths and laid across three point bending stand with bar gap set at one millimetre. A Warner Bratzler shear probe was used to break the samples. The resulting curve was evaluated using testXpert II v1.41 software (Zwick GmbH & Co., August-Nagael-Strasse) to measure the maximum force (N) and strain at maximum force (mm). Test

conditions for compression used a preload of 1N, and a pre-load speed of 50mm/min for up to 60 sec.. Force was zeroed after pre-load, cycle speed was positioned controlled 10mm/min, standard travel set to 25mm, the upper force limit 1KN and maximum test duration was 1min.

4.3.4 Expansion ratio

Expansion ratio was calculated for the extrudates as the cross sectional area of the extrudate divided by the cross sectional area of the die outlet. Each measurement was taken ten times and the results were averaged.

4.3.5 Bulk density

Unlike expansion ratio, bulk density accounts for expansion of the product in all directions (Falcone and Phillips, 1988) and is measured as mass of product residing in a specified unit of volume (Obatolu et al., 2006). Ten 4mm samples from each extrusion run were weighed and divided by the approximate volume of the sample to calculate the bulk density.

4.3.6 Shear strength

Shear strength is measured as the shear force required to break a product relative to its cross sectional area and is typically indirectly related to the expansion ratio (Chinnaswamy and Hanna, 1988). Ten measurements of shear strength were taken for each sample to obtain an average result.

4.3.7 Sensory evaluation

Sensory evaluation of extrudates was done following the consumer texture profile method outlined by Szczesniak et al. (1975) with some modifications. In summary, the method combines the use of texture terminology to describe a product with a scaling technique to provide directional information about the product. Sixty three panellists were recruited; the results of 56 which were complete were used for calculations. Panellists were presented with a ballot and asked to assess 3 tangible products made using whole yellow pea flour including: a formulation with 50% pea flour, 0% hull and 50% starch processed at barrel temperatures of 30,70,90, 135 and 135°C, and a formulation with 50% pea flour, 0% hull 50% starch at 30,70,90, 120 and 120°C and a formulation made with 50% pea flour, 10% pea hull, 40% starch and processed at barrel temperatures of 30,70,90, 135 and 135°C. In addition an intangible “ideal” product, the characteristics of which vary from panellist to panellist based on their opinion was used to get the perception of the product by each panellist of an ideal snack food. Predetermined characteristics evaluated included: toothpack, bad texture, hard, puffiness, soft, crispiness and good texture on a continuous scale with 7 anchor points (a copy of the ballot can be found in Appendix K) with the far left anchor representing the absence of the characteristic in the product and the far right anchor indicating a strong prevalence for the characteristic in the product. The difference between the sample characteristic tested and the ideal value for the characteristic was taken for the opinion for each panellist. The average of the difference was taken for all panellists and the results averaged.

4.3.8 Statistical analyses

Extrudates were evaluated for significant difference at $p < 0.05$ using Statistical Analysis Software (SAS, version 9.1) and differences located using a Tukey test. Each blend was run once with exception to the mid point blend (40% pea flour and 5% pea fibre) which was run in triplicate. Ten samples were used for texture analysis and the results were recorded as an average. Optimization of pea blend formulations was done using Design Expert software (Version 7.1, Minneapolis, Minnesota). The criteria for optimization required that flour incorporation was a maximum between 30 and 50% with an importance of +++, hull incorporation was a maximum between 0% and 10% with an importance of +++, bulk density was minimized but in the range of 0.050g/cm^3 to 0.197g/cm^3 given an importance of +++, shear strength in the range of 8.86N/cm^2 to 31.24N/cm^2 with an importance of +++ and expansion ratio was to be in the range of 8.00 to 17.83, values which were chosen to reflect results from commercial samples analyzed (Full optimization results are shown in Appendix D). Statistical analyses are shown in Appendices L through O.

4.4 Results and discussion

4.4.1 Expansion ratio

The expansion ratio of pea based snacks ranged from 5.53 for the 50-10 green pea formulation to 14.76 for the 30-0 green pea formulation as seen in Figure 4. Generally, a greater expansion ratio is more desired in puffed snack foods as this is correlated with a lighter, crisper product. Optimal expansion of corn starch was

studied by Chinnaswamy and Hanna (1988). They found that by testing 25% amylose corn starch and a 3mm die opening with a temperature range of 120-180°C the best conditions for expansion ratio of corn starch was at 140°C 14% moisture (db), 150 rpm screw speed and feed rate of 60g/min yielded an expansion ratio value of 16.1. Expansion ratio will depend on the extrusion conditions as well as the blend formulations which are being tested.

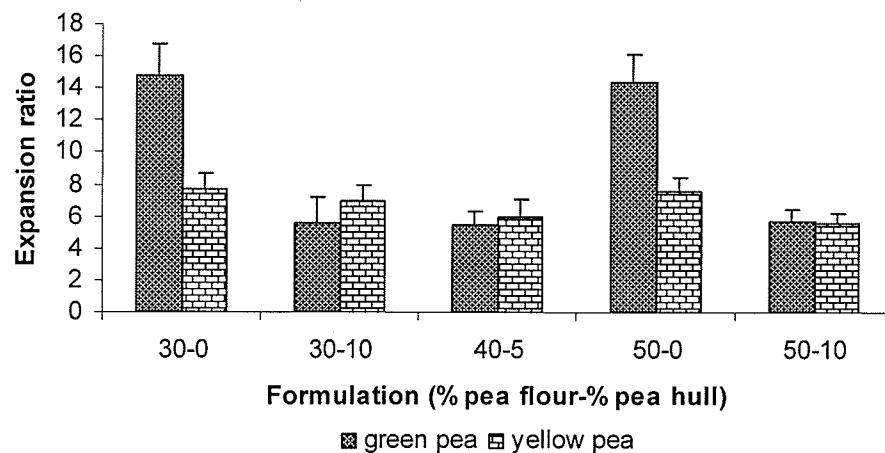


Figure 4: Expansion ratio of yellow and green pea puffed, extruded products varying in concentrations of pea flour and pea hull (n=10 for all formulations except for the 40-5 samples where n=30)

As compared to literature values for other pulse crop extrudates, Berrios et al. (2008) achieved expansion ratios of 10.50 to 12.13 for garbonzo beans (chickpeas) and by using fine pin milled black bean flours at 160°C, a feed rate of 25kg/h and an 18% moisture content produced an expansion ratio of 6.74 ± 0.86 . Using twin screw extrusion Berrios et al. (2004) extruded black beans at 200 rpm with an 80g/min feed rate and a 20% total moisture content expanded to a ratio of 6.70 but this was increased to 13.45 with the addition of 0.5% sodium bicarbonate. These

values are similar to the range obtained in this study which uses pea flour and pea hull in conjunction with pea starch. This is expected considering the similar nature of beans, chickpeas and peas in terms of protein, starch and fibre. Using a twin screw extruder, 160°C process temperature, 500rpm screw speed, 25kg/h feed rate, and with two 3.5mm die openings the expansion ratio of whole pea was 12.45 while the ratio for whole pea with corn starch (Hylon V at 20% of the formulation) increased to 16.46, a value slightly higher than compared to the current study (Berrios et al., 2008). Split pea flour expanded to a ratio of 20.72 and increased to 24.21 with 20% Hylon V corn starch added (Berrios et al., 2008). The differences between the whole pea and split pea flours may be explained by the presence or absence of hull in the flours.

A level of 30% pea flour was not found to be significantly different than a level of 50% pea flour or the starch control in terms of expansion ratio as seen in Table 9. Level of pea flour did not have as great an influence on the expansion ratio as the level of pea hull did. When pea hull concentrations were increased from 0% (11.9 expansion ratio) to either 5% or 10%, expansion was significantly reduced to 5.70 and 5.94 respectively (Table 10). Pea type (Table 11) also had a significant effect on expansion ratio; however, this is likely confounded by the presence of pea hulls in whole yellow pea flour which expanded significantly less (6.53 expansion ratio) compared to hull-less split green flour (8.11 expansion ratio).

Table 9: The effect of pea flour on bulk density, shear strength and expansion ratio

Pea flour	Bulk Density (g/cm ³)	Shear Strength (N/cm ²)	Expansion Ratio
30% ◀	0.11±0.02 ^{bc}	18.12±6.61 ^{bc}	8.77±3.84 ^a
40% ▲	0.12±0.02 ^b	21.56±6.11 ^b	5.70±1.10 ^b
50% ◀	0.10±0.03 ^c	15.80±7.91 ^c	8.30±3.80 ^a
Starch ▼	0.21±0.04 ^a	34.69±6.96 ^a	6.78±0.99 ^{ab}

Values given as an average ± standard deviation, different letters within the same column indicate significant difference (p<0.05) between the different levels of pea flour

◀ n=40; 2 pea types × 2 formulations × 10 replications

▲ n=60; 2 pea types × 1 formulation × 3 trials × 10 replications

▼ n=10; 10 replications

Table 10: The effect of pea hull on bulk density, shear strength and expansion ratio

Pea hull	Bulk Density (g/cm ³)	Shear Strength (N/cm ²)	Expansion Ratio
0% ◀	0.10±0.03 ^c	12.72±6.98 ^c	11.12±3.75 ^a
5% ▲	0.12±0.02 ^b	21.56±6.11 ^b	5.70±1.10 ^b
10% ◀	0.11±0.02 ^{bc}	21.20±4.86 ^b	5.94±1.19 ^b
Starch ▼	0.21±0.04 ^a	34.69±6.96 ^a	6.78±0.99 ^b

Values given as an average ± standard deviation, different letters within the same column indicate significant difference (p<0.05) between the different levels of pea hull

◀ n=40; 2 pea types × 2 formulations × 10 replications

▲ n=60; 2 pea types × 1 formulation × 3 trials × 10 replications

▼ n=10; 10 replications

Table 11: The effect of pea type on bulk density, shear strength and expansion ratio

Pea Type	Bulk Density (g/cm ³)	Shear Strength (N/cm ²)	Expansion Ratio
Split green ▶ (n=70)	0.11±0.03 ^c	16.71±7.65 ^b	8.11± 4.32 ^a
Whole yellow ▶ (n=70)	0.12±0.02 ^b	21.16±5.97 ^b	6.53±1.27 ^b
Starch ▼ (n=10)	0.21±0.04 ^a	34.69±6.96 ^a	6.78±0.99 ^{ab}

Values given as an average ± standard deviation, different letters within the same column indicate significant difference ($p < 0.05$) between the different levels of pea type

▶ n=70; 1 pea type × 5 formulations × 10 replications (+ 2 extra trials of the midpoint 40-5 sample × 10 replications)

▼ n=10; 10 replications

When Rampersad et al. (2003) extruded cassava and pigeon pea flour, lower expansion ratios were obtained as compared to those found in this study. Using a single screw extruder, a blend moisture content of 12%, temperature profile of 120-125°C, 520 rpm screw speed and 300g/min feed rate, expansion ratios of 1.68, 1.55, 1.38 and 1.18 were obtained for 0%, 5%, 10% and 15% added cowpea flour to cassava flour. Clearly, as cowpea flour was incorporated, expansion ratio decreased, an effect that was not as clearly seen in this study, as expansion ratio was more strongly related with the addition of pea hull than with pea flour. Differences between the level of fibre in cassava and cowpea, differences in the amylose and amylopectin ratios of starch as well as differences in the processing parameters and equipment may explain the discrepancies found between Rampersad et al. (2003) and the current study.

Fibre addition significantly affects the expansion ratio of lentils (Berrios et al., 2008). Without apple fibre addition, the expansion ratio was 30.7 while with added fibre this value was only 6.6-8.2 depending on starch source used (Berrios et al., 2008). Therefore, the effect of fibre was greater than that of starch source used for lentil extrudates. It was speculated that this effect was due to the decreased level of starch content in the dough due to the replacement of starch with fibre (Berrios et al., 2008).

This effect of fibre was also observed by Jin et al. (1995) while investigating the extrusion outcomes of yellow corn meal, soy fibre and cane sugar. It was found that using twin screw extrusion with a final barrel temperature of 121°C, a 3.08mm die opening, 45.4kg/h feed rate, total moisture content of 20% and a 325rpm screw speed that as fibre content increased from 0%-20%, the extrudate texture was more compact and less expanded. It was also observed that air cells were smaller and more numerous when observed with scanning electron microscopy, and cell walls of which were seen to be thinner at lower bran contents of 10% than compared to the thicker cell walls observed at 30% fibre. The effect of fibre was more thoroughly explained as the presence of bran causing a limiting effect on the expansion and extensibility of air cell walls, causing them to be incapable of steam retention and thus at a precarious point, the air cell bursts (Jin et al., 1995).

The effect of temperature (Table 12) also had a significant effect on expansion ratio. It was found that as temperature of the final two barrels was decreased from 135°C to 120°C to 110°C; the expansion ratio was significantly increased with increments of 6.64, 9.01 and 11.28, respectively, as seen in Table 12.

Falcone and Phillips (1988) investigated the extrusion of sorghum and cowpea blends under different conditions. Formulations of 100% sorghum, 67% sorghum-33% cowpea, 33% sorghum-67% cowpea, 100% cowpea, and a temperature range of 160°C -205°C with moisture contents ranging from 13-25% for single screw extrusion with a 7mm circular die opening using a screw speed of 180 rpm were studied. From this study the role of temperature on extrusion can be seen, as expansion tended to be greatest at 175°C, however, this was dependent on the formulation of the blend.

Table 12: The effect of extrusion processing temperature on bulk density, shear strength and expansion ratio of pea extrudates

Temperature of processing	Bulk Density (g/cm ³)	Shear Strength (N/cm ²)	Expansion Ratio
110°C	0.13±0.02 ^a	22.42±3.73 ^a	11.28±1.63 ^a
120°C	0.11±0.02 ^b	20.63±7.24 ^a	9.01±2.29 ^b
135°C	0.12±0.02 ^b	19.68±6.77 ^a	6.64±1.28 ^c

n=20

Berrios et al. (2008) investigated specifically the extrusion of pea flour using 160°C, 500 rpm, 25 kg/h feed rate and two die openings of 3.5mm diameter and were able to achieve an expansion ratio of 12.45 for whole pea flour, similar to results obtained for this study, while for split pea flour, an expansion ratio of 20.72 was achieved, greater than what was obtained for this study. This difference could be due to processing conditions and equipment differences as well as particle size, composition of peas in terms of fibre, protein and starch content and quality as

amylopectin exerts a positive effect and amylose a negative effect on expansion ratio (Falcone and Phillips, 1988).

4.4.2 Bulk density

Expansion ratio and bulk density generally are correlated; as expansion ratio increases, bulk density decreases. However, a lack of correlation between bulk density and expansion ratio may occur considering that bulk density accounts for expansion in all planes while expansion ratio only accounts for expansion in one direction (Falcone and Phillips, 1988). Bulk density of the pea extrudates ranged from 0.06g/cm³ for the 50-0 green pea sample to 0.140g/cm³ for 30-0 yellow pea samples (Figure5). Berrios et al. (2004) used a twin screw extruder with the parameters of 200 rpm screw speed, 80g/min feed rate and 20% total moisture content for the extrusion of black bean flour to produce a bulk densities of 0.35, 0.32, 0.33, 0.28, 0.26 and 0.24 g/cm³ for control, 0.1, 0.2, 0.3, 0.4 and 0.5% sodium bicarbonate addition respectively. These values were all greater than the range of bulk density incurred in the current study utilizing pea flour, pea fibre and pea starch for extrusion. A lower bulk density is generally more desirable considering that it indicates a lighter, crisper final product.

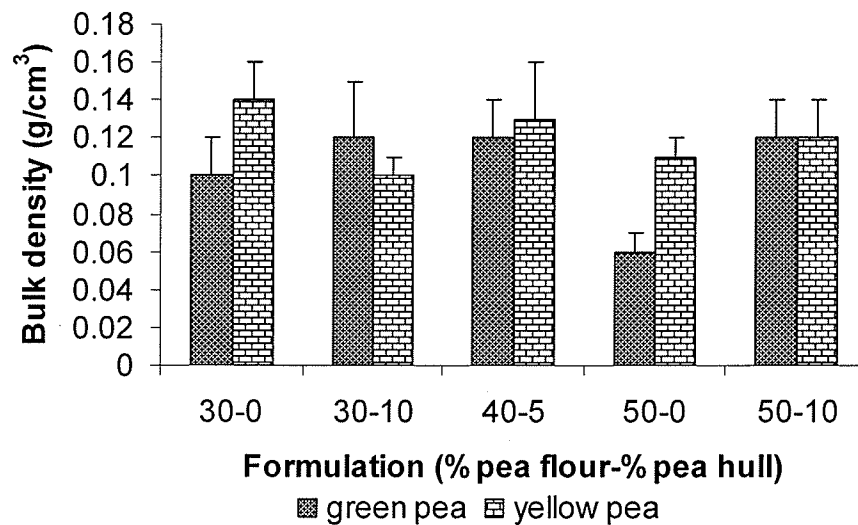


Figure 5: Bulk density of yellow and green pea puffed, extruded products varying in concentrations of pea flour and pea hull (n=10 for all formulations except for the 40-5 samples where n=30)

As seen in Table 9, as the concentration of pea flour used in blend formulations was increased, the bulk density as compared to the control was significantly reduced, suggesting that the addition of pea flour had a positive effect on the final extruded product. However, the only significant difference found in the addition of pea flour was between 40% and 50%, where 50% pea flour was significantly lower in bulk density than the 40% formulations. Cassava flour and pigeon pea flour were blended in ratios of 100:0, 95:5, 90:10, 85:15 and extruded with a single screw extruder with a moisture content of 12% (db) at 120-125°C and a 520rpm screw speed using a 300g/min feed rate (Rampersad et al., 2003). It was found that bulk density increased with increasing pigeon pea flour addition; 0%, 5%, 10%, 15% had 0.27, 0.29, 0.30 and 0.33 g/cm³ bulk density respectively (Rampersad et al., 2003). The opposite effect was found in this study, in that the addition of pea flour decreased the bulk density of the extrudates. This could be due

to the functionality of cassava as compared to pea starch as the base for extruded snacks as well as differences between pigeon pea and dry field peas in terms of fibre, starch and/or protein content as interactions between these components. Processing conditions may also have had an effect. The moisture content used in the study by Rampersad et al. (2003) was considerably lower (12%) than the 15% moisture content used in the current study.

Pea hull was found to be more influential than pea flour inclusion in terms of bulk density. The lowest bulk density observed (0.06g/cm^2) was in a sample where pea flour was added but additional pea hull was not incorporated; (50-0 green pea sample as seen in Table 10). This reflects the results from expansion ratio. The study by Jin et al. (1995) using yellow corn meal, soy fibre, pure sugar cane in a twin screw extrusion process and a final barrel temperature of 121.1°C , 3.08mm die opening, 45.4kg/h feed rate and moisture content of 20% with screw speed of 325 rpm found that as fibre level increased from 0-20%, bulk density decreased however, further increase to 40% fibre caused an increase in bulk density.

The significant differences for bulk density values found between the types of pea used may again be attributed to the presence of hull in the whole yellow pea flour compared to the absence of hull in split green pea flour (Table 11). However, when either flour was added to pea starch, a positive effect on bulk density was demonstrated as compared to the bulk density of extruded pea starch.

As temperature decreased from 135°C and 120°C to 110°C , bulk density became significantly greater as seen in Table 12. In this case, bulk density and expansion ratio were not correlated, as explained through the rationale that

expansion ratio only accounts for expansion from the cross sectional area while bulk density accounts for expansion in all directions. The effect of temperature depends greatly on the material which is being extruded and its physical properties; for instance gelatinization temperatures as well as amylopectin and amylose content will cause different properties of extrudates at different processing temperatures. In the Falcone and Phillips (1988) sorghum and cowpea experiment, of the parameters which were discussed in the previous section, the lowest bulk density results of 0.26g/cm^3 was obtained for 100% sorghum at 175°C and 20.5% moisture content. In this study, it appears that as the percent of cowpea incorporated in the blend increases, the temperature required achieving the lowest bulk density also increases. However, the lowest overall bulk density was achieved at a lower temperature using 100% sorghum.

When bulk density of the test samples was compared to bulk density of commercial samples, one commercial sample had a bulk density of $0.39\pm 0.10\text{g/cm}^3$ while another sample had a bulk density of $0.18\pm 0.02\text{g/cm}^3$. The test samples were generally less than both of these samples indicating that the samples produced in this study had a structure that was less dense than those products typically found on the market.

4.4.3 Shear strength

Shear strength of samples ranged from 5.20 to 25.01 N/cm^2 for samples 50-0 green pea and 50-10 green pea (24.53 N/cm^2 for the 40-5 yellow pea sample)

respectively as seen in Figure 6. Extruded pea starch in comparison had much greater shear strength than all treatments at 34.69 N/cm^2 (Table 9).

As pea flour was added to the formulation in increasing amounts, shear strength was reduced, 50% pea flour was significantly lower than 40% and the pea starch (Table 9). The addition of pea fibre on the other had a much greater effect. The samples with added flour, but with no added pea hull had significantly lower shear strength as seen in Table 10. Jin et al. (1995) also indicated the effect of fibre on shear strength. Yellow corn meal, soy fibre, pure sugar cane were subjected to twin screw extrusion, a temperature of 121.1°C , 3.08mm die opening, 45.4kg/h feed rate, moisture content of 20% and a 325 rpm screw speed. A Warner Bratzler shear blade was used to cut through the cross sectional area of the samples and indicated that shear strength increased with increasing sugar and fibre content. Breaking strength was related to microstructure suggesting that thicker cell walls resulted in greater shear force.

With respect to pea type used, there was no significant difference between using the shear strength for whole yellow pea flour or the split pea flour (Table 11). In terms of temperature, no significant difference was found between extrudates processed at 110°C , 120°C and 135°C (Table 12).

Commercially available samples had a very large difference in terms of their shear strength. One product had shear strength of $8.86 \pm 4.40 \text{ N/cm}^2$ while the other product had shear strength of $31.24 \pm 11.84 \text{ N/cm}^2$. The test samples in this study had shear strengths which were generally intermediate to this. Falcone and Phillips (1988) compared their sorghum/cowpea blended extrudate force at failure (N) to

that of fried or baked commercial corn snacks. The force at failure (N) that was comparable to fried/baked commercial corn snack ($\sim 23\text{N}$) and was obtained with samples made of 67% sorghum and 33% cowpea processed at 190°C and 23% moisture content (27N) as well as the 33% sorghum sample blended with 67% cowpea processed at 190°C using a 23% moisture content.

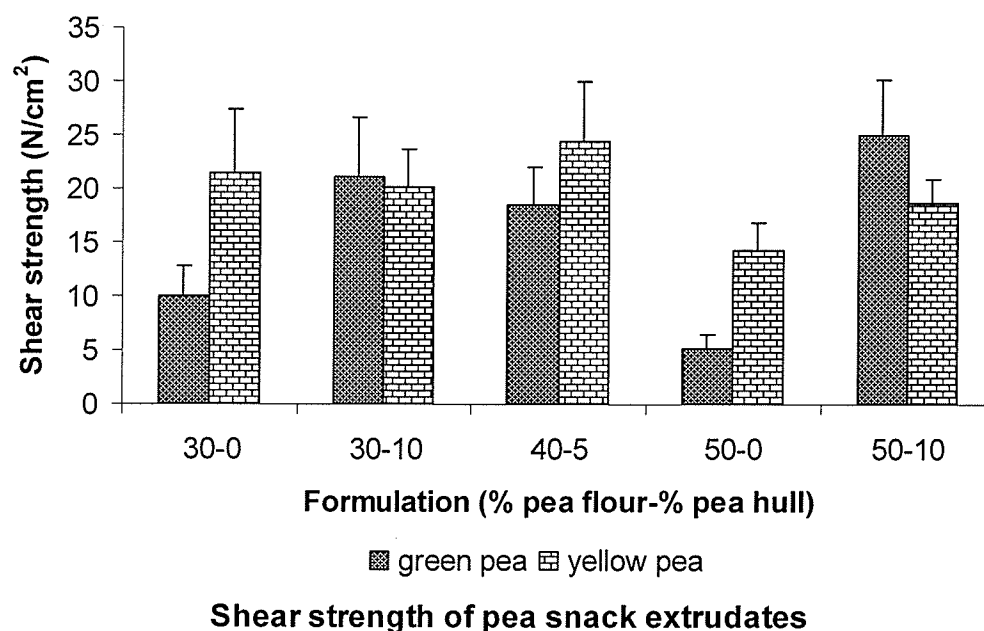


Figure 6: Shear strength (N/cm^2) of yellow and green pea puffed, extruded products varying in concentrations of pea flour and pea hull ($n=10$ for all formulations except for the 40-5 samples where $n=30$)

4.4.4 Optimization of formulations

When using response surface methodology for the optimization of extruded blends, three different outcomes were suggested to attain a minimum bulk density in the range of 0.08 to $0.28 \text{ g}/\text{cm}^3$, shear strength 8.86 to $31.24 \text{ N}/\text{cm}^2$ and an expansion ratio of 6.0 to 17.8 so that they would be comparable to commercial products: the

results may be found in Table 13. The outcomes suggest the use of 50% green pea flour, 7.34 % pea fibre made up to 100% using pea starch. This formulation yields predicted values for bulk density, shear strength and expansion ratio of 0.109 g/cm^3 , 18.590 N/cm^2 and 8.00 respectively. A second formulation option was given as 50% green pea flour with 6.74% pea fibre and made up to 100% with pea starch. This formulation resulted in predicted values for bulk density, shear strength and expansion ratio of 0.105 g/cm^3 , 17.439 N/cm^2 and 8.54 respectively. The final suggested formulation incorporated a lower amount of pea fibre using 50% green pea flour, 6.60% pea fibre and made up with pea starch. The result of this formulation gave predicted values of 0.105 g/cm^3 , 17.174 N/cm^2 and 8.67 for bulk density, shear strength and expansion ratio respectively. The optimization of the formulation reinforces the more significant effect that pea fibre has on the characteristics of the extrudates. Where green pea flour may be incorporated at its maximum tested value of 50%, pea fibre restricts the formulation and it may be seen that increasing the level of pea fibre caused an increase in bulk density and shear strength and lowered the expansion ratio of the predicted values in the product.

Table 13: Optimization of extrusion formulations for pea flour and pea hull (made up to 100% with pea starch) for bulk density (g/cm^3), shear strength (N/cm^2) and expansion index using design-Expert software

Pea type	Pea Flour %	Pea Hull %	Bulk Density (g/cm^3)	Shear Strength (N/cm^2)	Expansion Index
Green	50	7.34	0.108	18.590	8.00
	50	6.74	0.105	17.439	8.54
	50	6.60	0.105	17.174	8.66
Yellow	50	7.34	0.113	18.650	6.64
	50	6.74	0.112	18.343	6.72
	50	6.60	0.112	18.273	6.74

The optimization of whole yellow pea flour in extrudates was unable to achieve the parameters set for the optimization of green pea flour with regard to setting expansion index to be at least a ratio of 8. With this parameter compromised, the outcomes of optimization may be seen in Table 13. When 50% whole yellow pea flour was formulated with 7.34% pea fibre, bulk density, shear strength and expansion ratio were predicted values of 0.113g/cm^3 , 18.650N/cm^2 and 6.64 respectively. As the level of pea fibre was reduced to 6.74%, the bulk density, shear strength and expansion ratio was 0.112g/cm^3 , 18.343N/cm^2 and 6.72 respectively. Further reduction of pea fibre addition to 6.60% did not cause any large changes in the predicted values for bulk density, shear strength and expansion ratio which were 0.112g/cm^3 , 18.272N/cm^2 and 6.74.

4.4.5 Sensory evaluation: effect of pea flour, pea hull and temperature

Hardacre et al. (2006) made use of a Just-About-Right scale to assess the texture of expanded snack food wafers made of corn, lentil and other ingredients. Thirty-seven subjects were recruited to give their preference of characteristics of different wafers including colour (too light to too dark), hardness (too soft to too hard), taste (too strong to too bland) and toughness (very tough to very brittle) on a 1-5 scale with the 3 point indicating that the characteristic was at the just right level. A similar approach was used in this study with panellists indicating on a 1-7 point scale at what intensity pre-determined characteristics were present in each of three yellow pea samples while the ideal, intangible product characteristics were assessed separately from the actual samples. Whole yellow pea flour was used in the sensory analysis for two reasons, firstly, the acreage grown for yellow peas is much greater than for green peas in Canada, and therefore, would be more feasible to produce at a large scale. Secondly, the yellow pea extruded products are closer to what is seen in the market in terms of appearance for colour and although colour was not assessed in this sensory evaluation, it was felt that the use of green pea would confound negative opinions about colour in the texture data.

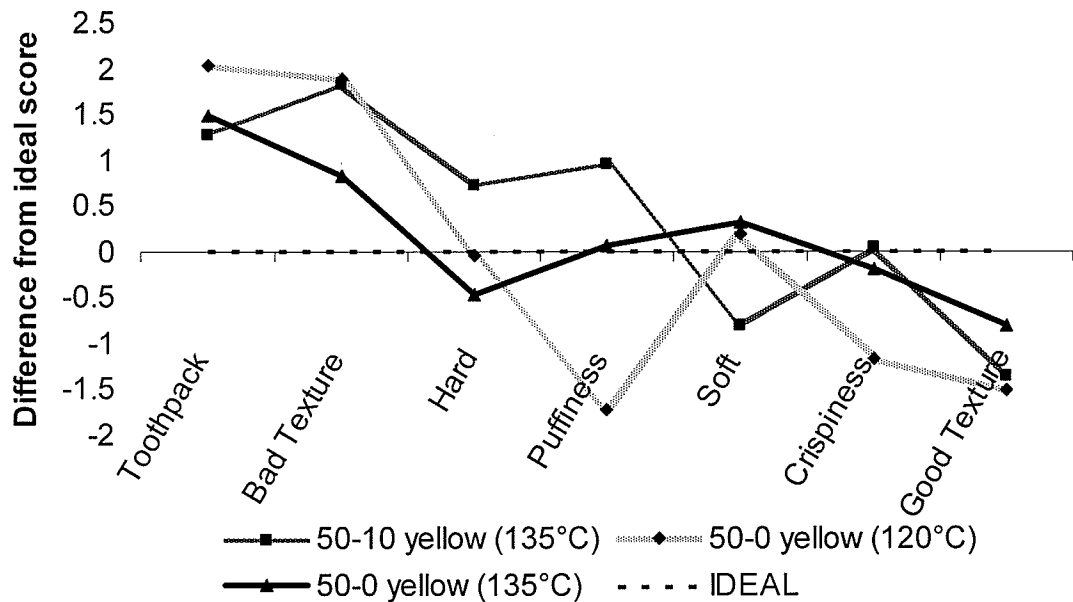


Figure 7: Sensory evaluation of texture characteristics of yellow pea extruded products (n=56 panellists)

From the sensory evaluation results in this study, it is possible to see which samples either over performed (positive score) or underperformed (negative score) what panellists considered being ideal for each textural characteristic (Figure 7). Raw data from the sensory panellists is given in Appendices P-S. For toothpacking, it was found that all samples had more toothpacking than what would be considered ideal. The 50-0 yellow pea sample processed at 120°C had a greater degree of toothpack than the other two samples. The lower temperature of processing likely caused this effect considering that the final product would have higher moisture content and would stick to the teeth more when chewed. The samples also had more of a bad texture than compared to an ideal sample. Whereas the 50-10 yellow pea sample at 135°C and the 50-0 yellow pea sample at 120°C had the same degree of bad texture compared to the ideal sample, the 50-0 at 135°C was less severe and closer to the ideal sample. When extrudates were tested for how hard they are, it

was found that while the 50-10 at 135°C sample over performed or was harder than what panellists consider being the ideal level of hardness, but the 50-0 at 135°C sample underperformed in terms of hardness. The 50-0 sample at 120°C however, was nearly ideal in terms of its hardness value. The samples varied greatly in terms of their puffiness. Where the 50-10 at 135°C sample was considered to be too puffy, the 50-0 sample at 120°C was considered to not be puffy enough. However, the 50-0 at 135°C sample was considered to be nearly ideal in terms of puffiness. The attribute soft reflected the hardness of the samples, where the 50-10 at 135°C sample underperformed or was not soft enough while the other two samples were slightly too soft but they were both very close to the ideal level of softness desired for puffed snack food products in the opinion of the panellists. In terms of crispiness of the products, the 50-10 at 135°C sample was almost exactly ideal while the 50-0 at 135°C sample was slightly less crispy than the ideal and 50-0 at 120°C sample was much less crispy than it should have been ideally. When samples were assessed for their degree of good texture, panellists indicated that 50-0 at 135°C sample had the closest texture to what they consider to be the ideal texture while the other two samples were similar in their degree to which they underperformed in terms of good texture. Generally, the product deviated the least from the ideal sample was the 50-0 whole yellow pea flour extruded at 135°C. However, it should be recognized that whole yellow pea flour was used to make the yellow pea flour meaning that a natural percentage of pea hull was incorporated in this product.

4.5 Conclusions

The use of pea flour, pea fibre and pea starch has potential to be used in many food products as demonstrated here as a puffed, extruded snack food. Images of these products are shown in Appendix T. Not only is the use of pea fractions technologically feasible, sensory evaluation indicates that the product characteristics closely resemble what consumers indicate to be ideal in terms of specified characteristics. Product characteristics could also be altered by changes in particle size of flours, protein content and level of starch degradation, factors that were not investigated in the current study. The product could be further characterized by investigating peak frequency of the compression curve and bubble frequency and size. Future work in terms of shelf life stability, scale up as well as market research is necessary to create a final marketable product.

CHAPTER 5: ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENT OF FOOD PRODUCTS FORMULATED WITH PEA FLOUR

5.1 Abstract

Tortillas and puffed extruded snack food products were made with varying concentrations of pea flour and pea fibre. For tortillas, the antioxidant activity was measured for the blends and the processed product using ABTS and DPPH antioxidant methods while total phenolics were measured through the Folin-Ciocalteu method. The extrudates and blends used in extrusion were measured for their antioxidant activity using the ABTS and ORAC methods while total phenolic content was again measured using the Folin-Ciocalteu method. Processing of composite pea flours to tortillas caused a reduction in antioxidant activity and total phenolic content. Extrudate antioxidant activity as measured by the ABTS method was reduced by processing, while increases were observed for the antioxidant activity using the ORAC method. Extrudate total phenolic content was reduced with processing. The results of the methods were compared through a correlation analysis which indicated limited correlation between methods especially when correlating different products and processing conditions.

5.2 Introduction

Growing concerns over heart disease and cancer are causing consumers to consciously increase their consumption of antioxidative compounds. Antioxidants are important because they encourage the scavenging of reactive oxygen species (ROS) which contribute to the degeneration of tissues such as proteins, lipids and DNA (Wuet al., 2004) causing degenerative diseases through oxidative damage

(Troszyńska et al., 2007). For instance, in an epidemiological study discussed by Xu et al. (2007), the increased consumption of foods high in phenolic, antioxidant compounds such as fruits, vegetables, legumes and cereals was related to a decreased instance of disease such as cancer, aging and cardiovascular disease. This new antioxidant initiative has lead to the development of functional foods and nutraceuticals which contain an increased level of antioxidants (Cheung et al., 2006). The incorporation of peas in food products, such as tortillas, may increase the consumption of antioxidants and thus, lower the instances of degenerative diseases.

Peas contain elevated levels of antioxidative compounds, particularly in the hull component of the pea where these compounds are needed by the plant to ward off oxidative damage from oxygen, light and other environmental stresses (Troszyńska et al., 2002). However, the antioxidant content of peas varies due to a number of factors including varietal differences, growing and harvesting conditions, growing location as well as environmental factors (Troszyńska et al., 2002).

In plants, the major contribution to antioxidant activity is believed to be through the dominant group of flavonoids, phenolic compounds (Wu et al., 2004). Research has identified common phenolic compounds present in pea to include *p*-hydroxybenzoic acid, protocatechuic acid, vanillic acid, *p*-coumaric acid, ferulic acid and apigenin-8C-glucoside (Dueñas et al., 2004, Lopez Amores et al., 2006 and Troszyńska et al., 2002). The concentration of these compounds found was dependent on the fraction of the pea used in the analysis (hull vs. cotyledon) as well as the variety of pea that was used. Dueñas et al. (2004) found protocatechuic acid in concentrations of 2.77 µg/g and 19.82 µg/g in the cotyledon of ZP-849 and

Fidelia variety peas, respectively, while its concentration in the pea hull was found to be 50.15 µg/g and 76.99 µg/g for ZP-849 and Fidelia variety peas respectively.

Previous research has identified some of the common phenolic compounds that contribute to antioxidant activity. However, research on the change in antioxidant activity following food processing is limited. Some previous research has been done of the change in total phenolic levels in processed beans (Granito et al., 2007). Li et al. (2007) studied the change in total phenolics and antioxidant activity of muffins baked using purple wheat bran and Mexican blue corn antioxidant activity following processing into tortillas and chips was studied by Del Pozo-Insfran et al. (2006).

Wu et al. (2004) looked at how processing affects the AOA of foods. Although previous studies have indicated that some vegetables result in an increased AOA following cooking, these results are not consistent among all foods. Wu et al. (2004) summarized these results indicating that foods more resistant to thermal processing contain active polyphenolic flavonoids rather than vitamins and related compounds, which will suffer a greater depreciation of antioxidant activity.

This research will focus on determining the antioxidant activity and total phenolic content of food products made with pea flour and pea hull as ingredients. These food products include tortillas as well as extruded puffed snack foods. Tortillas will be evaluated for their antioxidant activity using the ABTS and DPPH methods as well as total phenolic content using the Folin-Ciocalteu method. Extrudate antioxidant activity will be measured using the ABTS and ORAC methods while total phenolic content will be measured using the Folin-Ciocalteu

method. Differences between the activity of raw blends and the final processed foods will be evaluated.

5.3 Materials and Methods

5.3.1 Pea flour, pea hull and wheat flour blends for tortillas

Whole dry field peas were obtained from Tom Warkentin at the Crop Development Center in Saskatoon, Saskatchewan. Four varieties of yellow pea (Alfetta, Eclipse, SW Midas and CDC Mozart) as well as 2 varieties of green pea flour (Camry and Cooper) were used. Peas were split and dehulled according the CIGI Special Crops method. Three kilograms of peas were weighed into a small pail. The weight was recorded and the moisture content was measured using a Seedburo® moisture meter (Seedburo Equipment Company, automatic moisture meter model 1200A, 1022 west Jackson Blvd, Chicago Ill.). Peas were processed through a Pitting machine (SK Engineering and Allied Works Bahraich -271801-India) with a gap width of 5/8" after a handful of the corresponding pea was run through the equipment to flush out any remaining pulses. The pitted peas as well as the pitting dust were weighed. Tempering of the peas to 14% moisture content followed pitting based on the equation:

$$(\text{mL of water to add}) = ((100 - mc_i) / (100 - mc_f) - 1) * W$$

Where mc_i represents the initial moisture content (%), mc_f represents the final moisture content (%) and W represents the weight of the sample (g). Water was added slowly to the peas while continuously hand stirring. The peas sat in the closed pail for 1h before they were stirred again and left for an additional 4h (total

of 5h tempering time). Moisture content and weight were recorded following tempering. Peas were transferred to a heater (SK Engineering and Allied Works Bahraich -271801- India) for 20 minutes once the temperature reached 70°C. Peas were stirred often with a wooden spoon during heating. Peas were transferred back to a pail following heating and then moved to cooling towers (SK Engineering and Allied Works Bahraich -271801- India) to cool overnight.

Dehulling and splitting of the peas took place the following day (16h). A plastic bag was used to collect the flow of peas from the bottom of the cooling tower. The peas were again weighed and the moisture content measured. A sheller (SK Engineering and Allied Works Bahraich -271801- India) was used to remove the hulls from the cotyledon. Shelled peas were weighed and then passed through an aspirator (SK Engineering and Allied Works Bahraich -271801- India) to separate the cotyledon fraction from the hull fraction. The cotyledon fraction as well as the hull fraction was weighed and the cotyledon yield was determined for each cultivar. The split cotyledon fraction was milled into flour using a hammer mill (Jacobson Inc, Minneapolis MN) with a screen size of 1.5/64". The pea hull fraction was ground using a coffee grinder (Black and Decker) to a particle size of less than 850 µm.

CIGI also provided a straight grade Canada Western Red Spring wheat flour (Laura var.) that was used as the control and base of the composite flours. The protein content of the flour was 13.26% (combustion nitrogen analysis, N×5.7) with a moisture content of 13.41% and wet and dry gluten at 34.4 and 11.2% respectively. The characteristics of the wheat flour were predetermined by CIGI.

Five composite flours were made of varying concentrations of pea flour, ground pea hull fraction and wheat flour. The composition of these flours is illustrated in Table 14. The levels of pea flour used ranged from 15% to 35% while pea hull was incorporated at levels ranging from 0% to 5%. Composite flours were made on an as is basis by weight, were well mixed and stored in Ziploc® bags at 4°C until used.

Table 14: Composite flour formulations for yellow and green peas mixed with CWRS (Laura var.) wheat flour

Code	Pea flour %	Pea hull %	Wheat flour %
0-0 (control)	0	0	100
15-0	15	0	85
15-5	15	5	80
25-1.5	25	1.5	73.5
35-0	35	0	65
35-5	35	5	60

All composite flours were made on an as is basis % by weight

5.3.2 Tortilla procedure

Moisture content for all composite flours was determined using the AACC Moisture Air-Oven Methods (44-15A) (AACC, 1999)

Farinograph absorption was determined for all composite flours using the AACC method 54-21 (small 50g bowl). (AACC, 1982)

Tortillas were made following the method described by Ambalamaatil et al. (2006). One hundred grams of flour (14% moisture basis) was mixed with 1.5g of baking powder (Magic, commercial brand), 1.5g salt (Fisher Scientific) and 9 g of shortening (Crisco vegetable commercial) in a 200g mixer (National MFG. Co.,

Lincoln, Nebr.) for 2 minutes. Distilled water was then added and mixed for a total of 7, 6.2 and 3 minutes for 15%, 25% and 35% pea flours respectively. Volume of water varied depending on the Farinograph absorption value (mL of water required to achieve 500 farinograph units) for the flour, 10mL less the FAB was used for the tortilla dough formulation. Dough was formed into 35g balls, placed in plastic containers, covered with a damp cloth and allowed to rest for 5 min. Dough was pressed for 8 sec with a Doughpro press (Proprocess Corporation, CITY) to a thickness level in between the “thick” and “thin” setting (approximately 1mm final thickness) at 93°C, transferred to a 220°C frying pan for 30 sec, flipped and cooked for 40 seconds, flipped and cooked for a final 10 sec. Tortillas were cooled on a wire rack for 1 min before being placed in an open polyethylene bag to cool overnight at 25°C.

Tortillas were freeze dried (VirTis Genesis, Gardiner, NY) before all chemical analyses. All tortilla results were recorded on a dry weight basis.

5.3.3 Pea flour, pea fibre and pea starch blends for extrusion

Whole yellow pea flour (Eclipse variety), split green pea flour without hull and finely ground pea fibre (pea hull) were donated from Best Cooking Pulses of Portage la Prairie, Manitoba. A native, food grade pea starch made from yellow Canadian field peas was donated by Nutri-Pea Limited (Portage la Prairie, Manitoba) (Specification sheets may be found in Appendices F, G and H). Five different formulations with varying concentrations of pea flour, pea fibre and pea starch totalling 2.5kg were made for both yellow and green pea flours on an as is

basis. The formulations included using 30, 40 and 50% flour combined with 0, 5 and 10% pea fibre, the formulations are shown in Table 15. Each formulation was run singularly with the mid point (40-5) processed in triplicate. Straight pea starch was run as the control.

Moisture content of formulations was determined using the AACC moisture air oven method 44-15A (AACC, 1999).

5.3.4 Extrusion method

Blends were extruded with an APV co-rotating twin screw extruder (MPF19-25, 2.2kW motor, 19/25D, APV Baker Ltd. Peterborough England) and a circular die hole with a 4.5mm diameter under a high shear screw configuration. Prior to extrusion, blend feed and moisture injection rate were calibrated. Total moisture content of the blends was adjusted to 15% through the addition of water while the first three of five temperature barrels of extrusion were set to 30°C, 70°C, and 90°C. The effect of temperature was investigated by changing the final two barrel temperatures; temperatures were tested at 110°C, 120°C and 135°C. Screw speed was kept at a constant 240rpm. Following extrusion, the extrudates were dried in a convection oven at 135°C for 5 minutes, allowed to cool and placed in a polyethylene bag overnight until texture analysis the following day. A table of extrusion conditions and screw configuration can be found in Appendices I and J.

Table 15: Formulations for extrusion of yellow and green pea flour pea fibre and pea starch blends

Sample Code	Pea flour %	Pea hull %	Pea Starch %
30-0	30	0	70
30-10	30	10	60
40-5	40	5	55
50-0	50	0	50
50-10	50	10	40

5.3.5 Total phenolic content (TPC)

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method as described by Singleton and Rossi (1965) with modifications by Gao, et al. (2002). 0.2 g of sample was extracted with 4 mL acidified methanol at room temperature for 2h on a rotary shaker (84rpm). The mixture was centrifuged for 10 min on a table centrifuge (GLC-1, Sorval, Newton, CT) at 3000 rpm (906×g). The supernatant was decanted into polypropylene tubes and stored at -40°C until analysis. Results were recorded as mg ferulic acid equivalents (FAE)/g (dry weight).

5.3.6 Antioxidant activity (ABTS method)

The 2,2'-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS) method as described by Re et al., 1999 with some modifications was used to determine antioxidant activity of samples. Two tenths of a gram of sample was extracted in 10mL of methanol at 150 rpm for 2 hours on a model OS31 rotary shaker (Fermentation Design Inc., Allentown PA). Following which, the samples were centrifuged at 13000 rpm (11337×g) for 10min using a Sorval SS-34 rotor. Supernatant was decanted and stored at -40°C until analysis where 1mL of extract was added to 3.9mL of diluted ABTS solution (88µl of 140mM K₂S₂O₈ added to

5mL of 7mM ABTS solution and kept in darkness for 12-16h with absorbance adjusted to 0.7 at 734nm by the addition of the ABTS solution drop wise to 50% methanol), incubated at 30°C in a water bath for 6 min and absorbance measured at 734 nm using 50% methanol as the reference. Absorbance of samples was compared to a standard curve of Trolox. Results were recorded as μmol Trolox Equivalents (TE)/100g sample (dry weight).

5.3.7 Antioxidant activity (DPPH method)

The 2,2,-diphenyl-2-picryl-hydrazyl (DPPH) method was also used to measure the antioxidant activity of the samples following the method of Brand-Williams et al., (1995) with some modification. A 0.3g sample was extracted with 3mL of methanol by shaking for 2h on a wrist action shaker (RKVSD Laurel MD) At 84 rpm. Samples were then centrifuged for 10 min on a table centrifuge (GLC-1, Sorval, Newton, CT) at 3000 rpm (906 $\times$ g). 0.1 mL of supernatant was added to 3.9 mL of DPPH working solution (0.0025g/100mL methanol). Absorbance was measured at 515nm at t=0min and again at t=30min. The % decolouration was calculated as $(1 - ((\text{abs } t=30) / (\text{abs } t=0))) \times 100$ and was compared to the % decolouration of known concentration of a Trolox standard curve. Results were recorded as μmol TE/100g sample (dry weight).

5.3.8 Antioxidant activity (ORAC method)

Antioxidant activity was analyzed using the ORAC method as described by Li et al. 2007. A fluorescent probe, fluorescein was used in the assay to measure the

antioxidant ability of the compounds present in the samples. A strong antioxidant will be capable of inhibiting the loss of fluorescence of the probe when it is exposed to the 2,2'-azobis(2-amidopropane) dihydrochloride (AAPH) radical. Solutions prepared from extracting 0.2g of sample in 10 mL of methanol which were shaken for 2 h at room temperature were transferred automatically from a 96 well polystyrene microplate (Corning Incorporated, Corning, NY, USA) using a Precision 2000 Automated Microplate Pipetting System (BIO-TEK Instruments, Inc.). Fluorescence filters with an excitation wavelength of 485/20nm and an emission wavelength of 528/20nm were prepared for an FLx800 800 microplate reader (BIO-TEK Instruments, Inc., Winooski, VT). Software was used from KC4 3.0. Firstly, 120µl of the fluorescence working solution was added to each of the 96 well in the microplate. Twenty µl of the buffer solution (blank), Trolox standard, diluted sample and 20 µM of rutin control was then transferred to assigned wells. The microplate was then incubated for 20 min at 37°C. Next, 60 µl of AAPH (solution was added to the wells of the microplate and the plate was covered with an adhesive sealing film. The covered plate was placed in the FLx800 microplate. Fluorescence was measured for 50min at 37°C at one minute intervals. Fluorescein was the substrate in the reaction where AAPH produces the peroxy radical during measurement. Each sample was measured in quadruplicate to produce ORAC values as the area under the curve, which is calculated as;

$$AUC=0.5+f_1/f_0+f_2/f_0+\dots+f_{49}/f_0+0.5(f_{50}/f_0)$$

where f_0 = initial fluorescence reading at 0 min and f_i = fluorescence reading at time i min. ORAC results are recorded as TE µmol/g.

5.3.9 Statistical analysis

All data were recorded as means $\pm$ standard deviation and analyzed by SAS (ver 9.1) using Proc GLM and Tukey comparisons to test significant differences ($p < 0.05$).

5.3.10 Chemicals

Chemicals and reagents obtained from Sigma-Aldrich (St. Louis MO) included ferulic acid, potassium persulfate ($K_2S_2O_8$), ABTS, Trolox, DPPH, Folin-Ciocalteu. Fisher scientific (Nepean, Ontario) methanol and HCl were also used.

5.4 Results and Discussion

5.4.1 ABTS

Using the ABTS method, pea hull generally was higher in antioxidant activity than pea flour as seen in Table 16. In terms of varietal differences for pea flour, Midas (185.5 ± 6.3 $\mu\text{mol TE}/100\text{g}$) had a significantly greater antioxidant activity than most other varieties while the varieties with the lowest antioxidant activity were Mozart, Alfetta and Eclipse (133.8 , 134.0 , 143.3 $\mu\text{mol TE}/100\text{g}$ respectively). No significant differences were found between the different pea varieties for antioxidant activity in pea hull.

Table 16: Pea hull and pea flour antioxidant activity measured using the ABTS ($\mu\text{mol TE}/100\text{g}$) method

Variety	Pea flour antioxidant activity ($\mu\text{mol TE}/100\text{g}$)	Pea hull antioxidant activity ($\mu\text{mol TE}/100\text{g}$)
Alfetta Yellow Pea	134.0 $\pm$ 11.1 ^c	182.3 $\pm$ 17.6 ^a
Eclipse Yellow Pea	143.3 $\pm$ 8.0 ^{bc}	182.73 $\pm$ 7.4 ^a
Midas Yellow Pea	185.5 $\pm$ 6.3 ^a	188.0 $\pm$ 9.2 ^a
Mozart Yellow Pea	133.8 $\pm$ 13.8 ^c	174.8 $\pm$ 10.1 ^a
Camry Green Pea	166.4 $\pm$ 8.8 ^{ab}	171.1 $\pm$ 9.4 ^a
Cooper Green Pea	159.9 $\pm$ 12.3 ^b	171.7 $\pm$ 14.3 ^a

n=4, different letters within the same column represent significant differences between varieties at $p<0.05$

When pea flour and pea hull were blended with wheat flour to make tortillas, the greatest antioxidant activity was found to be in those formulations with the greatest amount of pea flour and hull, while the lowest concentration was found for the wheat flour control (60.2 $\mu\text{mol TE}/100\text{g}$) as indicated in Table 17. Following processing, the antioxidant concentration was greatest in both the control tortilla as well as the tortillas with a higher concentration of pea flour with and without pea hull. However, when looking at the percentage of antioxidant activity reduction due to tortilla processing, it was found that the wheat flour control increased in antioxidant concentration following processing while the pea flour and hull blends decreased in their antioxidant activity following tortilla processing conditions. This may indicate a difference in the stability of the antioxidants present in wheat as compared to those found in pea as measured through the ABTS method. No difference was found between green and yellow pea varieties.

Table 17: Tortillas and blend antioxidant activity measured using the ABTS ($\mu\text{mol TE}/100\text{g}$) method with formulations varying in pea hull and pea flour concentration

Formulation	Blend ($\mu\text{mol TE}/100\text{g}$)	Tortilla ($\mu\text{mol TE}/100\text{g}$)	% AOA Retained
Control	60.2 $\pm$ 3.8 ^{▶d}	69.3 $\pm$ 14.9 ^{▲a}	116.0
15-0	99.7 $\pm$ 20.9 ^{▲c}	50.1 $\pm$ 10.7 ^{▼c}	50.3
15-5	109.8 $\pm$ 17.9 ^{▲c}	52.2 $\pm$ 14.1 ^{▼cb}	47.6
25-1.5	111.8 $\pm$ 12.3 ^{▲bc}	57.7 $\pm$ 8.6 ^{▼b}	51.6
35-0	126.3 $\pm$ 17.0 ^{▲ab}	68.0 $\pm$ 16.5 ^{▼a}	53.9
35-5	127.1 $\pm$ 19.5 ^{▲a}	71.4 $\pm$ 11.9 ^{▼a}	56.2

Different letters within the same column represent significant differences between formulations at $p < 0.05$

▶n=4; ▲n=24, ▼n=48; 6 varieties $\times$ 2 tortilla replications $\times$ 4 ABTS replications

The processing conditions of pea extrudates for the whole yellow pea blends had an antioxidant activity which ranged from 35.8 to 84.9 $\mu\text{mol TE}/100\text{g}$ (30-0 and 40-5 formulations respectively) (Table 18). No significant differences were found in the antioxidant activity of the blends except for the case of the 30-0 blend, which was significantly lower. Following extrusion processing, the 50-10 sample had the greatest amount of antioxidants, significantly greater amount than the other samples with the exception of the 30-10 sample. This trend could be due to the presence of increased hulls, the antioxidants of which are thought to provide protection of the seed from oxidation from the elements during plant growth. The control sample, on the other hand, increased in antioxidant activity when subjected to extrusion processing. Using split green pea flour, the antioxidant activity of the blends decreased with decreasing concentrations of pea flour and pea hull. Following extrusion processing, similar reductions in the antioxidant activity of split green pea

extrudates as compared to whole yellow pea extrudates were observed. Again, the greatest retention of antioxidant activity was by samples containing greater amounts of added pea hull.

Looking more specifically at the effects of extrusion temperature on antioxidant activity of pea flour extrudates, Table 19 illustrates that as the temperature of the two final barrels increased from 110° to 135°C, the antioxidant activity levels significantly increased (with the exception of the whole yellow pea sample where no significant difference is observed between the 135° and 110°C samples). This indicates a change in the antioxidant profile of the samples under the high temperature, high pressure conditions induced by extrusion resulting in an increase in the measurable antioxidant activity of the samples. During tortilla processing at a lower temperature, the antioxidant activity was generally lower than the results obtained from extrusion processing.

Table 18: Extrudates and blends antioxidant activity measured using the ABTS ($\mu\text{mol TE}/100\text{g}$) method with formulations varying in pea hull and pea flour concentration

Formulation	Yellow blend ($\mu\text{mol TE}/100\text{g}$)	Yellow extrudate ($\mu\text{mol TE}/100\text{g}$)	% AOA retained	Green blend ($\mu\text{mol TE}/100\text{g}$)	Green extrudate ($\mu\text{mol TE}/100\text{g}$)	% AOA retained
Control	1.0 ± 0.4^c	11.50 ± 4.4^{bc}	1116.5	1.0 ± 0.4^c	11.5 ± 4.4^{bcd}	1116.5
30-0	35.8 ± 6.6^b	2.76 ± 0.1^d	7.7	64.7 ± 2.7^b	5.1 ± 0.5^d	7.8
30-10	77.2 ± 3.3^a	17.24 ± 1.5^{ab}	22.4	72.8 ± 12.2^b	31.3 ± 0.8^a	43.0
40-5	84.9 ± 7.7^a	9.08 ± 0.0^{cd}	10.7	78.6 ± 8.2^{ab}	17.6 ± 1.5^{bc}	22.3
50-0	63.8 ± 2.0^a	10.22 ± 1.0^{bcd}	16.0	76.9 ± 9.6^{ab}	10.7 ± 1.9^{cd}	14.0
50-10	79.6 ± 10.2^a	22.95 ± 0.0^a	28.8	104.1 ± 4.5^a	20.2 ± 2.5^b	19.4

Different letters within the same column represent significant differences between formulations for extrudates and blends at $p < 0.05$, $n = 4$

Table 19: Effect of temperature on the antioxidant activity of extruded snack foods using the ABTS method

Sample formulation	Extrusion Temperature	Antioxidant Activity ($\mu\text{mol TE}/100\text{g}$)
control	110	-0.4 ± 3.5^d
	120	10.1 ± 1.5^{bc}
	135	11.5 ± 4.4^{abc}
Green pea 50-10	110	9.8 ± 2.0^{bc}
	120	17.8 ± 0.0^{ab}
	135	20.2 ± 2.5^a
Yellow pea 50-0	110	4.8 ± 1.9^{cd}
	120	12.7 ± 1.0^{abc}
	135	10.2 ± 1.0^{bc}

Different letters within the same column represent significant differences between barrel temperatures in extrusion at $p < 0.05$
 $n=2$

5.4.2 DPPH

Using the DPPH method, pea flour was found to be lower in antioxidant activity than pea hull as seen in Table 20. In terms of varietal differences, Eclipse and Midas pea flours were found to have a significantly greater antioxidant activity than Mozart and Camry samples while Midas pea hulls were significantly higher in antioxidant activity compared to the other pea hull samples. These results generally reflected those of using the ABTS method for antioxidant analysis however, the AOA from the ABTS method were much higher than those of the DPPH method and no significant difference were found in the pea hull samples using the ABTS method.

Using the DPPH method, Xu et al., (2007) found the AOA of yellow and green peas were 2.0 $\mu\text{moleTE/g}$ and 1.5 $\mu\text{molTE/g}$ respectively but found no significant difference between green and yellow peas. However, yellow peas contained a range of AOA from 0.6-2.7 $\mu\text{moleTE/g}$, with SW Capri containing a significantly greater concentration of AOA than other varieties tested (Eclipse lowest at 0.6 $\mu\text{molTE/g}$). The range of values for green peas was 1.0-2.3 $\mu\text{molTE/g}$ with the K-2 variety being significantly greater in AOA than other green pea varieties when tested using the DPPH method.

Table 20: Pea hull and pea flour antioxidant activity measured using the DPPH ($\mu\text{mol TE}/100\text{g}$) method

Variety	Pea flour antioxidant activity ($\mu\text{mol TE}/100\text{g}$)	Pea hull antioxidant activity ($\mu\text{mol TE}/100\text{g}$)
Alfetta	51.1 $\pm$ 4.9 ^{ab}	76.5 $\pm$ 3.4 ^b
Eclipse	58.7 $\pm$ 4.7 ^a	71.1 $\pm$ 6.8 ^{bc}
Midas	59.5 $\pm$ 3.8 ^a	96.6 $\pm$ 1.9 ^a
Mozart	35.3 $\pm$ 4.3 ^c	70.8 $\pm$ 2.7 ^{bc}
Camry	44.2 $\pm$ 4.8 ^{bc}	66.3 $\pm$ 6.2 ^c
Cooper	53.1 $\pm$ 4.0 ^{ab}	70.5 $\pm$ 3.0 ^{bc}

Different letters within the same column represent significant differences between variety at $p < 0.05$, $n=4$

The results for this study were lower, due possibly to the differences in the pea varieties used or the growing conditions or storage conditions of the peas or the extraction method or sample preparation differences between the studies.

Yellow pea DPPH values were also found to be in the range of 0.0-2.6 $\mu\text{molTE/g}$ and 0.0-1.3 $\mu\text{molTE/g}$ for green peas (Xu and Chang, 2007). In the same study, it was also found that the extraction solvent had a major impact in the DPPH results in that yellow pea concentration values were ranked by extraction solvent as 70% methanol > 70% ethanol > 50% acetone while for green peas, the AOA of DPPH was greatest for 80% acetone > acidic 70% acetone > 50% acetone. The differences in the most efficient extraction solvents leads one to believe that the antioxidants present in yellow peas differs from those which are found in green peas and may explain the differences in the results between the two pea types.

The blends used to make tortillas had no significant difference in antioxidant activity when measured using the DPPH method and ranged from 44.5 to 53.2 $\mu\text{mol TE/100g}$ (Table 21). Following processing into tortillas, the DPPH antioxidant activities generally were reduced, with the exceptions of the control tortilla as well as the 35-5 tortilla. However, the percent of reduction was not as great as when measured using the ABTS method.

When the results for DPPH and ABTS were correlated, an r^2 value of 0.4948 is obtained (Figure 8). The correlation is low due mostly to the variation in samples from pea flour and pea hull.

Table 21: Antioxidant activity of tortillas and blends formulated with pea flour and pea hull blended with wheat flour using the DPPH method

Formulation	Blend antioxidant activity ($\mu\text{mol TE}/100\text{g}$)	Tortilla antioxidant activity ($\mu\text{mol TE}/100\text{g}$)	% AOA retained
Control	42.3 $\pm$ 4.8 ^{▲a}	66.4 $\pm$ 2.9 ^{▲a}	157.1
15-0	46.9 $\pm$ 13.9 ^{▲a}	36.7 $\pm$ 8.6 ^{▼c}	78.2
15-5	53.2 $\pm$ 11.5 ^{▲a}	44.8 $\pm$ 8.4 ^{▼bc}	84.2
25-1.5	44.5 $\pm$ 14.9 ^{▲a}	41.4 $\pm$ 14.3 ^{▼c}	93.1
35-0	51.0 $\pm$ 14.6 ^{▲a}	40.0 $\pm$ 11.9 ^{▼c}	78.4
35-5	50.4 $\pm$ 19.3 ^{▲a}	52.5 $\pm$ 8.4 ^{▼ab}	104.3

Different letters within the same column represent significant differences between formulations at $p < 0.05$

▲n=4, ▲n=24, ▼n=48

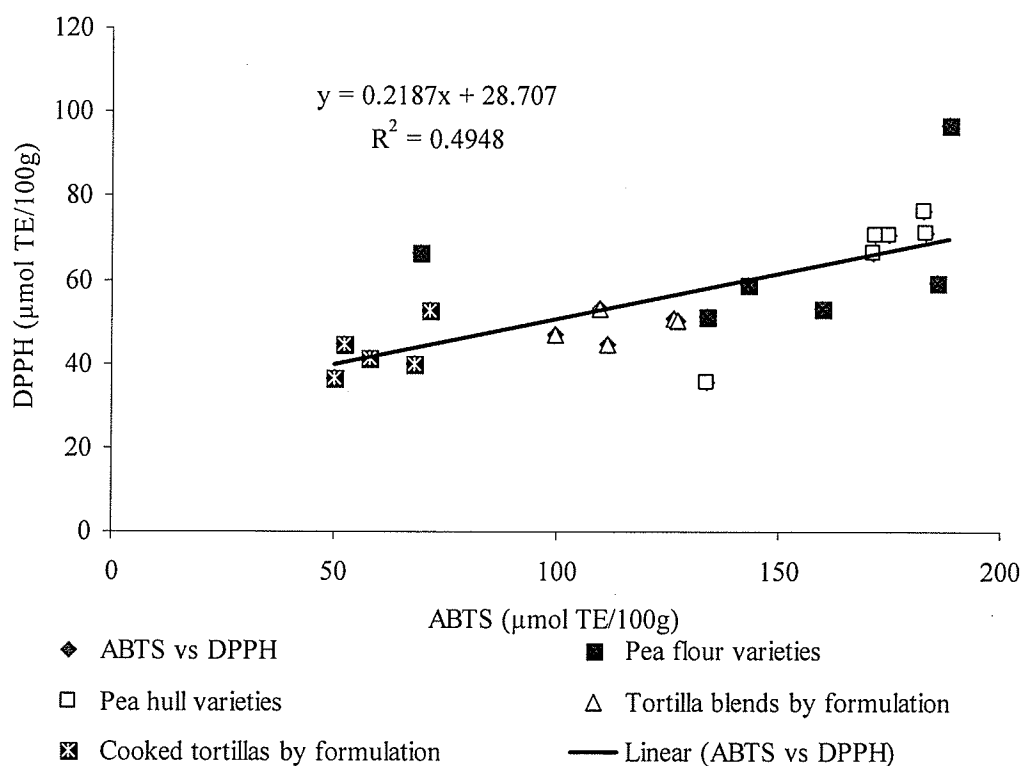


Figure 8: Correlation of ABTS and DPPH methods to measure the antioxidant activity of pea flour and pea hull tortillas and composite flours

5.4.3 ORAC

Antioxidant activity of samples based on ORAC values was also used to analyze blends used for extrusion and the extruded products. The results from this analysis may be found in Table 22. The whole yellow pea blends ranged in antioxidant activity from 72.7 to 80.0 TE $\mu\text{mol/g}$. There were no significant differences between the sample formulations which varied in their concentrations of pea flour and pea fibre. Following extrusion processing, the antioxidant activity for whole yellow pea flours increased; although the 30-0 sample was the only one which was significantly greater in antioxidant activity than the control. The green pea blends also had no significant differences in antioxidant activity when the concentrations of pea flour and pea hull were changed in the formulations. Following extrusion processing, the green pea extrudates, with the exception of the 30-10 and the 50-0 samples, tended to increase in antioxidant activity. The 30-0 sample had a significantly greater antioxidant activity than the control, 30-10 sample, 50-0 and 50-10 samples.

In a study by Xu et al. (2007) it was found that yellow peas had an antioxidant activity of 8.4 $\mu\text{mol TE/g}$ using the ORAC method while for green peas the antioxidant activity was 5.9 $\mu\text{mol TE/g}$ which was considerably lower than the values obtained for this study.

The USDA (2007) also issued data indicating the antioxidant content of peas obtained via the ORAC method and found that for “peas, split mature seeds raw”, the hydrophilic ORAC and total ORAC were both 524 $\mu\text{mol TE/100g}$ while for “peas, yellow, mature seeds, raw” these values were both 741 $\mu\text{mol TE/100g}$, split

peas generally means that the hull or seed coat has been removed, there is no indication of the removal of the seed coat in the latter sample in the USDA study. The presence of a seed coat would explain the higher antioxidant activity of the “peas, yellow, mature seeds, raw” samples. Again, the values obtained for this study were higher than those listed in the USDA database. The higher values may be due to the pea starch used in the samples which resulted in greater ORAC values than expected (77 $\mu\text{mol TE/g}$).

The effect of temperature on antioxidant activity of extrudates is shown in Table 23. In this case, the lower processing temperatures tended to yield greater antioxidant activity values except for the control sample.

Li et al. 2007 used the ORAC method to determine the antioxidant activity of muffins made with purple wheat bran. It was found that producing muffins from purple wheat bran resulted in an 89% decrease in ORAC AOA.

Table 22: Extrusion antioxidant activity using the ORAC method Extrudate and blend ORAC values

Formulation	Yellow blend TE $\mu\text{mol/g}$	Yellow extrudate TE $\mu\text{mol/g}$	% retained	Green blend TE $\mu\text{mol/g}$	Green extrudate TE $\mu\text{mol/g}$	% retained
Control	75.0 $\pm$ 2.4a	91.2 $\pm$ 3.2b	121.6	75.0 $\pm$ 2.4a	91.2 $\pm$ 3.2bc	121.6
30-0	79.8 $\pm$ 1.9a	103.2 $\pm$ 1.9a	129.2	71.5 $\pm$ 7.1a	107.7 $\pm$ 2.2a	150.8
30-10	80.0 $\pm$ 3.5a	97.5 $\pm$ 6.5ab	121.9	72.8 $\pm$ 5.5a	63.7 $\pm$ 5.8d	87.5
40-5	78.1 $\pm$ 0.8a	97.6 $\pm$ 2.8ab	125.0	70.9 $\pm$ 2.1a	101.7 $\pm$ 3.5ab	143.5
50-0	77.2 $\pm$ 4.7a	99.4 $\pm$ 2.1ab	128.7	71.8 $\pm$ 6.2a	60.1 $\pm$ 1.4d	83.7
50-10	72.7 $\pm$ 0.4a	100.4 $\pm$ 5.8ab	138.0	72.3 $\pm$ 6.9a	84.6 $\pm$ 6.9c	117.0

Different letters within the same column represent significant differences between formulation at $p < 0.05$
n=3

Table 23: Effect of temperature on extrudate antioxidant activity ORAC values

Sample formulation	Temperature	ORAC
Control	110	82.1±2.9d
	120	95.7±7.2bc
	135	91.2±3.2bcd
Green pea 50-10	110	88.5±2.5bcd
	120	84.3±1.8cd
	135	84.6±6.9cd
Yellow pea 50-0	110	108.2±3.6a
	120	109.6±2.7a
	135	99.4±2.1ab

Different letters within the same column represent significant differences between barrel temperature used for extrusion processing at $p < 0.05$, $n=3$

The correlation analysis between antioxidant activity using the ABTS method and the ORAC method may be seen in Figure 9. Little correlation exists between the methods (r^2 value of 0.447, Figure 9) due largely to the increase in antioxidant activity of extrudates following processing as measured through the ORAC method, something that was not evident with the ABTS data. This may be due to different components being measured in one assay as compared to the other.

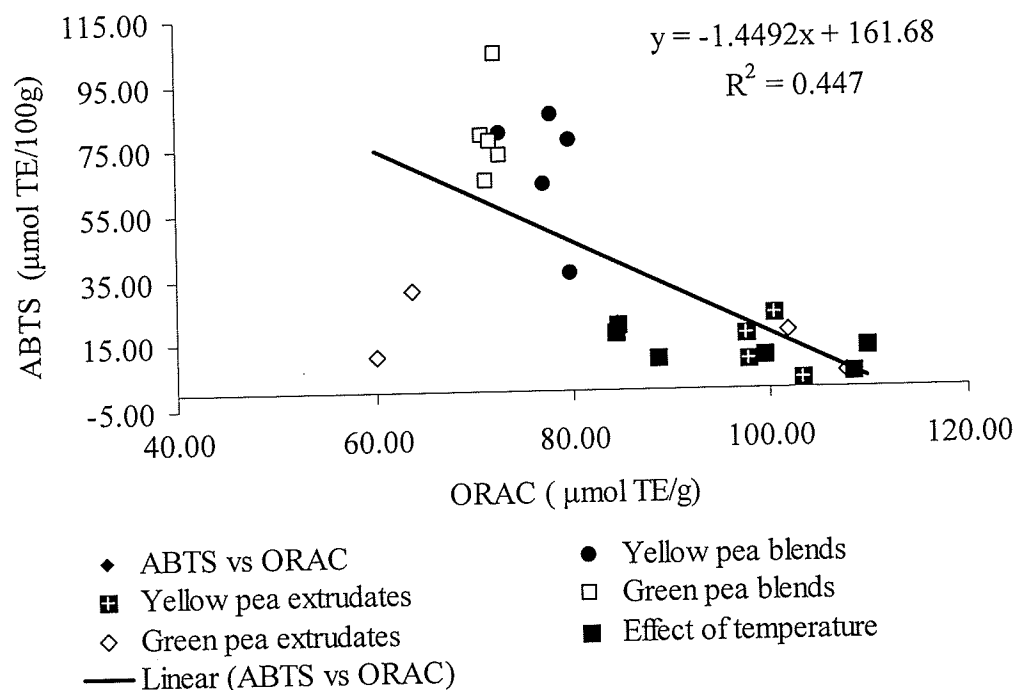


Figure 9: Correlation of antioxidant activity measured using the ORAC method with antioxidant activity measured using the ABTS method for yellow and green pea blends and extrudates

As indicated, the antioxidant activity of phenolics is highly variable depending on the structure of the compounds; flavonoids as compared to non-flavonoids are stronger in AOA and conjugated forms (glycosides) are lower in AOA than free forms (Lopez-Amores et al., 2006). Although a low polyphenolic content may be observed in a food, such as the Mexican blue corn used in a study by Del Pozo-Insfran et al., 2006, this does not necessarily correlate to the AOA of the sample, considering that this sample contained greater antioxidant capacity relative to American Blue and White corn genotypes. This may be due to the strong peroxy radical scavenging activity of anthocyanins as compared to cinnamic acid derivatives. Also, it is thought that interaction between the constituents present may

also affect the AOA of a sample. There are many factors which may alter the effectiveness of the antioxidant free radical scavenging ability including interactions with other compounds endogenous to the food, the charge associated with the food, the nature of the radical as well as the type of substrate protected by the antioxidant (Del Pozo-Insfran et al., 2006). With the processing of Mexican and American blue corn into tortillas, a 54% loss of anthocyanins was observed. This anthocyanin concentration correlated to the AOA of the sample with an r value of 0.94. That study also found a protective effect for antioxidants following acidified nixtamalization processing. This study found that generally, antioxidant activity retention was greater for those samples which contained additional pea hull, however this effect should be looked at more carefully in future research.

5.4.4 Total Phenolic Content

The total phenolic content of pea flour and pea hull for the pea varieties is shown in Table 24. For pea flour, the total phenolic content ranged from 18.0 to 22.7 mg FAE/g. Only the Midas variety was significantly greater in total phenolic content as compared to the other varieties tested. For pea hull, the Mozart yellow pea variety was significantly greater in total phenolic content than the other varieties with the exception of the Cooper green pea variety. Pea hull total phenolic concentration ranged from 26.3 to 34.0 mg FAE/g.

Based on the literature, differences were found between the concentrations of phenolics present in green and yellow pea types. Xu and Chang (2007) found that green pea variety Cruiser, TPC to be 1.3 ± 0.0 mg GAE/g while a yellow pea

variety, SW Capri, was found to have 1.4 ± 0.0 mg GAE/g when extracted with 70% methanol. This same trend was found by the USDA (2007) total phenolic food values compilation stated that yellow and green dry peas contained 83mg GAE/100g and 74mg GAE/100 g respectively while a study by Xu et al. (2007) found that TPC of yellow peas ranged from 0.9-1.1 mg GAE/g and green pea TPC ranged from 0.7-1.0 mg GAE/g.

Table 24: Pea hull and pea flour total phenolic content measured using the Folin method (mg FAE/100g) method

Variety	Pea flour mg FAE/g	Pea hull mg FAE/g
Alfetta	20.3 ± 1.0^b	26.3 ± 0.8^d
Eclipse	19.9 ± 1.3^b	30.6 ± 0.9^{bc}
Midas	22.7 ± 0.8^a	31.0 ± 1.4^b
Mozart	19.8 ± 1.7^b	34.0 ± 1.1^a
Camry	18.0 ± 0.6^b	28.1 ± 1.3^{cd}
Cooper	20.1 ± 0.8^b	32.5 ± 1.9^{ab}

Different letters within the same column represent significant differences between varieties at $p < 0.05$, $n=4$

With respect to changes due to formulation, the tortilla blends did not have a significantly different total phenolic content when different concentrations of pea flour and pea hull were used. The pea flour pea hull blends were also not significantly different than the control wheat flour with regard to total phenolic content (Table 25). Following processing into tortillas, no significant affects due to the inclusion of pea flour were found. However, the total phenolic content of all samples increased following processing into tortillas. Wheat flour accounted for

the majority of the formulation of the pea composite flour tortillas discussed. The study by Gao et al. (2002) found that Canada Western Red Spring wheat contained 1g FAE/kg (dry wt) when extracted with acidified methanol which were somewhat lower than the results found in this study. The difference may be due to extraction differences.

Table 25: Total phenolic content of tortillas and blends formulated with pea flour and pea hull blended with wheat flour using the Folin-Ciocalteu method

Formulation	Tortilla blend total phenolic content mg FAE/g	Tortilla mg FAE/g	% TPC retained
Control	8.0±9.4 ^{a▶}	11.1±3.1 ^{a▼}	138.8
15-0	7.9±7.7 ^{a▲}	11.6±2.6 ^{a◄}	147.9
15-5	8.6±7.9 ^{a▲}	12.4±3.0 ^{a◄}	143.6
25-1.5	7.9±8.5 ^{a▲}	11.6±2.3 ^{a◄}	146.6
35-0	8.4±8.3 ^{a▲}	11.2±2.4 ^{a◄}	133.3
35-5	8.2±8.9 ^{a▲}	10.9±1.9 ^{a◄}	134.1

Different letters within the same column represent significant differences between formulations at p<0.05

▶n=3, ▼n=16, ▲n=18, ◄n=48

When the total phenolic contents of extrudate blends were analyzed, the total phenolic content increased with an increase in the concentration of pea flour and pea hull as seen in Table 26. The yellow pea blends ranged from 12.2 to 21.2 mg FAE/g (30-0 to 50-10 respectively) for total phenolic content. Following extrusion processing, the total phenolic content was reduced from what was present in the blends. However, the extrudates with the greatest concentration of pea flour and

pea hull still tended to have a significantly greater total phenolic content than those samples with lower concentrations of pea flour and pea hull. The percentage of remaining total phenolic content had the most significant losses for the samples with no added hull (30-0 and 50-0). For split green pea flour extrudates and blends, the results were similar. The green pea blends increased in total phenolic content with increasing concentration of pea flour and pea hull that was used in the formulation. The green pea flour blends ranged from 11.8 to 18.5 mg FAE/g for total phenolic content. Following extrusion processing, the total phenolic content of the blends was decreased. Like the yellow pea extrudates, those samples which had a greater concentration of green pea flour and pea hull were significantly higher in total phenolic content. The 50-10 sample had a significantly greater total phenolic content than the other samples with 12.8 mg FAE/g; it also retained the greatest percentage of total phenolic content at 69.3% compared with the other samples (with the exception of the control which retained 83.6%). The samples with added pea fibre tended to retain more of the total phenolic content than those samples without added fibre.

Table 26: Extrudate blend and extrudate total phenolic content of yellow and green pea flour and pea hull formulations

Formulation	Yellow blend mg FAE/g	Yellow extrudate mg FAE/g	% TPC retained	Green blend mg FAE/g	Green extrudate mg FAE/g	% TPC retained
Control	4.8±0.5 ^e	4.0±0.1 ^d	83.6	4.8±0.5 ^d	4.0±0.1 ^d	83.6
30-0	12.2±1.2 ^d	5.8±0.8 ^d	47.6	11.8±0.9 ^c	5.6±0.2 ^c	47.6
30-10	14.2±1.1 ^c	8.2±0.4 ^c	57.5	14.1±1.2 ^b	9.2±0.6 ^b	65.6
40-5	16.2±0.6 ^b	10.8±0.8 ^b	66.6	15.5±0.9 ^b	9.7±0.4 ^b	62.5
50-0	19.9±0.7 ^a	9.8±0.4 ^{bc}	49.1	17.6±1.0 ^a	9.0±0.6 ^b	51.1
50-10	21.2±1.0 ^a	13.0±1.7 ^a	61.4	18.5±0.5 ^a	12.8±0.9 ^a	69.3

Different letters within the same column represent significant differences between formulations at $p < 0.05$, $n=4$

The total phenolic content measured for the samples was affected by the temperature of extrusion as seen in Table 27. Although the control pea starch did not indicate any significant differences in total phenolic content processed under different extrusion temperatures; both the yellow pea and green pea flour samples had significantly greater total phenolic content with 135°C processing than at the lower temperatures.

The effect of processing of beans on TPC was studied by Granito et al. (2007). The TPC of raw bean and cooked bean was 1917 and 854 mg/100g dry matter, equivalent to a 55.45% reduction, which was similar to the reduction in the composite pea flours after processing into tortillas. The reduction in total phenolics was likely due to the binding or damage of aromatic structures at high temperatures, resulting in the inability of the quantification of phenols with the Folin-Ciocalteu reagent (Granito et al., 2007).

In Li et al. (2007), muffins were baked using purple wheat bran. It was found that the muffins made of purple wheat bran and the control wheat bran were much lower in TPC as compared to untreated or heat treated purple wheat bran, indicating that the addition of ingredients used in muffin mix had a dilution effect on the TPC and/or the baking process caused the reduction in TPC. Specifically, in the study by Li et al., 2007, methanol extracts of the purple wheat bran, heat treated purple wheat bran, the purple wheat bran muffin and wheat bran muffin had a TPC of 3.34, 3.68, 0.26 and 0.35 mg FAE/g respectively. As mentioned previously, the TPC is also correlated to the antioxidant activity (AOA) of a sample; however, different phenolic compounds differ in their antioxidant activity based on their structure.

In a study by Randhir et al. (2007), it was speculated that the soluble conjugate and insoluble bound forms of phenolics are the majority of the total phenolics found in cereals. Following thermal processing, Randhir et al. (2007) concluded the increase in phenolics was likely due to the breakdown of cell walls and other cell components. After the alterations of constituents, polymerization/oxidation of phenolics from heat processing may result in the increase of total phenolic through the formation of phenolics which were not originally found in the seed (Randhir et al. 2007). For example, conjugated polyphenolics such as tannins may be broken down into a more simple phenolic form and change the total phenolic content/ antioxidant activity (Randhir et al., 2007).

Table 27: Effect of temperature on extrusion on total phenolic content of pea flour, pea hull and pea starch extruded formulations

Sample	Extrusion Temperature	Total phenolic content mg FAE/g
control	110	3.1±0.3 ^e
	120	3.9±0.1 ^e
	135	4.0±0.1 ^e
Green pea 50-10	110	9.7±0.3 ^c
	120	11.2±0.5 ^b
	135	12.8±0.9 ^a
Yellow pea 50-0	110	1.7±0.2 ^f
	120	6.9±0.6 ^d
	135	9.8±0.4 ^c

Different letters within the same column represent significant differences between barrel temperatures used during extrusion processing at $p < 0.05$, $n=4$

When the results for total phenolic content are correlated to the antioxidant activity results obtained using the ABTS method, the correlation (r^2) value is 0.6263 (Figure 10). The correlation suggests that the antioxidant activity of the samples tested is at least, partially due to the phenolics present in the samples.

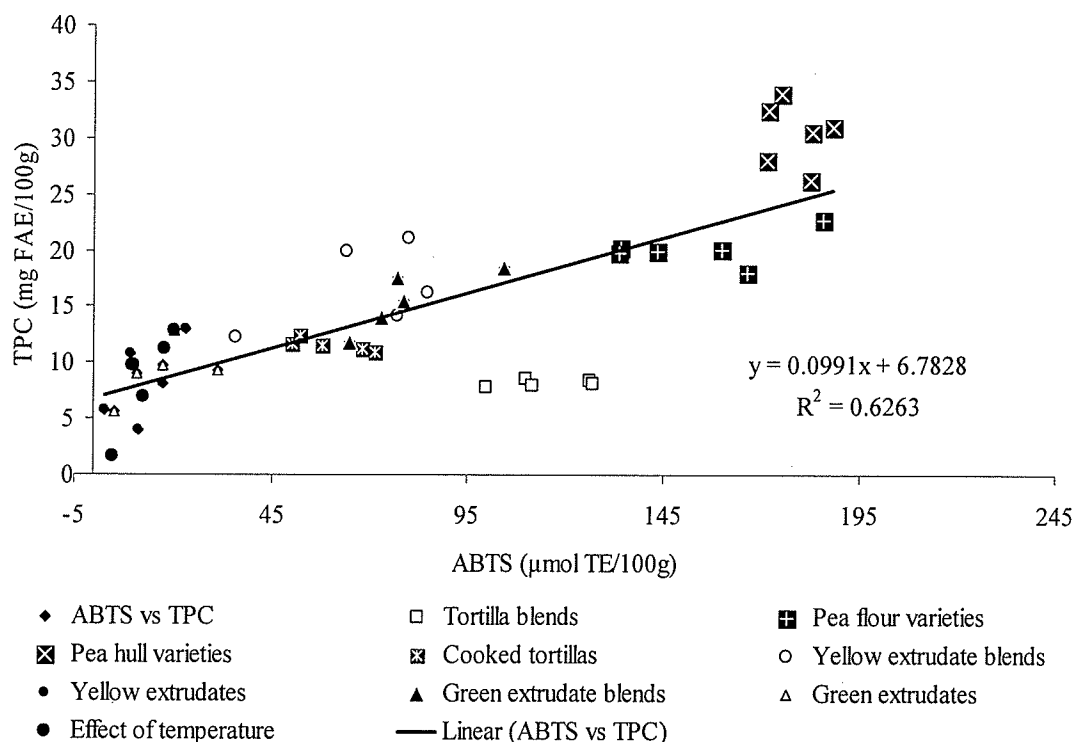


Figure 10: Correlation of total phenolic content of samples measured using the Folin method to the antioxidant activity of samples measured through the ABTS method

When total phenolic content results were correlated with antioxidant activity results using the DPPH method, the correlation (r^2) was 0.2877 (Figure 11). Outlier values from the results of one pea hull and one pea flour point reduced the correlation between the methods. This correlation is not as strong as the correlation between ABTS and total phenolic content which (r^2 of 0.6263) (Figure 10). This may be due to

different compounds evaluated between the DPPH method as compared to the ABTS method.

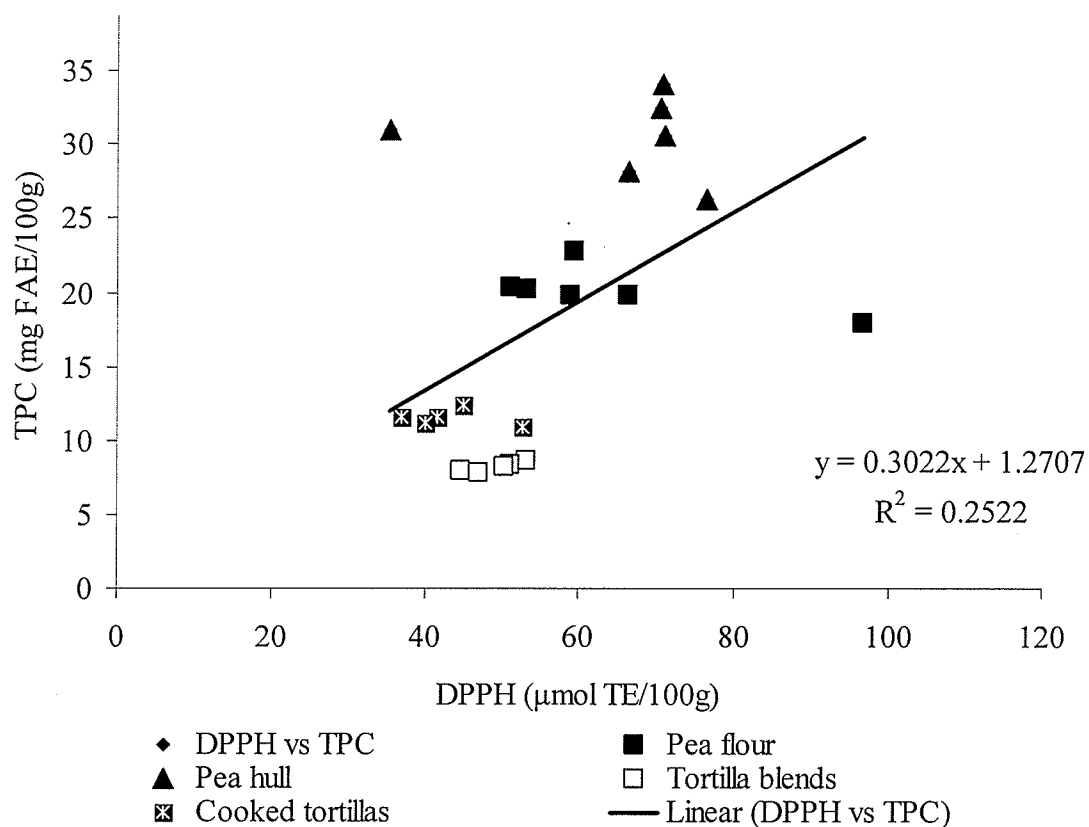


Figure 11: Correlation of total phenolic content measured using the Folin method with the antioxidant activity of samples measured using the DPPH method

Figure 12 illustrates the correlation between the total phenolic content as measured by the Folin method of samples and the antioxidant activity of samples using the ORAC method. Again, little correlation exists between the methods, suggesting that components other than phenolics contribute significantly to the antioxidant activity of the samples.

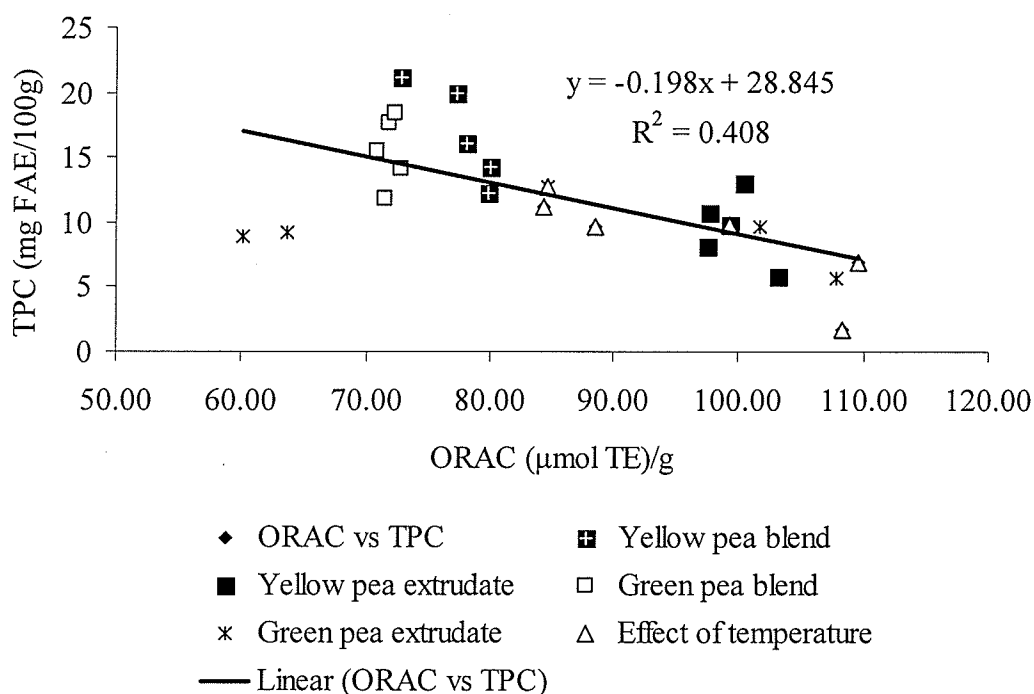


Figure 12: Correlation of antioxidant activity of pea blend and final products using the ORAC method as compared to the total phenolic content of the samples measured using the Folin method

5.5. Conclusion

Food products containing pea flour and pea hull were successfully formulated in this experiment. It was found that the raw blends used to make both tortillas and extruded products had a greater antioxidant activity than the final products when measured using the ABTS and DPPH methods. Total phenolic contents were also greater for the blends than for the extruded products. However, antioxidant activity analysis using the ORAC method suggested an increase in antioxidant activity following processing which was not in accordance with the other methods. This suggests that different components were being measured between the different methods.

Chapter 6: General Discussion, Conclusion and Future Research Opportunities

6.1. Discussion

As the concentration of pea flour included in tortillas increased, the texture of the product was compromised. The cohesiveness values were more affected than the firmness values for the tortillas when texture were analyzed using a TA.XT2 texture analyzer. This effect was a result of a reduction in the gluten protein in wheat which is responsible for the texture of wheat flour tortillas. Likely there is a balance between the level of gluten and the protein network that it creates which is responsible for the elasticity of the tortilla. This explains why the gluten abundant control tortilla had the greatest extensibility. Even with a smaller diameter than the composite pea flour tortillas, the control tortillas were capable of stretching to a greater degree when force was applied in the penetration test. With the addition of pea flour to tortilla formulations, the diameter of the final product decreases. These tortillas have a smaller diameter due to a lack of gluten protein and interferences of the gluten network with pea flour and fibre which prevents the extensibility of the tortilla. The lack of extensibility was verified through the compression/penetration test. Rollability scores of tortillas were also correlated to the thickness of tortillas, which was thicker as a higher concentration of pea flour was added. Therefore, it was observed that at a high concentration of pea flour (~35%), a tortilla which was thick, firm, dry with little extensibility was being rolled around a wooden dowel and resulting in poor rollability scores with obvious cracking and breaking. The addition of hull on the other hand did not have as great an effect on the physical characteristics of tortillas due partially to the limited amount of hull that was incorporated but also likely due to the tendency of fibre

to absorb a greater amount of water than flour, a trait that is beneficial likely for the plasticizing effect of water on the textural properties of tortillas. Thicker, smaller diameter tortillas were the result when an increased level of water was used. These tortillas also had similar or better rollability as compared to the control due likely to the plasticizing effect of wheat protein by the water to improve fluid like properties as suggested previously (Srinivasan et al., 2000). With a reduction in gluten as well as the interference of the gluten network by other components present in pea, it appears that composite flours for tortillas may contain approximately 26% pea flour with another 5% pea hull made up with a strong CWRs wheat flour. The interference of the gluten network was previously observed as additional insoluble fibre had a stronger gluten structure than when soluble fibre was added. The gluten network was physically disrupted by insoluble fibre particles which weakened air bubble walls and caused the collapse of air bubbles and decreased shelf stability (Seetharaman et al., 1997). Air bubbles and channels present were smaller, contributing to the dense crumb of the tortilla. Larger diameters, higher moisture contents and shorter shelf life were characteristic of tortillas containing insoluble fibre (Seetharaman et al., 1997).

In extruded snack foods, it was found that pea flour was capable of being incorporated at a level of 50% depending on the amount of added hull that was used in the formulation. Overall, pea flour did not have as great an influence on the expansion ratio as the level of pea hull did. This is because peas contain about 50% starch, of which the majority is amylopectin which is beneficial in terms of expansion properties, due likely to the branched nature of the polymer, while amylose has a more negative functionality in terms of expanded extrusion, due likely to the alignment of linear chains

preventing the formation of bubble nucleation (Falcone and Phillips 1988). Pea hull (fibre) on the other hand, limited expansion and extensibility of air cell walls and prevented steam retention which lead to bubble collapse at a critical point (Jin et al., 1995). Although the air cell size was decreased, the number of air cells was increased (Jin et al., 1995). The effect of temperature on extrudate texture seems to depend on the material which was being extruded and its physical properties, as previous research has indicated optimum conditions for specified food products which are different from those presented in this study. When tested under sensory evaluation, the product which deviated the least from the characteristics of the consumer ideal product was the 50-0 whole yellow pea blend processed at 135°C. Consumers perceived the sample processed at a lower temperature using the same formulation to be too puffy while the sample using a greater percentage of added pea fibre was not puffy enough.

Following processing, the antioxidant concentration was greatest in both the control tortilla as well as the tortillas with a higher concentration of pea flour with and without pea hull. However, when looking at the percentage of antioxidant activity reduction due to tortilla processing, it was found that the wheat flour control increased in antioxidant concentration following processing while the pea flour and hull blends decreased in their antioxidant activity following tortilla processing conditions. This may indicate a difference in the stability of the antioxidants present in wheat as compared to those found in pea as measured through the ABTS method. No difference was found between green and yellow pea varieties.

The AOA of the DPPH method was found to be lower than the antioxidant activity when measured using the ABTS method. Between the different methods, the

ABTS assay tended to have more consistent results on a day to day basis with greater stability in the free radical. The ORAC method on the other hand showed an increase in antioxidant activity of extruded samples as compared to the blends. This trend was not seen in the ABTS method which was also used to analyze the antioxidant activity of extrudates and extrudate blends. The difference may be due to the breakdown in products which ORAC is capable of measuring the antioxidant activity, but ABTS is not.

6.2. Conclusion

In conclusion, pea flour and fractions were able to be successfully incorporated into tortillas as well as in expanded, extruded snack food products. In the case of tortillas, the amount of pea flour capable of being incorporated in the final product is somewhat limited due to an interruption of the gluten network which prevents the necessary rollability of the final product. Pea hull however, did not appear to have the same influence and may be incorporated in higher levels in tortillas than pea flour. In extruded snack foods, it was apparent that the incorporation of pea flour improved the texture of extrudates as compared to using straight pea starch, the texture of which appeared much harder than commercial samples that were tested. The effect of pea fibre on extrudate texture tended to limit the size of the air cells in the final expanded product while increasing the number of air cells. Although the product generated with and without fibre had different structures and textures, there was no indication that one of these products was much better received by the sensory panel. When addressing the specific hypotheses for this experiment

- As the concentration of pea flour in tortillas increased, interruption of the gluten network occurred at the critical point of approximately 25% pea flour.
- Pea fibre addition increased the water absorption of samples, pea fibre did not have as great an effect on texture as pea flour.
- The antioxidant activity of composite pea flours was similar among all composite flour formulations, suggesting that pea antioxidants are thermally unstable
- As the concentration of pea flour increases in extruded snack foods, the expansion of the products increased as compared to the control sample
- The increased level of pea fibre added to extrusion formulations will limited the air cell size in extruded snack foods.

6.3. Future Research Opportunities

From this study the feasibility of incorporating pea flours and fractions as ingredients can be seen. However, research is still required in many areas in order to maximize the potential of using peas as an ingredient. A more extensive genetic by environment study is required to identify the effect of growing conditions on the quality of peas used for food use purposes. Also, the methods to process pea flour as well as pea fractions may play a significant role on end product quality, therefore the effect of particle size, milling methods and starch extraction methods may be analyzed for their effect on the final product. Also, if pea flours and fractions are to be used in foods as a

substitute for a percentage of wheat flour, it would be beneficial to test the effect of using different protein contents and qualities of wheat flours.

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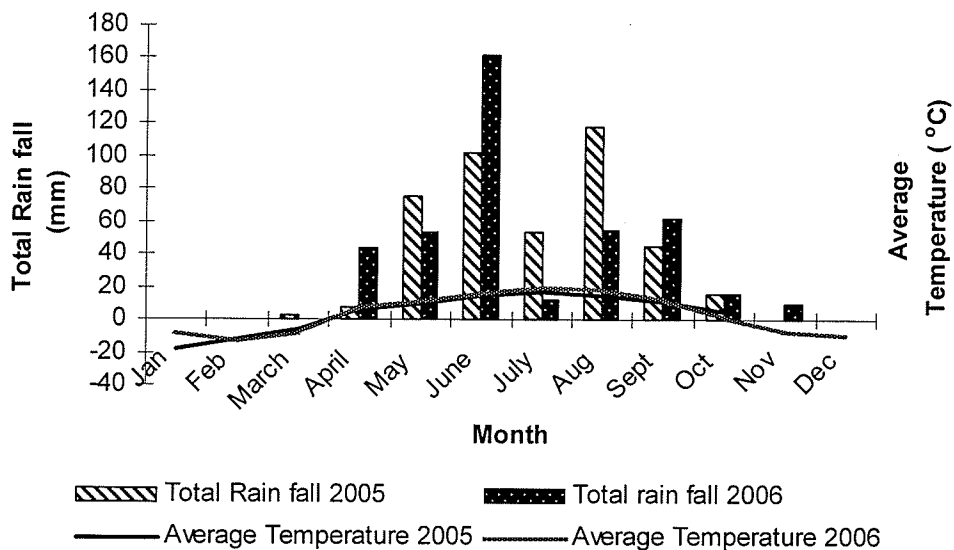
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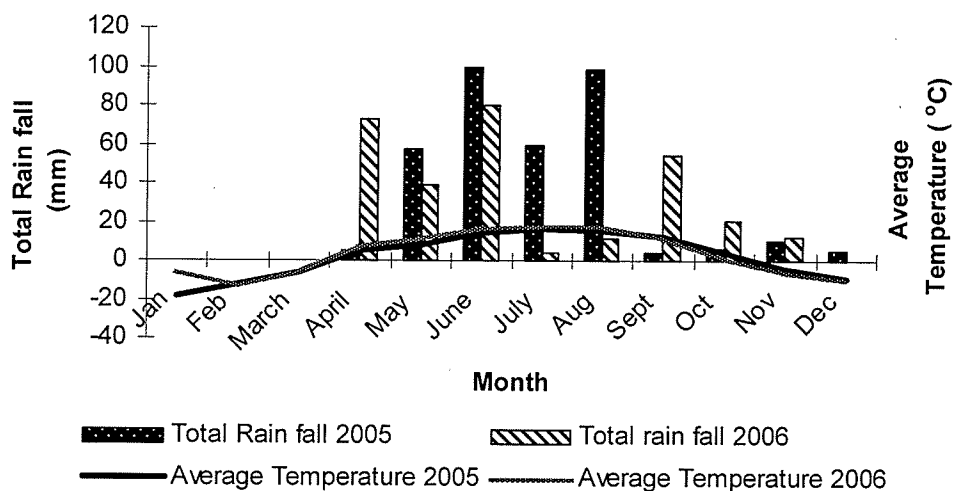
APPENDIX A

Climate information of growing locations and crop years: Davidson, Indian head, Langham** and Saskatoon**



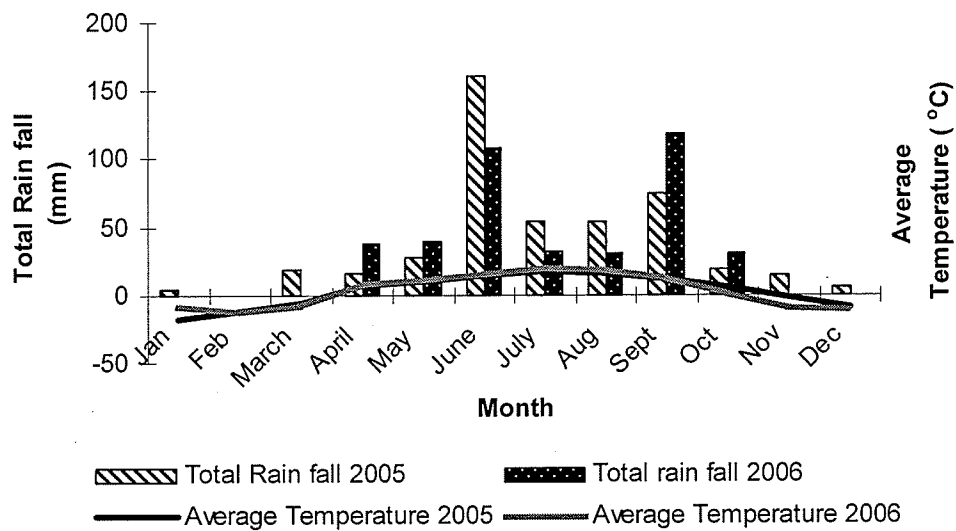
Climate information for Davidson

Data adapted from Environment Canada Monthly data report for 2006
http://climate.weatheroffice.ec.gc.ca/climateData/monthlydata_e.html



Climate information for Indian Head

Data adapted from Environment Canada Monthly data report for 2006
http://climate.weatheroffice.ec.gc.ca/climateData/monthlydata_e.html



Climate information for Saskatoon

**Rosthern is 63.5km from Diefenbaker International Airport in Saskatoon

Data adapted from Environment Canada Monthly data report for 2006
http://climate.weatheroffice.ec.gc.ca/climateData/monthlydata_e.html

APPENDIX B
Pea acreage values for Manitoba and Saskatchewan pea varieties for the 2005 and 2006 crop years

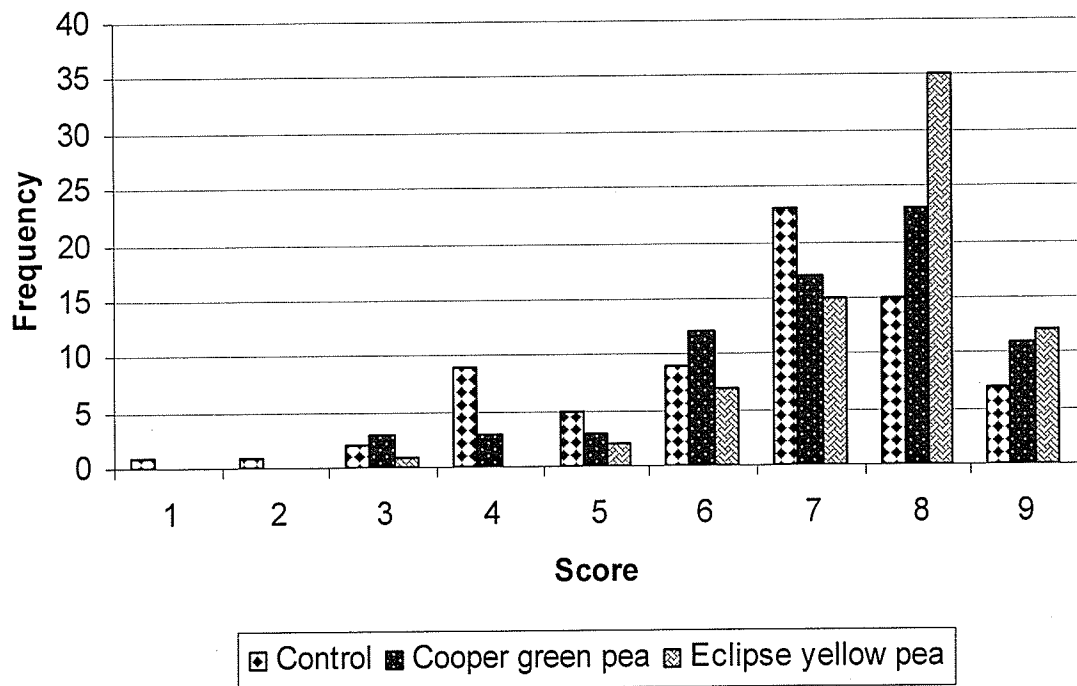
Variety	Type	MB06 Acres	MB05 Acres	SK06 Acres	SK05 Acres
Alfetta	Y	4297	5085	320	665
CDC Mozart	Y	2807	5989	15275	19960
Eclipse	Y	14671	20567	35943	31421
SW Midas	Y	1738	226	2850	
Cooper	G	480	113	520	
Camry	G	157	226	150	
total pea acres		85,000	120,500	2,100,000	similar to '06

* Obtained from Bruce Brolley of Manitoba Pulse Growers' Association

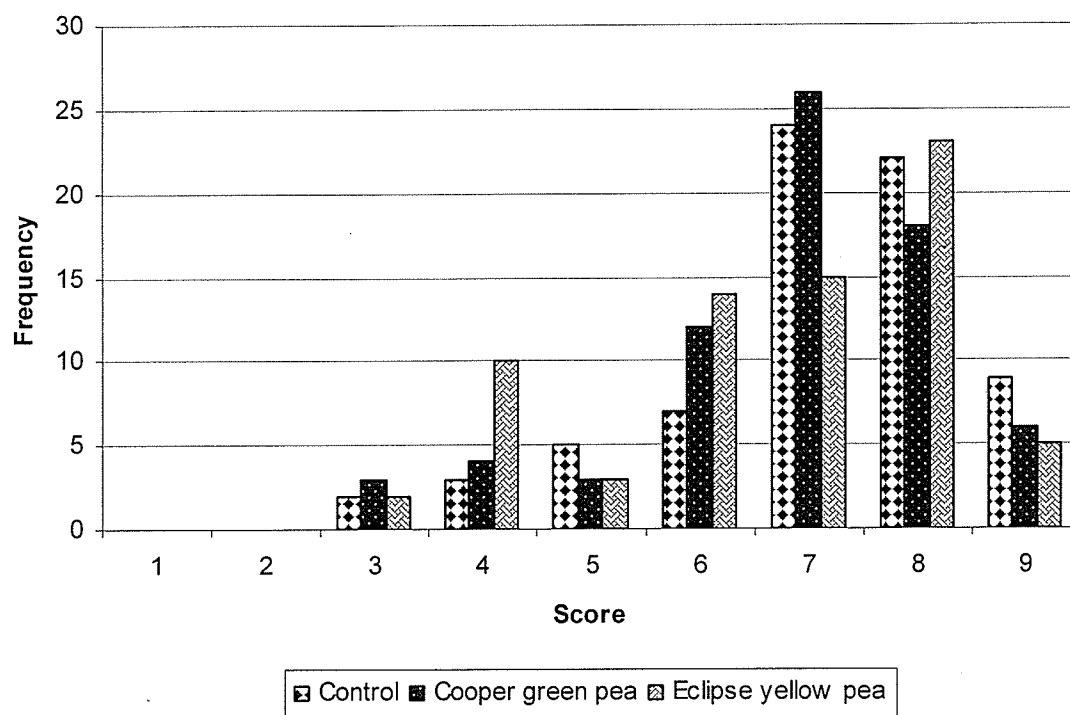
APPENDIX C

Frequency scores of tortilla sensory attributes appearance, flavour, texture, overall opinion and potential purchase intent

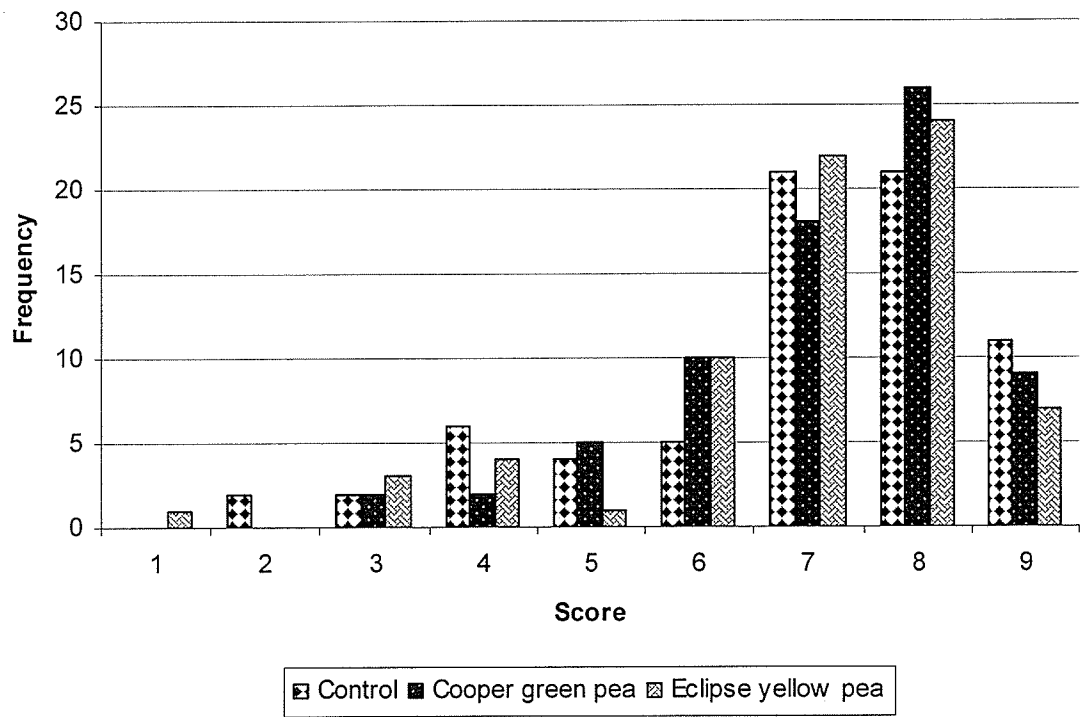
Appearance sensory scores of pea composite flour tortillas



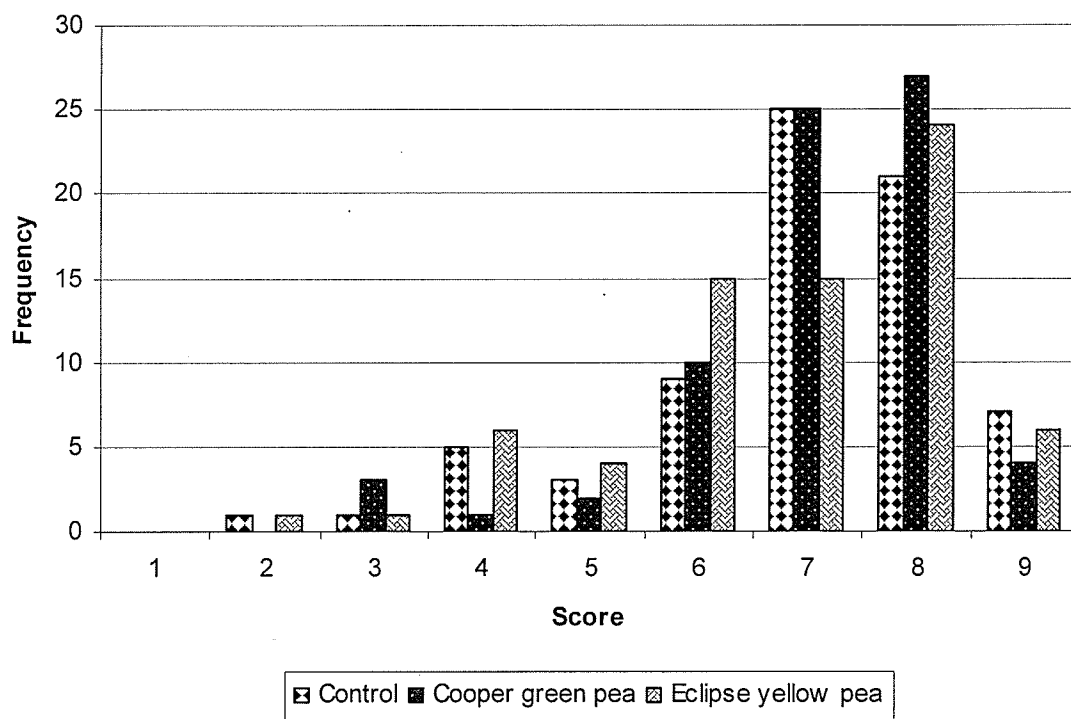
Flavour sensory scores of pea composite flour tortillas



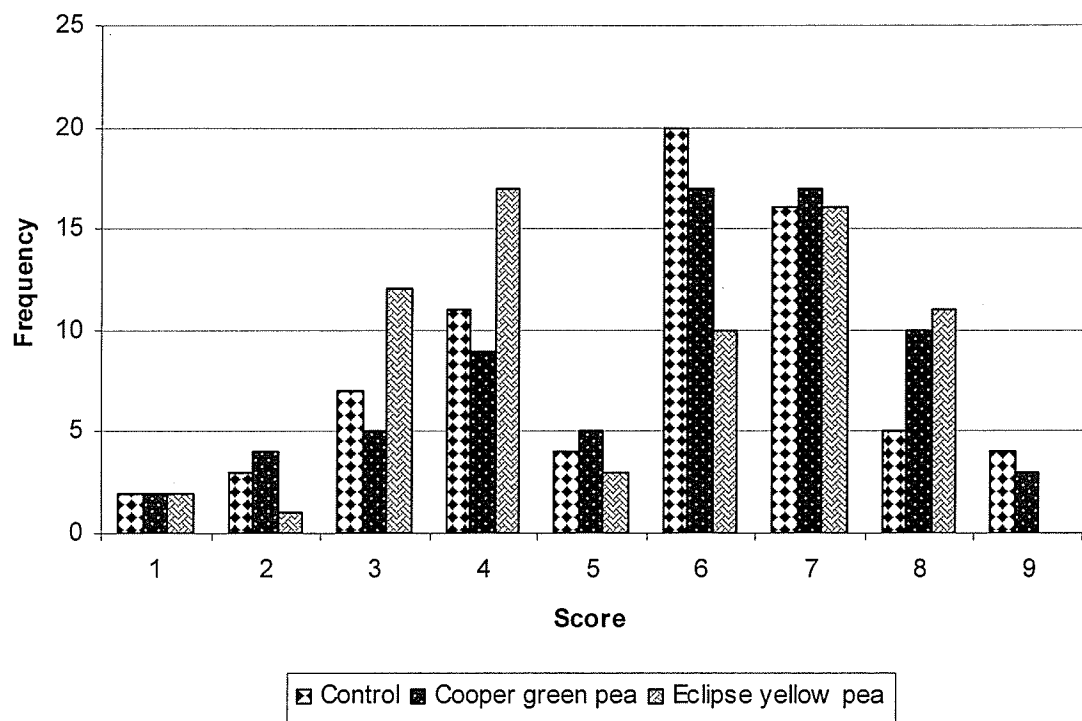
Texture sensory scores of pea composite flour tortillas



Overall opinion scores of pea composite flour tortillas



Potential Purchase scores of pea composite flour tortillas



APPENDIX D
Optimization results Green and Yellow Pea tortillas 2005/2006 crop year

	Variety	Crop Year	% pea flour	% pea hull	peak force	area	desirability	peak force final equation (coded factors)	area final equation (coded factors)
Yellow Pea	Mozart	2005	26.89	5	722.631	3269.508	53.65%	peak force = 789.76-142.39A-41.34B+5.96AB	area = 3747.11-1101.81A-287.56B+96.40AB
			27.04	5	720.571	3254.328	53.65%		
			26.65	5	725.845	3293.199	53.65%		
			27.14	5	719.187	3244.131	53.64%		
			26.35	5	730.028	3324.025	53.62%		
	Mozart	2006	26.07	3.93	651.024	3120.780	48.60%	peak force = 699.39-134.36A-60.19B+8.49AB	area = 3504.40-1212.78A-458.45B+147.26AB
	Midas	2005	25.46	4.95	773.304	3669.070	48.60%	peak force = 823.97-136.13A-45.12B-3.84AB	area = 4036.91-1077.82A-326.30B+35.51AB
			27.08	5	629.940	2960.910	58.90%		
			27.25	5	627.640	2941.500	58.80%		
	Midas	2006	26.89	5	632.670	2983.900	58.80%	peak force = 700.25-114.66A-45.15B-25.27AB	area = 3498.47-1050.22A-291.91B-128.63AB
			27.08	5	629.940	2960.910	58.90%		
			27.25	5	627.640	2941.500	58.80%		
	Eclipse	2005	25.56	5	722.874	3320.660	50.30%	peak force = 779.60-154.25A-45.50B-46.33AB	area = 3730.56-1224.97A-330.52B-193.71AB
			25.63	5	721.416	3310.340	50.30%		
	Eclipse	2006	26.69	4.6	617.856	2871.950	50.50%	peak force = 688.88-154.12A-54.69B+6.55AB	area = 3491.22-1329A-500.45B+179.75AB
	Alfetta	2005	25.45	5	730.812	3447.180	53.50%	peak force = 779.47-138.73A-41.63B-17.37AB	area = 3861.93-1305.57A-355.05B-21.22AB
			25.81	4.97	629.654	2997.930	49.60%		
	Alfetta	2006	25.96	4.95	627.789	2982.640	49.60%	peak force = 685.10-133.85A-44.65B-6.45AB	area = 3470.94-1201.49A-384.46B+48.55AB
			25.96	4.95	627.789	2982.640	49.60%		

	Variety	Crop Year	% pea flour	% pea hull	peak force	area	desirability	peak force final equation (coded factors)	area final equation (coded factors)
Green Pea	Camry	2005	25.9 26.31	5 5	623.000 618.000	2824.540 2780.270	53.60% 53.50%	peak force = 668.74- 138.46A-33.08B+1.13AB	area = 3192.16-1133.67A- 269.98B+48.83AB
	Camry	2006	25.84	5	625.577	3106.100	52.10%	peak force = 677.75- 160.98A-37.38B-14.55AB	area = 3517.87-1370.45A- 295.15B-13.45AB
	Cooper	2005	26.07	4.12	623.328	2788.600	45.80%	peak force = 663.11- 128.88A- 42.50B+23.11AB	area = 3168.69-1088.70A- 434.95B+269.51AB
	Cooper	2006	26.17	3.96	610.904	2980.660	48.40%	peak force = 667.03- 158.83A- 65.67B+11.44AB	area = 3449.87-1376.90A- 554.2B+224.08AB

APPENDIX E
Sensory evaluation forms and sensory ethics approval

****Printed on food science letterhead**

Recruitment Letter - Test 1 Tortillas

The Department of Food Science

Date:

Dear Colleague,

We are looking for volunteers who are willing to participate in a sensory study to determine the acceptability of wheat flour tortillas which contain pea flour and pea hull. The research is being funded by the Saskatchewan Pulse Growers' Association in order to increase consumption of dry field peas.

Volunteers must be familiar with wheat flour tortillas and consume them a minimum of four times a year. You will be asked to taste 3 tortillas and check a descriptor of your overall opinion of the product on a nine point scale and whether you would purchase them (nine point scale). The one time session will last for approximately 30 minutes. You will receive a snack following your participation.

Allergy to one of the food ingredients may pose a risk to individuals involved in the study. A questionnaire regarding allergies completed by those interested in participating in the study will inform the researcher of any possible risk. Information regarding the project objectives as well as results will be sent to participants within a month of the data collection.

Sessions will take place in the Food Science Building (Ellis) in room 221 during the days of XXX to XXX during a time of your convenience as indicated in the questionnaire.

If you are interested in participating in this research, please read and fill out the required consent form as well as the questionnaire attached to this letter and return it to Heather Maskus by XXXX. If you agree to participate you will be contacted to arrange your session time and date. Any questions may be directed to Heather at 612-9957 or 474-9878.

Thank-you for your time and assistance with this project,

Sincerely,

Heather Maskus, Research Coordinator
University of Manitoba
Department of Food Science
M.Sc. Student

Recruitment Poster – test 1



UNIVERSITY
OF MANITOBA

Department of Food Science
Ellis Building
University of Manitoba, Winnipeg, MB
R3T 2N2 Canada

Sensory Analysis Study

The Department of Food Science at the University of Manitoba is developing food products made partially with pea flours. A sensory analysis is being conducted to determine the consumer acceptability of wheat flour tortillas which include pea flour and pea hull.

The study is open to those people 18 years and older who consume tortillas at least 4 times a year

Commitment required for a one time session of approximately 30 minutes.

No experience is required.

Volunteers will be compensated for their participation

Please contact Heather Maskus (principle investigator) at ummasku2@cc.umanitoba.ca if interested

** Printed on Food Science Letterhead

Written Consent Form – Test 1 tortillas

Research Project Title: Incorporation of pea in food products: Sensory Evaluation of tortillas

Researcher(s): Heather Maskus and Dr. S. Arntfield

This consent form, a copy of which will be left with you for your records and reference, is only part of the process of informed consent. It should give you the basic idea of what the research is about and what your participation will involve. If you would like more detail about something mentioned here, or information not included here, you should feel free to ask. Please take the time to read this carefully and to understand any accompanying information.

The study is being done to evaluate the consumer acceptance of tortillas made using a percentage of pea flour and ground pea hull. The research is being funded by the Saskatchewan Pulse Growers' Association in order to increase consumption of dry field peas.

The criteria necessary for each volunteer is that they must be familiar with wheat flour tortillas and consume them at least 4 times a year. You will be asked to taste 3 tortillas and check a descriptor of your opinion of the product on a nine point scale and whether you would purchase them (nine point scale). The one time session will last for approximately 30 minutes. You will receive a snack following your participation.

Food allergies may pose a risk to some individuals. A questionnaire regarding allergies will be used to screen for this potential risk.

All data collected relating to personal information and results obtained will be kept in a locked filing cabinet for 5 years or until the data is published, whichever comes first. Access to information will be limited to the researchers listed above. All data will be shredded after time has expired.

Information regarding the project will be sent to participants within a month of completion of the data collection.

You will be offered a small snack and drink (pop or juice) following your participation as compensation.

By signing this form you will indicate that you understand, to your satisfaction, the information regarding your participation in the research project and agree to serve as a subject. This does not equate to waiving your legal rights nor release the researchers, sponsors or involved institutions from their legal and professional responsibilities. You may leave out answering any questions that you choose without consequence and are free to cease your participation in the study at any time without judgement or consequence. Feel free to ask for clarification or new information throughout your participation. This study is being conducted by Heather Maskus (University of Manitoba Master's Student), 474-9878 or 612-9957 under the supervision of Dr. S. Arntfield 474-9866.

This research has been approved by the Joint-Faculty Research Ethics Board at the University of Manitoba. If you have any concerns or complaints about this project you may contact any of the above named persons or the Human Ethics Secretariat at 474-7122 or e-mail Margaret_bowman@umanitoba.ca. A copy of this consent form will be given to you to keep for your records and reference.

Participant's Signature

Date

Telephone Number

E-mail Address

Researcher and/or Delegate's Signature

Date

Questionnaire – Test 1

Incorporation of pea in food products: Sensory Evaluation of Tortillas

This information is confidential and will only be viewed by the principal researcher and the supervisory professor.

Name _____

1. Are you allergic to any food products? Yes ____ No ____
If yes, please list them below

2. Are there any foods specifically, or food flavours and textures in general that you would prefer not to evaluate?

3. Are you on a restricted diet? Yes ____ No ____; if yes please explain below

4. Please indicate the day and time that would be most convenient for you to attend the sensory session (use a 1 to indicate your first choice and a 2 to indicate a second choice)

Week of _____

Time slot	Monday	Tuesday	Wednesday	Thursday	Friday
10:30 to 11:30					
12:00 to 1:00					
2:30 to 3:30					
4:30 to 5:30					

Thank-you for completing this questionnaire

Panellist No. 76

Ballot
Sensory Evaluation of Tortilla

So that we know your familiarity with the commercial tortilla samples, please indicate how often you consume wheat flour tortillas

- ☐ At least once a week
- ☐ At least once a month
- ☐ At least four times a year
- ☐ Other; please explain _____

For each sample, according to the three digit code given **510** please check the box beside the phrase which best describes your opinion of the sample for each characteristic (appearance, flavour, texture and overall opinion). Then indicate how often you would purchase this sample knowing that it contains added nutritional benefits compared with commercially available tortillas and is a comparable price.

Please rinse your mouth with water between tasting of different samples.

Appearance	Flavour	Texture	Overall Opinion
☐ Like extremely	☐ Like extremely	☐ Like extremely	☐ Like extremely
☐ Like very much	☐ Like very much	☐ Like very much	☐ Like very much
☐ Like moderately	☐ Like moderately	☐ Like moderately	☐ Like moderately
☐ Like slightly	☐ Like slightly	☐ Like slightly	☐ Like slightly
☐ Neither like nor dislike	☐ Neither like nor dislike	☐ Neither like nor dislike	☐ Neither like nor dislike
☐ Dislike slightly	☐ Dislike slightly	☐ Dislike slightly	☐ Dislike slightly
☐ Dislike moderately	☐ Dislike moderately	☐ Dislike moderately	☐ Dislike moderately
☐ Dislike very much	☐ Dislike very much	☐ Dislike very much	☐ Dislike very much
☐ Dislike extremely	☐ Dislike extremely	☐ Dislike extremely	☐ Dislike extremely

How often would you purchase this sample?

- ☐ Every opportunity
- ☐ Very often
- ☐ Frequently
- ☐ Now and then
- ☐ If available
- ☐ On an occasion
- ☐ Hardly Ever
- ☐ If no other choice
- ☐ Never



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AMENDMENT APPROVAL

11 January 2008

TO: Heather Dawn Maskus
Principal Investigator

FROM: Wayne Taylor, Chair
Joint-Faculty Research Ethics Board (JFREB)

Re: Protocol #J2007:128
"Incorporation of Pea (Pisum Sativum) in Food Products: Sensory Evaluation"

This will acknowledge your e-mail dated January 9, 2008 requesting amendment to the above-noted protocol.

Approval is given for this amendment. Any other changes to the protocol must be reported to the Human Ethics Secretariat in advance of implementation.

Appendix F

Specification Sheet of Best Cooking Pulses Whole Yellow Pea Flour



PRODUCT SPECIFICATION SHEET

BEST Whole Yellow Pea Flour

PRODUCT NAME	BEST Whole Yellow Pea Flour	
DESCRIPTION	identification / ingredients product use	pulverized whole yellow peas functional food ingredient
PHYSICAL CHARACTER	flavour colour aroma grind	mild pea light cream mild fine
TYPICAL ANALYSIS	calories moisture crude protein nitrogen carbohydrate fat ash	3.66kcal/g ✓ ^{100g} 7% 20.6% ✓ 3.29% 69% ✓ 0.85% ✓ 2.6% ✓
MINERALS	potassium calcium iron sodium	9,540mg/kg ✓ 1310mg/kg ✓ 131mg/kg ✓ 85.9mg/kg ✓
PACKAGING	multi-wall kraft bags tote bags	50 lb or 25 kg 2,000 lb
STORAGE	temperature humidity shelf life	cool dry 1 year

Information in this specification is a typical analysis based on randomly selected samples. Actual analytical data may vary. 17/11/06

BCP PORTAGE (flour/fLOUR): 124 - 10th Street NE Portage la Prairie
Manitoba Canada R1N 1B5 TEL (204) 867-4451 FAX (204) 239-6885
BCP ROWATT (peas): RR#3, Site 10, Module 3, Compartment 13, Rowatt
Saskatchewan, Canada S4P 2Z3 TEL (306) 586-7111 FAX (306) 586-4848
www.bestcookingpulses.com

Appendix G

Specification Sheet for Nutri-Pea Native Pea Starch (Accu-Gel)



Nutri-Pea Limited
Natural Pea Ingredients

Accu-Gel
Native Pea Starch

Accu-Gel™ is a food-grade, native vegetable starch offering excellent gel strength, white color and bland flavor. The raw materials, Golden Canadian field peas are not genetically modified. Accu-Gel™ is therefore suitable for a GMO-free label.

Nutrition Facts Per 100 g serving (typical, on dry basis)

Calories	400 Kcal ✓
Fat (AOAC 7.060, 14 th Ed)	<0.1 g ✓
Monosaturated	N/A, Saturated N/A
Polyunsaturated	N/A, Trans 0.00g
Cholesterol	0 mg
Sodium	49 mg ✓
Carbohydrate	>98.7 g ✓
Fibre	N/A
Sugars	N/A
Starch	> 95.0g
Protein	<1.0 g ✓

Minerals

Potassium	320 ppm
Magnesium	43 ppm
Calcium	71 ppm
Phosphorus	79 ppm
Manganese	<1 ppm
Iron	6 ppm
Zinc	<1 ppm
Copper	<0.1 ppm

Origin

kernel of Canadian Yellow Peas (100% GMO-free)

Compositional & Physical Data

Moisture (16 hrs ± 100°C ± 5°C)	< 12%
Ash (AOAC 14.006, 14 th Ed)	<0.2%
Flavor	very bland
Color	white
pH (10% solution)	6.5 – 7.5
Particle Size (through a 80 mesh Tyler)	>95%
Microns	175

Microbiology

Total Plate Count (AOAC 46.015, 14 th Ed)	<10,000/g
E.Coli (AOAC 46.016, 14 th Ed)	negative
Salmonella (AOAC 46.117, 14 th Ed)	negative
Yeasts and Molds (AOAC 997.02, 16 th Ed)	<100/g

Applications

Accu-Gel™ provides unique properties unlike that of wheat, corn and potato starches. Its superior gelling properties allow it to be used at a 20-30% lower usage level offering economic advantage to meat and confectionery industries in particular. It offers good body and mouthfeel without altering flavor. Accu-Gel™ exhibits excellent heat, shear, and acid stability similar to many modified starches. This native starch provides food manufacturers with "clean labeling" opportunities.

- meat and fish products • noodles • soups • canned food
- light sour cream • batters • extruded snacks • pie fillings

Ingredient Declaration

Pea Starch
Native Pea Starch
Unmodified Vegetable Starch

Shelf Life

2 years (dry and cool conditions)

Packaging

25 kg multi-walled, kraft paper bags

Appendix H

Specification Sheet for Best Cooking Pulses Pea Flour and Pea Fibre



5000 Plymouth Avenue North Minneapolis MN 55427
1-800-245-5615 (763) 764-4453 Fax: (763) 764-4010

Final Report

Report Date: February 06, 2007
Date Submitted: January 23, 2007
Company Code: CANADIANINTL01

Elaine Sopiwnyk
Canadian Intl Grains Institute
1000-303 Main Street
Winnipeg, MB R3C 3G7 Canada

Library Number: 2007-00598
PO Number: MASTERCARD

Medallion Sample ID:	2007004523	Whole Pea Flour	
Customer Sample ID:	SC117-06	Elaine Sopiwnyk, Canadian Intl Grains Institute	
Assay	Component	Results	Units
Calories (FBDG Subtracted)	Calories	306 ✓	Calories/100 g
Calories from Fat	Calories	17	Calories/100 g
Calories from Saturated Fat	Calories	3	Calories/100 g
Fatty Acid Analysis w/Profile	Total Fat	1.84 ✓	%
	Saturated Fat	0.34 ✓	%
	Monounsaturated Fat	0.44 ✓	%
	cis-cis Polyunsaturated Fat	0.98 ✓	%
	trans Fat	0.00	%
Carbohydrates, Available	Carbohydrates	50.7 ✓	%
Carbohydrates, Total	Carbohydrates	66.3 ✓	%
Fiber, Group	Total Dietary Fiber	17.6 ✓	%
	Insoluble Fiber	15.6 ✓	%
	Soluble Fiber	2.0 ✓	%
Protein by Dumas (N x 6.25)	Protein	21.6 ✓	%
Moisture by Forced Air (1 hr)	Moisture	7.74 ✓	%
Ash, Overnight (16 hr)	Ash	2.550 ✓	%

Medallion Sample ID:	2007004524	Pea Fibre	
Customer Sample ID:	SC118-06	Elaine Sopiwnyk, Canadian Intl Grains Institute	
Assay	Component	Results	Units
Calories (FBDG Subtracted)	Calories	113 ✓	Calories/100 g
Calories from Fat	Calories	6	Calories/100 g
Calories from Saturated Fat	Calories	2	Calories/100 g
Fatty Acid Analysis w/Profile	Total Fat	0.62 ✓	%
	Saturated Fat	0.19 ✓	%
	Monounsaturated Fat	0.15 ✓	%
	cis-cis Polyunsaturated Fat	0.26 ✓	%
	trans Fat	0.00 ✓	%
Carbohydrates, Available	Carbohydrates	18.7	%
Carbohydrates, Total	Carbohydrates	84.6 ✓	%
Fiber, Group	Total Dietary Fiber	75.9 ✓	%
	Insoluble Fiber	65.9 ✓	%
	Soluble Fiber	10.0 ✓	%
Protein by Dumas (N x 6.25)	Protein	8.30 ✓	%
Moisture by Forced Air (1 hr)	Moisture	3.56	%
Ash, Overnight (16 hr)	Ash	2.964 ✓	%

Dry wt basis

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Medallion's services, including this report, are provided subject to all provisions of Medallion's Standard Terms and Conditions, a copy of which appears at www.medlabs.com

APPENDIX I
Extrusion settings

Sample ID	MC (%)	feed rate equation (x=setting)	feed rate kg/h	moisture injection rate equation (x=setting)	total mc %	Water input setting	torque	RPM	T profile (30, 70, 90...)	Tf (°C)	Die pressure (bar)
30-10 green pea	10.1	y=56.837x-13.09	2.62	y=1.018x-0.85	15.78	3	45	242	135, 135	153	20
50-0 green pea	10.7	y=60.663x-14.53	2.77	y=0.955x+0.26	14.70	2	64	240	135, 135	154	25
50-10 yellow pea	10.7	y=69.423x-17.36	3.12	y=0.981x+0.12	15.49	3	62	241	135, 135	154	17
40-5 green pea	10.5	y=59.7x-14.56	2.71	y=0.990x-0.41	15.28	3	54	242	135, 135	154	18
40-5 green pea	10.4	y=50.167x-12.12	2.28	y=0.995x-0.29	16.39	3	41	244	135, 135	153	18
50-10 green pea	10.4	y=66.39x-16.66	2.98	y=0.979x-0.08	15.26	3	58	242	135, 135	155	18
	10.4	y=66.39x-16.66	2.98	y=0.979x-0.08	15.26	3	64	242	120, 120	135	24
	10.4	y=66.39x-16.66	2.98	y=0.979x-0.08	15.26	3	70	242	110, 110	125	30
40-5 green pea	10.8	y=66.47x-16.94	2.97	y=1.002x-0.30	15.45	3	58	242	135, 135	156	17
50-0 yellow pea	12.1	y=67.467x-17.93	2.97	y=0.944x+0.16	15.62	2	60	242	135, 135	154	16
	12.1	y=67.467x-17.93	2.97	y=0.944x+0.16	15.62	2	64	242	120, 120	134	25
	12.1	y=67.467x-17.93	2.97	y=0.944x+0.16	15.62	2	70	242	110, 110	122	32
40-5 yellow pea	11.0	y=67.343x-17.24	3.01	y=0.982x-0.43	15.98	3	56	241	135, 135	153	14
30-0 yellow pea	11.5	y=56.663x-13.14	2.61	y=0.989x-0.02	15.25	2	51	242	135, 135	153	19
40-5 yellow pea	11.8	y=55.233x-14.30	2.46	y=0.976x-0.03	15.72	2	51	240	135, 135	155	17
30-10 yellow pea	11.4	y=62.777x-16.04	2.80	y=0.980x-0.04	14.93	2	57	240	135, 135	155	17
30-0 green pea	11.1	y=58.247x-13.79	2.67	y=0.975x-0.15	14.54	2	55	240	135, 135	153	24
40-5 yellow pea	11.8	y=65.323x-17.31	2.89	y=0.996x-0.44	14.60	2	54	242	135, 135	153	15
Control- starch 100%	10.3	y=39.083x-9.90	1.75	y=1.010x-0.59	14.52	2	62	242	135, 135	157	11
	10.3	y=39.083x-9.90	1.75	y=1.010x-0.59	14.52	2	65	243	120, 120	136	16
	10.3	y=39.083x-9.90	1.75	y=1.010x-0.59	14.52	2	80	243	110, 110	126	16

Appendix J

Extrusion Screw Configuration

High Shear Screw Configuration

8D Feed Screw

1½D 30° forward paddle

6D Feed Screw

¼D Paddle

1D Single Lead Screw

½D 60° Forward Paddle

½D 60° Reverse Paddle

1D Single Lead Screw

¾D 60° Forward Paddle

1D Single Lead Screw

½D 60° Forward Paddle

1D 60° Reverse Paddle

2D Single Lead Screw

1D Single Lead Screw

Appendix K **Sensory Evaluation Ballot for Extrusion**

Panellist No. _____

Ballot – Test 2 Puffed Snack Foods

So that we know your familiarity with commercial puffed snack food samples, please indicate how often you consume puffed snack food products (eg. Cheetos, etc)

- a. at least once a week _____
- b. at least once a month _____
- c. at least four times a year _____
- d. other _____ please explain _____

For each coded sample please indicate your perception of each given attribute (Puffiness, Bad texture, Hard... etc) along the scaled line by marking an X. For the "Ideal product" category, please indicate along the scale for each attribute what you would expect from a similar ideal product. Taste as much of the product as necessary to form an opinion. Rinse with water before evaluating each sample.

Sample 113

	Not at All						Very Much So
	1	2	3	4	5	6	7
Puffiness	◆	◆	◆	◆	◆	◆	◆
Bad Texture	◆	◆	◆	◆	◆	◆	◆
Hard	◆	◆	◆	◆	◆	◆	◆
Good Texture	◆	◆	◆	◆	◆	◆	◆
Soft	◆	◆	◆	◆	◆	◆	◆
Toothpack	◆	◆	◆	◆	◆	◆	◆
Crispiness	◆	◆	◆	◆	◆	◆	◆

Additional Comments

Appendix L
ANOVA Partial sum of squares - Type III of extruded snack texture for bulk density

Source	Sum of Squares	df	Mean Square	F Value	p-value	
Model	0.033	5	6.585E-003	15.61	< 0.0001	significant
<i>A-pea variety</i>	5.145E-003	1	5.145E-003	12.20	0.0007	
<i>B-flour</i>	2.446E-003	1	2.446E-003	5.80	0.0174	
<i>C-hull</i>	1.507E-003	1	1.507E-003	3.57	0.0609	
<i>AC0.014</i>	1	0.014	32.68	< 0.0001		
<i>BC0.011</i>	1	0.011	25.13	< 0.0001		
Curvature	7.834E-003	2	3.917E-003	9.29	0.0002	significant
Residual	0.056	132	4.218E-004			
<i>Lack of Fit</i>	8.889E-004	2	4.444E-004	1.05	0.3513	not significant
<i>Pure Error</i>	0.055	130	4.214E-004			
Cor Total	0.096	139				

Std. Dev.	0.021	R-Squared	0.3716
Mean	0.11	Adj R-Squared	0.3478
C.V. %	17.88	Pred R-Squared	0.3038
PRESS	0.062	Adeq Precision	15.556

Coefficient		Standard	95% CI	95% CI		
Factor	Estimate	df	Error	Low	High	VIF
Intercept	0.11	1	2.296E-003	0.10	0.11	
A-pea variety	8.019E-003	1	2.296E-003	3.477E-003	0.013	1.75
B-flour	-5.529E-003	1	2.296E-003	-0.010	-9.869E-004	1.00
C-hull	4.341E-003	1	2.296E-003	-2.011E-004	8.883E-003	1.00
AC-0.013	1	2.296E-003	-0.018	-8.585E-003	1.00	
BC0.012	1	2.296E-003	6.968E-003	0.016	1.00	

Ctr Pt A[1]	0.019	1	4.960E-003	9.675E-003	0.029	1.37
Ctr Pt A[2]	8.789E-003	1	4.960E-003	-1.023E-003	0.019	

Final Equation in Terms of Coded Factors:

Bulk density = $0.11 + 8.02E-3 * A - 5.53E-3 * B + 4.34E-3 * C - 0.01 * A * C + 0.012 * B * C$

Final Equation in Terms of Actual Factors:

Green Pea Variety

Bulk density = $0.15 - 1.70E-3 * \text{flour} - 5.71E-3 * \text{hull} + 2.30E-4 * \text{flour} * \text{hull}$

Yellow Pea variety

Bulk density = $0.19 - 1.70E-3 * \text{flour} - 0.01 * \text{hull} + 2.30E-4 * \text{flour} * \text{hull}$

Appendix M

ANOVA Partial sum of squares - Type III of extruded snack texture for shear strength

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	3466.59	5	693.32	31.15	< 0.0001	significant
<i>A-pea variety</i>	221.87	1	221.87	9.97	0.0020	
<i>B-flour</i>	108.16	1	108.16	4.86	0.0292	
<i>C-hull</i>	1435.06	1	1435.06	64.48	< 0.0001	
<i>AC974.95</i>	1	974.95	43.80	< 0.0001		
<i>BC255.63</i>	1	255.63	11.49	0.0009		
Curvature	783.31	2	391.65	17.60	< 0.0001	significant
Residual	2937.87	132	22.26			
<i>Lack of Fit</i>	87.86	2	43.93	2.00	0.1390	not significant
<i>Pure Error</i>	2850.01	130	21.92			
Cor Total	7187.77	139				
Std. Dev.	4.72			R-Squared	0.5413	
Mean	18.93			Adj R-Squared	0.5239	
C.V. %	24.92			Pred R-Squared	0.4897	
PRESS	3268.34			Adeq Precision	17.655	

Coefficient	Standard	95% CI	95% CI			
Factor	Estimate	df	Error	Low	High	VIF
Intercept	16.96	1	0.53	15.92	18.00	
A-pea variety	1.67	1	0.53	0.62	2.71	1.75
B-flour	-1.16	1	0.53	-2.21	-0.12	1.00
C-hull	4.24	1	0.53	3.19	5.28	1.00
AC-3.49	1	0.53	-4.53	-2.45	1.00	
BC1.79	1	0.53	0.74	2.83	1.00	
Ctr Pt A[1]	3.29	1	1.14	1.04	5.55	1.37
Ctr Pt A[2]	5.90	1	1.14	3.65	8.16	

Final Equation in Terms of Coded Factors:

$$\begin{aligned} \text{Shear Strength} &= \\ +16.96 & \\ +1.67 & \quad * A \\ -1.16 & \quad * B \\ +4.24 & \quad * C \\ -3.49 & \quad * A * C \\ +1.79 & \quad * B * C \end{aligned}$$

Final Equation in Terms of Actual Factors:

$$\begin{aligned} \text{pea variety} & \quad \text{green} \\ \text{Shear Strength} & = \\ +19.37064 & \\ -0.29503 & \quad * \text{flour} \\ +0.11521 & \quad * \text{hull} \\ +0.035751 & \quad * \text{flour} * \text{hull} \end{aligned}$$

$$\begin{aligned} \text{pea variety} & \quad \text{yellow} \\ \text{Shear Strength} & = \\ +29.68326 & \\ -0.29503 & \quad * \text{flour} \\ -1.28118 & \quad * \text{hull} \\ +0.035751 & \quad * \text{flour} * \text{hull} \end{aligned}$$

Appendix N

ANOVA Partial sum of squares - Type III of extruded snack texture for expansion index

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	906.00	3	302.00	206.28	< 0.0001	significant
<i>A-pea variety</i>	<i>194.81</i>	<i>1</i>	<i>194.81</i>	<i>133.07</i>	<i>< 0.0001</i>	
<i>C-hull</i>	<i>537.39</i>	<i>1</i>	<i>537.39</i>	<i>367.07</i>	<i>< 0.0001</i>	
<i>AC281.43</i>	<i>1</i>	<i>281.43</i>	<i>192.23</i>	<i>< 0.0001</i>		
Curvature	386.69	2	193.35	132.07	< 0.0001	significant
Residual	196.18	134	1.46			
<i>Lack of Fit</i>	<i>11.29</i>	<i>4</i>	<i>2.82</i>	<i>1.98</i>	<i>0.1007</i>	<i>not significant</i>
<i>Pure Error</i>	<i>184.89</i>	<i>130</i>	<i>1.42</i>			
Cor Total	1488.87	139				
Std. Dev.	1.21			R-Squared	0.8220	
Mean	7.32			Adj R-Squared	0.8180	
C.V. %	16.54			Pred R-Squared	0.8051	
PRESS	214.79			Adeq Precision	36.340	

Coefficient		Standard	95% CI	95% CI		
Factor	Estimate	df	Error	Low	High	VIF
Intercept	8.53	1	0.14	8.26	8.80	
A-pea variety	-1.56	1	0.14	-1.83	-1.29	1.75
C-hull	-2.59	1	0.14	-2.86	-2.32	1.00
AC1.88	1	0.14	1.61	2.14	1.00	
Ctr Pt A[1]	-4.64	1	0.29	-5.21	-4.06	1.37
Ctr Pt A[2]	-1.04	1	0.29	-1.61	-0.46	

Final Equation in Terms of Coded Factors:

$$\begin{aligned} & \text{EI} \\ & +8.53 \\ & -1.56 \quad \quad \quad * A \\ & -2.59 \quad \quad \quad * C \\ & +1.88 \quad \quad * A * C \end{aligned}$$

Final Equation in Terms of Actual Factors:

$$\begin{aligned} & \text{pea variety} \quad \quad \quad \text{green} \\ & \text{EI} \quad \quad \quad = \\ & +14.56009 \\ & -0.89348 \quad \quad \quad * \text{hull} \end{aligned}$$

$$\begin{aligned} & \text{pea variety} \quad \quad \quad \text{yellow} \\ & \text{EI} \quad \quad \quad = \\ & +7.68793 \\ & -0.14324 \quad \quad \quad * \text{hull} \end{aligned}$$

Appendix O
Optimization results of puffed pea snacks

Constraints

Name	Lower Goal	Upper Limit	Lower Limit	Upper Weight	Weight	Importance
pea variety	is in range	green	yellow	1	1	3
flour maximize	30	50	1	1		3
hull maximize	0	10	1	1		3
Bulk density	minimize	0.0503	0.197	1	1	3
Shear Strength	is in range	8.86	31.24	1	1	3
EI is in range	8	17.8271	1	1		3

Solutions for 2 combinations of categoric factor levels

Number pea variety	flour	hull	Bulk density	Shear Strength	EI Desirability	
1 green	50.00	7.34	0.108832	18.5895	8.00001	0.761
2 green	50.00	6.74	0.10532	17.4364	8.54147	0.749
3 green	<u>50.00</u>	<u>6.60</u>	<u>0.104514</u>	<u>17.1716</u>	<u>8.66577</u>	<u>0.746</u>

3 Solutions found

Appendix P
Sensory Evaluation results 50% Whole Yellow Pea Flour 10% Pea Fibre extruded
with a final barrel temperature set to 135°

Panellist No.	Texture Descriptor						
	Puffiness	Bad Texture	Hard	Good Texture	Soft	Toothpack	Crispiness
1	7	2	4	6	1	3	6
2	7	1	1	6	3	2	6
3	7	1	3	5	3	5	6
4	7	7	1	5	7	3	7
5	7	5	5	6	1	6	7
6	3	6	6	3	2	4	6
7	7	5	4	3	1	5	7
8	4	1	1	7	5	1	2
9	7	5	6	2	1	2	6
10	6	1	2	7	5	4	6
11	6	3	3	4	4	4	6
12	4	1	1	6	5	7	7
13	7	1	1	6	6	2	6
14	3	4	6	4	2	2	6
15	6	1	1	7	1	1	6
16	5.5	3.5	2.5	5.5	4.5	5.5	5.5
17	7	5	5	3	3	7	6
18	6.5	2.25	2.25	6	3	5	6.5
19	5.5	4.5	4.5	3.5	3.5	5	5
20	6.25	4.25	6.75	4.75	1.75	4.75	6.5
21	6	3	4	5	4	3	5
22	6	1.25	3.75	4.5	2	3	5
23	3.5	4.5	4.5	4.5	3.5	4.5	2.5
24	7	4	4	5	1	2	6
25	5	5	6	3	3	3	6
26	3	3	4	5	4	4	6
27	7	2	4	5	4	3	7
28	6.5	4.5	3.5	2.5	3.5	4.5	5.5
29	5.75	1	6	5.75	1	3.5	6.5
30	7	5	6	5	2	6	6
31	6.25	1.75	3.5	1.5	3.5	2.5	6.5
32	7	3	5	6	1	2	7
33	6	5	3	6	5	2	6
34	6	2	2	5	5	2	6
35	6	2	2	6	4	1	7
36	7	2	4	6	2	3	7
37	6	2	4	6	1	2	5
38	4.5	1.5	3.5	3.5	3.5	4.5	3.5
39	6	1	2	6	4	2	5
40	6	2	3	6	2	2	7
41	6	2	5	6	2	2	5
42	7	6	5	6	3	2	5
43	7	5	5	2	4	6	6
44	7	1	4	7	2	4	6
45	4	3	4	6	6	4	6
46	6	5	4	3	4	5	5
47	6	4.75	5.5	5.25	3.5	4	4.5
48	7	1	7	5	1	6	7
49	7	5	5	3	4	3	6
50	5.75	1.25	4	6	2.5	1.5	6.5
51	7	6	7	2	1	3	7
52	7	4	6	2	1	4	7
53	6.5	4.25	4.25	3.5	1.5	3.5	6.75
54	5	5	6	3	3	6	6
55	7	2	1	6	7	1	6
56	7	1	3	7	7	3	6
57	7	2	6	6	1	3	6
58	6	4	3	4	5	2	5
59	7	3	5	6	3	5	7
60	7	4	1.75	4	4	5	5
61	7	2	5	5	2	4	6
62	7	4	5	3	3	3	7
63	6	6	6	4	3	5	6
492 - Average	6.1	3.2	4.0	4.8	3.1	3.5	5.9

Appendix Q
Sensory Evaluation results 50% Whole Yellow Pea Flour 0% Pea Fibre extruded
with a final barrel temperature set to 120°

Panellist No.	Puffiness	Bad Texture	Hard	Texture Descriptor		Toothpack	Crispiness
				Good Texture	Soft		
1	6	1	2	6	6	2	6
2	4.5	4	1	4	4	6.5	5
3	6	2	1	5	2	2	7
4	7	1	1	7	7	2	7
5	3	5	5	7	2	5	6
6	2	4	5	4	3	7	6
7	6	1	2	6	5	4	5
8	2	4	3	3	6	1	1
9	1	6	6	1	5	5	4
10	2	5	4	2	2	3	5
11	3	2	3	5	4	4	6
12	4	1	1	7	7	7	7
13	3	1	1	6	6	2	4
14	3	3	5	5	3	3	6
15	3	3	2	3	1	2	4
16	2.5	2.5	3.5	5.5	4.5	2.5	5.5
17	4	2	1	7	6	6	7
18	4.5	3.5	3.5	5	3	4.5	5.5
19	3.5	4.5	4.5	3.5	2.5	4.5	5
20	4.25	6.25	4.75	2.75	3.25	5.75	5.25
21	2	5	1	4	6	5	2
22	2	3.75	2	3	6	7	2
23	2.5	5.5	4.5	2.5	2.5	4.5	1.5
24	4	4	1	4	5	2	3
25	5	4	5	4	3	2	5
26	1	2	3	6	1	6	7
27	6	5	2	3	6	5	5
28	2.5	5.5	5.5	2.5	2.5	5.5	3.5
29	1	1	6.5	5.5	1	5	6.5
30	3	3	4	6	3	4	6
31	2.5	3.25	5.5	4.25	3.5	1.5	3.5
32	4	3	2	5	4	5	4
33	4	4	3	5	3	3	6
34	3	4	2	4	4	3	4
35	7	1	1	7	3	4	5
36	4	4	5	5	2	6	5
37	2	4	3	2	2	4	4
38	2.5	1.5	1.5	5.5	5.5	5.5	4.5
39	5	2	3	4	5	2	3
40	4	6	2	2	3	4	2
41	2	3	3	4	5	5	4
42	4	1	1	7	6	3	7
43	6	2	1	6	6	6	2
44	2	6	6	2	2	6	5
45	4	2	2	5	6	2	6
46	3	2	3	6	6	5	5
47	2.25	6.25	3.75	4.75	5.25	5.75	4.5
48	5	1	2	6	7	4	6
49	4	2	5	6	2	3	5
50	3	4	6.5	4	3	4.5	5
51	4	1	5	7	3	6	6
52	7	2	2	7	6	5	6
53	4.5	2.5	2.5	5.5	5.5	2.5	2.5
54	3	3	6	6	2	6	7
55	3	4	4	4	4	3	6
56	3	3	1	6	7	5	5
57	2	2	4	6	3	6	7
58	3	3	3	4	3	3	5
59	5	3	5	6	2	4	6
60	3	7	3	1	3	7	2
61	3	3	3	4	5	4	5
62	4	3	4	6	5	5	5
63	4	4	5	3	2	4	3
526 - Average	3.6	3.2	3.2	4.7	4.0	4.2	4.8

Appendix R
Sensory Evaluation results 50% Whole Yellow Pea Flour 0% Pea Fibre extruded
with a final barrel temperature set to 135°

Panellist No.	Texture Descriptor						
	Puffiness	Bad Texture	Hard	Good Texture	Soft	Toothpack	Crispiness
1	7	2	2	6	7	2	7
2	7	2	3	5	5	4	6
3	7	1	3	6	4	2	5
4	7	1	1	6	7	4	7
5	6	4	4	6	1	4	6
6	6	1	2	7	6	5	6
7	6	2	1	6	5	3	6
8	5	1	1	7	7	1	5
9	6	1	4	7	1	2	7
10	5	1	4	7	2	4	4
11	5	3	3	5	4	4	5
12	6	1	1	7	7	6	7
13	6	1	1	5	6	3	5
14	4	2	2	5	4	2	5
15	5	3	1	5	1	4	6
16	4.5	2.5	2.5	5.5	5.5	4.5	6
17	5	1	2	6	5	6	7
18	5.75	2.25	2.5	6.5	3.25	4.75	6.5
19	5	3.5	4	4.5	4	5	4.5
20	6	2.75	5.25	4.25	2.75	3.25	6.25
21	6	2	2	6	6	2	6
22	6	1.75	2	3.75	4.75	5	5
23	3.5	4.5	3.5	3.5	2.5	5.5	3.5
24	7	1	1	6	6	2	7
25	5	3	5	4	5	2	6
26	3	5	5	3	5	6	6
27	7	2	2	6	4	3	7
28	5.5	4.5	1.5	5.5	5.5	4.5	5.5
29	2.75	1	3.75	5.75	1	2.5	6.5
30	6	2	5	6	3	3	6
31	6.5	3.25	1.25	2.5	6.5	3.5	6.5
32	6	1	3	6	2	3	7
33	6	4	4	5	5	2	4
34	5	2	4	5	4	3	5
35	5	2	4	5	3	5	6
36	6	3	4	6	4	4	6
37	4	2	3	3	2	4	3
38	3.5	1.5	1.5	4.5	3.5	1.5	5.5
39	6	2	2	3	4	2	5
40	5	1	2	6	4	5	5
41	5	1	1	6	5	2	6
42	6	1	3	5	6	4	5
43	7	1	1	7	6	6	7
44	5	4	4	3	3	6	6
45	4	3	2	6	4	5	7
46	4	2	2	6	6.5	5	5
47	4.25	5.25	4	5	4.75	3.75	4.75
48	7	1	1	7	7	6	7
49	6	2	3	5	5	3	5
50	6.75	1	6	6	4	4	7
51	7	1	6	6	1	2	7
52	6	3	3	5	5	5	7
53	5.5	1	2.5	6.5	2.5	5.5	6.5
54	4	3	6	5	3	3	6
55	6	2	1	6	6	2	6
56	5	1	2	7	6	4	6
57	6	3	3	5	3	2	4
58	5	1	3	6	6	2	6
59	7	2	3	7	3	2	7
60	3	1	1	7	6	3	7
61	5	5	4	3	3	4	4
62	6	4	4	6	5	5	7
63	6	1	3	7	4	4	7
<u>113 - Average</u>	5.5	2.2	2.8	5.5	4.3	3.7	5.9

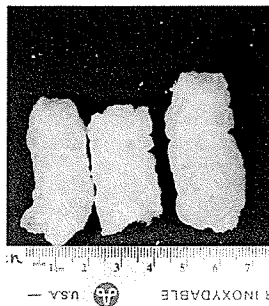
Appendix S

Sensory Evaluation results for an ideal product

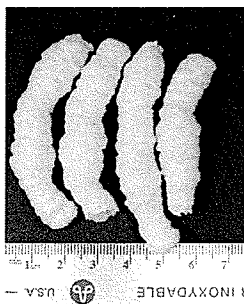
Panellist No.	Frequency	Puffiness	Bad Texture	Hard	Texture Descriptor		Toothpack	Crispiness
					Good Texture	Soft		
1	C	7	1.5	5	6.5	2	2	7
2	D	6.5	1	1	7	3	1	6
3	A	7	1	2	6	3	2	6
4	B	7	1	2	7	7	1	7
5	B	6	1	5	7	3	1	7
6	B							
7	A	7	1	2	6	5	1	7
8	B	4	1	1	7	7	1	1
9	C	4	1	7	7	1	2	7
10	D	4	1	2	7	5	2	4
11	B	5	1	2	6	4	2	6
12	B	6	1	1	7	7	7	7
13	B	6	1	2	7	7	1	6
14	B							
15	D	6	1	1	7	1	1	7
16	A	5.5	1.5	4.5	5.5	2.5	2.5	5.5
17	C	5	2	2	7	5	4	6
18	C	6.25	2.25	3.5	6.5	2.5	3.5	6.5
19	C	5	3	3	5	5	3.5	5
20	B	5.5	1.75	5.75	5.75	2.5	1.5	6
21	C	6	4	4	4	6	1	6
22	C	6	1	1	5	4	2	6
23	B	5.5	1.5	1.5	5.5	5.5	4.5	4.5
24	D							
25	B							
26	A							
27	C							
28	D	5.5	1.5	1.5	6.5	5.5	1.5	5.5
29	C	2.75	1	6.5	5	1.25	2.25	6.5
30	C							
31	C	5.5	1	3.5	6.75	4.5	1	5.5
32	C	6	1	3	7	2	1	7
33	C	7	1	1	7	7	1	4
34	B	6	1	3	6	4	2	7
35	D	6	1	3	7	4	3	6
36	D	7	1	4	7	2	2	6
37	C	6.5	1	1	6	4	1	6.5
38	C	5.5	1.5	1.5	6.5	6.5	1.5	6.5
39	C	2	1	5	5	2	3	5
40	C	6	1	2	7	2	1	6
41	A	6	1	2	7	4	2	6
42	C	7	1	1	7	6	2	7
43	C	6	1	3	6	5	3	7
44	B	7	1	4	7	4	2	7
45	C	4	2	6	6	2	1	7
46	C	4	2	3	6	6	2	6
47	C	4.25	2.5	5.5	4.75	4	5.25	5.5
48	B	7	1	3	7	5	4	7
49	C	3	1	6	7	2	1	6
50	B	5	1	4	7	2	2	6
51	C	6	1	5	1	3	4	5
52	C	5	1	3	7	5	2	6
53	C	4.5	1.5	3.5	6.5	2.5	1.5	4.5
54	C	3	1	6	7	2	5	6
55	C	6	2	2	6	6	2	6
56	C	5	1	5	7	4	3	7
57	B	3	1	4	7	4	1	6
58	C	5	2	3	6	6	2	6
59	B	6	2	4	6	2	2	6
60	C	4	1	3	7	4	2	6
61	C	6	1	5	6	3	3	6
62	C	5	1	5	7	4	4	6
63	C	6	1	2	7	4	2	7
IDEAL - Average		5.4	1.3	3.2	6.3	4.0	2.2	6.0

Appendix T

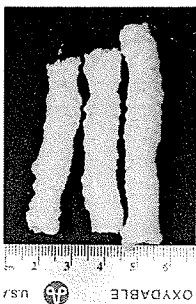
Images of final extruded products



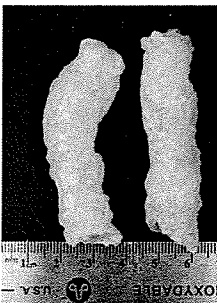
30% Green Pea flour, 0% pea fibre 135°C processing temperature



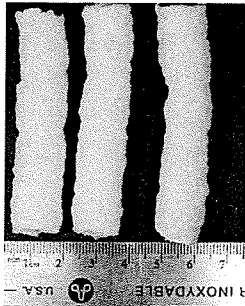
30% Green Pea flour, 10% pea fibre 135°C processing temperature



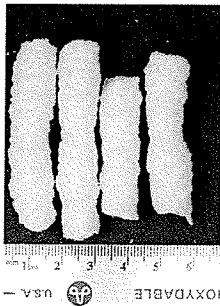
40% Green pea flour, 5% pea fibre 135°C processing temperature



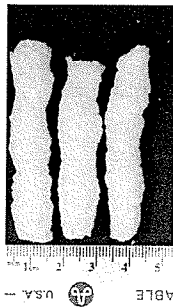
50% Green pea flour, 0% pea fibre 135°C processing temperature



50% Green pea flour, 10% pea fibre, processing temperature at 110°C



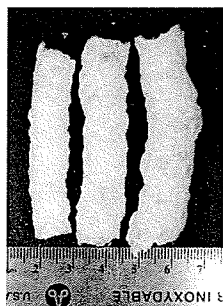
30% Whole yellow pea flour, 10% pea fibre, processing temperature of 135°C



40% Whole yellow pea flour, 5% pea fibre, processing temperature of 135°C



50% Whole yellow pea flour, 0% pea fibre, processing temperature 120°C



Pea starch processed at 135, 120 and 110°C from L to R