Use of Soybean (*Glycine max* (L.) Merrill) Presscake and Flours as Food Ingredients: Effect on Nutritional, Physical, Textural, Sensory Properties, Starch Digestibility and Glycemic Index

by

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ABSTRACT

Corn-based tortillas and wheat-based pizza crust were formulated using soybean presscake (SP) and defatted soy flour (SF); changes in nutritional properties, selected anti-nutritional factors, physical characteristics and consumer acceptance were evaluated as were *in vitro* and *in vivo* evaluation of glycemic index (GI) for tortillas at selected SP fortification levels. Protein quality and quantity improved by adding soy products. Fat levels increased with SP but decreased with SF. While levels of trypsin inhibitor and phytic acid generally increased, levels were considered acceptable. Texture of SP tortillas was more like the corn control than SF tortillas, likely because of higher fat content in SP. Flavour, texture and overall consumer acceptability were higher for tortillas containing soy products. *In vitro* analysis showed lower starch hydrolysis for the soybean tortillas, but *in vivo* GI values were not significantly different. Thus, incorporation of SP and defatted SF to fortify bakery products has potential.

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LIST OF ABBREVIATIONS

BAPNA	α-N-benzoyl-DL-arginine-p-nitroanilidehydrochloride
BBI	Bowman-Birk Trypsin Inhibitor
CMC	Carboxymethyl Cellulose
CVD	Cardiovascular Disease
DV	Daily Value
F	Familiar
FAO	Food and Agriculture Organization of United Nations
FDA	U.S. Food and Drug Administration
GI	Glycemic Index
LDL	Low-density Lipoprotein
MAFRD	Manitoba Agriculture, Food and Rural Development
PUFA	Polyunsaturated Fatty Acids
SF	Soy Flour
SP	Soy Presscake
SPC	Soy Protein Concentrate
SPI	Soy Protein Isolate
SSL	Sodium Stearoyl-2-lactylate
TIA	Trypsin Inhibitor Activity
TIU	Trypsin Inhibitor Unit
TSF	Textured Soy Flours
TSPC	Textured Soy Protein Concentrates
UF	Unfamiliar
USDA	United States Department of Agriculture

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is a member of the *Leguminosae* family, subfamily *Papilionoideae*, and the genus *Glycine*, L (Johnson & Bernard, 1963). Soybean is known as one of the most nutritious and economical agricultural commodities in the world due to its unique chemical composition and nutritional profile. Depending on different varieties and growing conditions, soybeans vary widely in their nutrient content. Typically, soybean contains approximately 35% to 40% protein, 15% to 20% fat and 23% carbohydrates, 5% minerals (ash), 4% fibre, 8% moisture, vitamins and other minor substances (Riaz, 2006; Ali, 2010).

Canada accounts for less than 2 percent of the world's soybean production, and Canada was the 7th largest soybean production country in 2011 and 2012. Soybeans produced in Canada are high-yield, high-quality food grade beans, and therefore, approximately 35 percent of Canadian produced soybeans are exported to premium markets such as Japan and Europe (Canadian Soybean Council, 2012).

Among all the cereals and legumes, soybean has the highest protein content up to 40%. In addition to the high protein content, soy protein also provides a complete range of all the essential amino acids that are needed for human growth, and health maintenance (Hoogenkamp, 2005; Endres, 2001). The consumption of soy protein may play an important role in lowering serum levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, and serum triacylglycerol (Urade, 2011). Therefore, in 1999, the United States Food and Drug Administration approved the following health claim for soy protein: "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease" (FDA, 2014). In addition, there is evidence showing that carbohydrates in foods such as legumes, pasta and whole grain cereals are slowly digested and absorbed, and are favorable in the dietary management of metabolic disorders (e.g. diabetes and hyperlipidemia) (Goni, Garcia-Alonso, & Saura-Calixto, 1997).

The use of soy ingredients in food products has been of interest for decades. However, very few studies have been conducted on relatively high levels of incorporation of soybeans (> 30%) as a food ingredient. In addition, the consumption or the use of soybean presscake, which is the by-product obtained from solvent-free oil extraction, as a food ingredient is still not common.

Therefore, this work was undertaken to increase the value of soybean crop, as well as to provide products that are superior in nutrition and acceptable by consumers at high levels of soy fortification. The effect of added soybean presscake and defatted soy flour on some nutritional, physical and sensory properties was investigated in terms of proximate analysis including moisture, fat, protein and ash contents, anti-nutritional factors such as trypsin inhibitor activity and phytic acid content, general physical characteristics including size and thickness, rollability, colour and texture determined instrumentally, and consumer acceptance of the soy fortified corn tortillas on appearance, flavour and texture. Additionally, the *in vitro* starch digestibility and *in vivo* glycemic responses of selected soy fortified tortillas were evaluated, and compared to control corn tortillas. Moreover, the effect of added soybean presscake and soy flour on some physical properties such as texture and colour was determined instrumentally for wheat-based pizza crust.

It is expected that levels of protein will increase in the finished products. In addition the rate of starch hydrolysis and glycemic index values are expected to be lower. In comparison to control corn tortillas, levels of anti-nutritional factors are expected to increase. Texture and colour of finished products are expected to change.

2. LITERATURE REVIEW

2.1 Soybeans

Soybean (*Glycine max* (L.) Merrill) is a member of the *Leguminosae* family, subfamily *Papilionoideae,* and the genus *Glycine,* L (Johnson & Bernard, 1963). It originated from China 4000 to 5000 years ago, and now has become an important crop at a global level due to the varied uses (Liu, 1997).

2.1.1 Soybean Production and Utilization in Manitoba, Canada

It is widely believed that the soybeans were first cultivated in China. Until 1954, China was the leading soybean producer and exporter in the world (Liu, 1997). However, soybean production developed rapidly in the United States in the 1950s. Since then, United States has become the largest soybean producing country (Liu, 1997).

Canada accounts for less than 2 percent of the world's soybean production, and Canada was the 7th largest soybean producing country in 2011 and 2012 (Table 2.1). Compared to the total production of soybeans, however, Canada had the third highest yield in the world in 2012 (29191 hectogram/hectare) following Egypt (44537 hg/ha) and Turkey (36394 hg/ha) (FAO, 2014). In 2006, soybeans were Canada's 5th most valuable field crop; the other four crops were canola, wheat, potatoes and corn (Canadian Soybean Council, 2012). Soybeans produced in Canada are high-yield, high-quality food grade beans, and therefore approximately 35 percent of Canadian produced soybeans are exported to premium markets` such as Japan and Europe (Canadian Soybean Council, 2012).

Table 2.1. The Production of Soybeans in Top 10 Countries in 2012 and 2011* (Adapted from FAO, 2014)

Country	Production (tonnes)	
	2012	2011
World Total	**241,841,416**	**262,352,402**
United States of America	82,054,800	84,191,930
Brazil	65,848,857	74,815,447
Argentina	40,100,197	48,878,771
China, mainland	12,800,000	14,485,000
India	11,500,000	12,214,000
Paraguay	8,350,000	8,309,793
Canada	4,870,160	4,246,300
Uruguay	3,000,000	1,830,000
Ukraine	2,410,200	2,264,400
Bolivia (Plurinational State of)	2,400,000	2,299,857

*Ranking is based on 2012 data.

Soybeans are primarily grown in Ontario, Manitoba and Quebec in Canada (Canadian Soybean Council, 2012) (Table 2.2). Manitoba produces approximately 10 to 18% of Canada's total soybean production, of which about 4% to 5% is non-GMO soybeans (MAFRD, 2011 & Canadian Soybean Council, 2014). Manitoba grown soybeans are exported to the United States, Japan and other countries in Asia and Europe, and also used as ingredients for a variety of food products (MAFRD, 2011). According to Oilseeds: Worlds Market and Trade (USDA, 2014), soybean meal production accounted for 67.5% of world total major protein meals, followed by rapeseed meal (13.7%) and sunflower seed meal (9.0%).

Table 2.2. Total Seed Area (Hectares) of Soybeans in Canada by Province from 2010 to 2013 (Adapted from Canadian Soybean Council, 2014)

Province	2010	2011	2012	2013
Quebec	262,000	300,000	292,000	288,506
Manitoba	210,400	232,700	341,829	424,929
Ontario	987,400	987,400	1,072,440	1,011,736
Maritimes	17,800*	22,300*	22,258*	34,803*
Saskatchewan	N/A	N/A	N/A	68,798

* Data is from the province of PEI only

2.1.2 Composition and Nutritional Profile of Soybeans

Soybean is known as one of the most nutritious agricultural commodities in the world due to its unique chemical composition and nutritional profile. Based on different varieties and growing conditions, soybeans vary widely in their nutrient content. Typically, soybean contains approximately 35% to 40% protein, 15% to 20% fat and 23% carbohydrates, 5% minerals (ash), 4% fibre, 8% moisture, vitamins and other minor substances (Riaz, 2006; Ali, 2010) (Table 2.3).

Table 2.3. Nutritional Value of Raw Soybean Mature Seeds (Adapted from the USDA National Nutrient Database for Standard Reference, Release 25, 2012)

Nutrient	Units	Value per 100 g
Proximate analysis		
Water	g	8.54
Energy	kcal	446
Protein	g	36.49
Total lipids (fat)	g	19.84
Carbohydrate, by difference	g	30.16
Fiber, total dietary	g	9.3
Sugars, total	g	7.33
Mineral	mg	3081
Calcium, Ca	mg	277
Iron, Fe	mg	15.7
Magnesium, Mg	mg	280
Phosphorus, P	mg	704
Potassium, K	mg	1797
Sodium, Na	mg	2
Zinc, Zn	mg	4.89
Lipids		
Fatty acids, total saturated	g	2.884
Fatty acids, total monounsaturated	g	4.404
Fatty acids, total polyunsaturated	g	11.255
Cholesterol	mg	0

Accessed in 2014

2.1.2.1 Protein

Typically, legumes contain about 20 to 30% proteins, and cereals contain about 8 to 15% proteins (Liu, 1997). Among all the cereals and legumes, soybean has the highest protein content up to 40%. In addition to the high protein content, the protein nutritional quality of soybeans has also been shown to be equal to the protein quality of milk, meat, fish and eggs (Riaz, 2006; Endres, 2001). Soy protein provides a complete range of all the essential amino acids that are needed for human growth, and health maintenance (Hoogenkamp, 2005; Endres, 2001) (Table 2.4). Like other legume plants, soy proteins are low in sulfur-containing amino acids (Liu, 1997). Therefore, methionine is the most limiting amino acid in soy protein. Soy proteins are rich in lysine, which is deficient in most cereal proteins (Liu, 1997). In addition, both human clinical studies and animal research have demonstrated the excellent digestibility of soy protein (Endres, 2001). Therefore, though soy protein lacks sulfur-containing amino acids, the sulfur-containing amino acid concentration in soy protein was not significantly less than that required by the human body due to the excellent digestibility (Kumar, Rani, & Chauhan, 2010).

Table 2.4. Amino Acid Composition of Soybeans (g/16 g Nitrogen). (Adapted from Berk, 1992)

Amino Acid	Soybeans
Essential Amino Acids	
Isoleucine	4.54
Leucine	7.78
Lysine	6.38
Methionine	1.26
Cysteine	1.33
Meth. + Cyst.	2.59
Phenylalanine	4.94
Tyrosine	3.14
Phe + Tyr.	8.08
Threonine	3.86
Tryptophan	1.28
Valine	4.80
Histidine	2.53
Non-Essential Amino Acids	
Arginine	7.23
Alanine	4.26
Aspartic acid	11.70
Glutamic acid	18.70
Glycine	4.18
Proline	5.49
Serine	5.12

Based on the biological function, seed proteins can be classified into metabolic proteins and storage proteins; and based on solubility patterns, legume seed proteins are categorized into albumins (soluble in water) and globulins (soluble in salt solution) (Liu, 1997). Soy proteins are mainly composed of albumin and globulins, of which approximately 90% are storage proteins and are mostly globulins (Kinsella, 1979). The remaining proteins are mainly intracellular enzymes including lipoxygenase, amylase, protein inhibitors and membrane lipoproteins (Kinsella, 1979). The principal components of soy storage proteins are the 7S (β-conglycinin) and 11S (glycinin) globulins, in which the subunits are associated mainly through hydrophobic and hydrogen bonding (Kinsella, 1979; Guerrero, 2010). β-conglycinin is composed of three unique peptides, α, α' and β, and these three peptides are associated as trimers, while glycinin is composed of 12 unique polypeptides (Murphy, 2008). The content of glycinin and β-conglycinin varies widely with soybean varieties and growing environments. It has been indicated that glycinin accounts for about 60% to 70% of total globulin contents (Kinsella, 1979; Murphy, 2008). The distribution of major soy protein components and fractions are classified and summarized in Table 2.5 according to protein sedimentation properties (Kinsella, 1979; Murphy, 2008; Mohamed & Xu, 2003).

Table 2.5. The Distribution of Major Soy Protein Components and Fractions*

Fraction	Content (%)	Principal Components
2S	8	Trypsin inhibitor, Cytochrome & Small molecular weight enzymes
7S	35	Globulins (β-conglycinin), Lipoxygenase, Amylase
11S	52	Globulins (glycinin)
15S	5	Polymers (dimer of glycinin)

*Kinsella, 1979, table II; Murphy, 2008; Mohamed & Xu, 2003

2.1.2.2 Lipid

Soybean has the second highest oil content among all the legume plants (~20%), and the highest oil content is found in peanut, which is about 48% (Liu, 1997). Soybean oil is mainly in the form of triglycerides, and most of the fatty acids are unsaturated (Table 2.6) (Pryde, 1980). The highest percentage of fatty acid in soybean oil is linoleic acid, followed by oleic, palmitic, linolenic, stearic acids and other minor fatty acids.

Table 2.6. Fatty Acid Composition of Soybean Oil (Adapted from Pryde, 1980)

Component acid	Fatty Acid Composition (wt%)	
	Range	Average
Triglycerides	> 95	
Unsaturated		
Palmitoleic	< 0.5	0.3
Oleic	20 – 50	22.8
Linoleic	35 – 60	50.8
Linolenic	2 – 13	6.8
Eicosenoic	< 1.0	---
Total	---	80.7
Saturated		
Lauric	---	0.1
Myristic	< 0.5	0.2
Palmitic	7 – 12	10.7
Stearic	2 – 5.5	3.9
Arachidic	< 1.0	0.2
Behenic	< 0.5	---
Total	10 – 19	15.0

Soybean is a good source of essential fatty acids – linoleic acid (~50%) and α-linolenic acid (~7%) – that human body cannot synthesize (Kumar et al., 2010; Liu, 1997; Emken, 1980). They are also known as omega-6 (n-6) and omega-3 (n-3) polyunsaturated fatty acids (PUFA) due to the presence of double-bonds at carbon 6 and carbon 3 and the presence of more than one unsaturated bond in the structure (Kumar et al., 2010). These essential fatty acids are involved in the biosynthesis of prostaglandins, which are important to the function of brain nerve, retinal and reproductive tissues (Kumar et al., 2010). Evidence has shown that PUFAs have the benefit of lowing blood cholesterol levels and coronary heart diseases (Emken, 1980). However, recent research indicated that an imbalanced n-6:n-3 ratio might also be the cause of many chronic disease such as diabetes and CVD (Kumar et al., 2010) Therefore, the type of PUFA rather than the total PUFA intake is being emphasized. An ideal ratio 2.3:1 for n-6: n-3 fatty acid has been recommended (Hill et al., 2008).

Soybean oil is comparable in its nutritive value to other vegetable oils such as canola oil and olive oil (Hill et al., 2008).

2.1.2.3 Carbohydrates

On average, soybeans contain approximate 35% carbohydrates on a dry matter basis. This includes non-structural (soluble) and structural (non-soluble) carbohydrates (Liu, 1997; Karr-Lilienthal et al., 2005). The soluble carbohydrates include trace amounts of monosaccharides (glucose and arabinose) and measurable amounts of di- and oligosaccharides (sucrose, raffinose and stachyose) (Liu, 1997). The insoluble carbohydrates in soybeans are structural components mainly found in cell walls, and include cellulose, hemicellulose, pectin and trace amounts of starch (Liu, 1997).

2.1.2.4 Minor Components

Mature raw soybean seeds contain around 5% ash (minerals), of which potassium is found in the highest concentration, followed by phosphorus, magnesium and calcium (Table 2.2). The minor minerals in soybeans include silicon, iron, zinc, copper, molybdenum, fluorine, chromium, selenium, cobalt, cadmium, lead, arsenic, mercury, and iodine (Liu, 1997).

Soybeans also contain vitamins. Both water-soluble and fat-soluble vitamins are present in soybeans. Water-soluble vitamins in soybeans include thiamin, riboflavin, niacin, pantothenic acid, and folic acid, and fat-soluble vitamins include vitamin A and E, with essentially no vitamins D and K (Liu, 1997). The water-soluble vitamins are not substantially lost during oil extraction, but were reported to have a remarkable loss in processes involving water, such as tofu making, whereas vitamin E goes with oil as a natural antioxidant (Liu, 1997).

Isoflavones belong to the group of flavonoids that share a basic structure consisting of two benzyl rings joined by a three-carbon bridge (Liu, 1997). This group of compounds includes the most diverse range of plant phenolics (Liu, 1997). There are four major forms of isoflavones in soybeans, and, in total, twelve isomers are present. The four forms of isoflavones are (1) as free aglycones (genistein, daidzein and glycitein); (2) as β-glucosides when sugar moiety is attached to aglycone; (3) as malonyl; and (4) as acetylated derivatives of β-glucosides (Kumar et al., 2010). Isoflavones vary in soybeans and soy products due to different genotypes, environmental conditions and different processing methods. Isoflavones have been reported to have positive effects on lowering the risk of chronic diseases including breast cancer, cardiovascular diseases, diabetes, renal diseases and osteoporosis due to their antioxidant and anti-carcinogenic properties (Kumar et al., 2010; Liu, 1997).

2.1.3 Anti-nutritional Factors

Anti-nutritional factors present in soybeans include protease inhibitor (trypsin inhibitor), phytic acid, oligosaccharides (raffinose and stachyose) and lectin.

Trypsin inhibitors can reduce bioactivity of trypsin, resulting in decreased protein digestibility and inducing pancreatic hypertrophy (Yuan et al., 2008). Trypsin inhibitors in soybeans consist of two types, Kuntz Trypsin Inhibitor (KTI), which acts specifically against trypsin, as well as Bowman-Birk Trypsin Inhibitor (BBI), which inhibits trypsin and chymotrypsin simultaneously at independent binding sites (Yuan et al., 2008 & Dia et al., 2012). Soybean and its products including soymilk, oilcake, soy protein concentrates and isolates are rich in BBI (Losso, 2008). Trypsin inhibitors are generally thermo-labile, and can be eliminated or inactivated through different methods including thermal treatment, chemical modification, enzymatic hydrolysis, and gamma irradiation (Yuan et al., 2008; Faris, Wang, &Wang 2008; Dia et al., 2012; Dixit et al., 2011).

Oligosaccharides present in soybeans are non-reducing sugars that cannot be digested by the enzymes in small intestine of humans. They directly pass into the lower intestine, and then are metabolized by microorganisms in the large bowel. Therefore, heat stable oligosaccharides, especially raffinose and stachyose have been linked to flatulence and abdominal discomfort (Deshpande & Damodaran, 1990; Dixit et al., 2010)

Phytic acid is the hexaphosphate of myo-inositol with three strongly bonded water molecules, and is the major phosphorus storage form in plants (Jaffe, 1981). Phytic acid is commonly found in plant seeds and known to be associated with protein bodies (Jaffe, 1981; Liu, 1997). Thus, phytic acid levels should be higher in food products with high protein content, such as soybeans. Phytic acid can form poorly soluble complexes with di- or trivalent metal ions and proteins by chelate formation (Jaffe, 1981). These formed complexes reduce the bio-availability of metal ions such as iron, calcium, magnesium and zinc, as well as lower the digestibility of the proteins in the human body (Jaffe, 1981; Liu, 1997). Phytic acid and its salts are known to be thermal stable, and various forms of complexes can be formed during processing. Conditions used to eliminate phytic acid during processing need to be carefully monitored.

Lectins, also known as hemagglutinins, are glycoproteins that are believed to have the ability to bind to cellular surfaces through specific oligosaccharides or glycopeptides (Douglas, Parsons, & Hymowitz, 1999; Liu, 1997). Evidence has shown that soybean lectin, along with the trypsin inhibitors, might account for growth-depressing effects and might affect the pancreatic functions of animals and humans (de la Barca, Vazquez-Moreno, & Robles-Burgueno, 1991). Soybean lectins can be inactivated through thermal processing, however, they have been shown to be resistant to dry heat (De Muelenaere, 1964).

2.1.4 Soy Protein Products

Due to the different uses, two different types of soybeans have emerged: the oil beans and food beans (Liu, 1997). Food beans are generally higher in protein and lower in oil, and usually have a lighter seed coat and a clear hilum (Liu, 1997). Food beans are selected for direct food consumption. For example, they are used as ingredients for the preparation of traditional soy foods, which include soymilk, tofu, bean sprouts, soy sauce, miso, natto and so on (Liu, 1997). However, most of the soy protein products are prepared from the oil beans after oil extraction, including defatted soy grits/flour, soy concentrates and soy isolates (Liu, 1997). The compositions of commercial soy protein products vary due to the use of different raw beans and processing methods. Table 2.7 shows the typical compositions of commercial soy protein products.

Table 2.7. Typical Compositions of Commercial Soy Protein Products (dry basis %)*

	Defatted Soy Flour/Grits	Soy Protein Concentrates	Soy Protein Isolates
Protein	56-59	65-72	90-92
Fat	0.5-1.1	0.5-1.0	0.5-1.0
Crude Fibre	2.7-3.8	3.5-5.0	0.1-0.2
Soluble Fibre	2.1-2.2	2.1-5.9	<0.2
Insoluble Fibre	17-17.76	13.5-20.2	<0.2
Ash	5.4-6.5	4.0-6.5	4.0-5.0
Moisture	0	0	0
Carbohydrates	32-34	20-22	3-4

*Endres, 2001, table 2.1; Hoogenkamp, 2005, table 2.1; Kinsella, 1979, table I

2.1.4.1 Soy Flakes/Meals/Presscake

Soybeans are dried, cleaned, cracked, dehulled, conditioned and then flaked before oil extraction. The flakes without being defatted are known as full-fat soy flakes. Defatted soy flakes are obtained by removing the oil using different press systems, solvent extractors or a combination of both (Aydeniz, Guneser & Yilmaz, 2014). Manufacturing cost, availability, material properties (oil content), and usage goals of the cake (meal) all play significant roles in selection of extraction methods (Aydeniz et al, 2014). The defatted soy flakes produced by solvent extraction contain about 30-35% residual hexane, and need to be desolventized before being processed into meals/press cake (Liu, 1997).

Screw press, a common mechanical press oil extraction method, is an old method that has been used since 1930 until 1950 in United States to extract oil from vegetables or fruits (Bredeson, 1978). Though the cold pressing technique can yield very pure, safe and nutritionally rich virgin oil that does not require further refining, the oil yield is lower than hot pressing and solvent extraction (Aydeniz et al, 2014).

Thus, some pretreatment can be done for whole oil beans to improve the oil yield, such as microwave treatment, steaming, enzyme application and pre-roasting (Aydeniz et al, 2014).

Soy meals are produced by grinding defatted and desolventized (solvent extraction) flakes. Soy meals/press cake is commonly used for animal feed as an important protein source, however, soy press cake for human consumption is still underutilized.

2.1.4.2 Soy Grits/Flour

Soy flour and grits are the least refined forms of soy protein products commercially available, including full-fat soy grits/flour, defatted soy grits/flour and sometimes lecithinated forms (Endres, 2001). Soy grits are produced by coarsely grinding the full-fat or defatted flakes, and soy flours are prepared by grinding the full-fat or defatted flakes into very fine particles (Liu, 1997; Raghuvanshi & Bisht, 2010). Soy grits can be classified into three types based on the particle sizes: coarse (10-20 mesh), medium (20-40 mesh) and fine (40-80 mesh), whereas 97% of the soy flour should be able to pass through 100-mesh screen (Berk, 1992). Defatted soy flours are more common in the market. Soy flour, including full fat and defatted flours, contain about 40 to 60% of protein on a moisture free basis, depending on the fat content. Defatted soy flours generally contain 56% to 59% of protein on a moisture free basis (Table 2.7), and also contain relatively high levels of fibre and carbohydrates (Endres, 2001; Kinsella, 1979).

2.1.4.3 Soy Protein Concentrates

Soy protein concentrate (SPC) is a soy protein product that contains at least 70% protein on a moisture-free basis (Berk, 1992; Mondor et al, 2004; Alibhai et al, 2006). However, the protein content of SPC in the market varies from 65% to 72% (Table 2.7), and is usually different with respect to particle size, functional properties, especially fat and water absorption, and flavor due to different processes. SPC is commonly prepared by three basic processes: aqueous ethanol (60-90%) extraction or acid leaching at an isoelectric pH (pH 4.0-4.8) or water leaching after moist heat treatment followed by centrifugation and/or filtration, and then drying (Hoogenkamp, 2005; Krishna Kumar, Yea, & Cheryan, 2003;Endres, 2001; Arce, Pilosof, & Bartholomai, 1991). New membrane technologies such as ultrafiltration and electro-acidification are also used to produce SPC with higher yield, better quality and functionality (Mondor et al, 2004).

2.1.4.4 Soy Protein Isolates

Soy protein isolate (SPI) is the most refined form of soy protein product used as a food ingredient, containing at least 90% of protein on a dry basis (Berk, 1992; Mondor et al., 2004; Alibhai et al., 2006; Deak & Johnson, 2006;). Soy protein isolates are generally prepared from defatted soy flakes or flours by alkali extraction followed by acid precipitation at their isoelectric points (pH=4.5), to remove both insoluble fibre and soluble sugar (Deak & Johnson, 2006). New membrane technologies can also be used in the SPI production.

2.1.4.5 Texturized Soy Protein Products

Texturized soy protein products, including textured soy flours (TSF) and textured soy protein concentrates (TSPC), are processed by extrusion technology (Hoogenkamp, 2005). By varying the extrusion conditions and mix, texturized soy protein products vary in structure, texture, shape, size and colour (Endres, 2001). Texturized soy protein products can absorb water and fat, which can change the physical properties of food products; they are able to provide a meat-like structure and texture (Hoogenkamp, 2005; Engres, 2001).

2.2 The Corn Tortillas

Corn tortilla, is the traditional Mexican and Central American flat bread made from an alkaline (e.g. lime stone) cooking of corn, known as nixtamalization (Herrera-Corredor et al., 2007). Nixtamalization is the process of alkaline cooking, steeping, and washing corn to produce nixtamal (Serna-Saldivar, Gomez, & Rooney, 1990). In Mexico, the average consumption of corn tortillas is more than 80 kg per person annually, and thus corn tortillas are the major sources of carbohydrates and calcium (from alkali cooking) for in the Mexican diet (Feria-Morales & Pangborn, 1983; Serna-Saldivar, 2012).

2.2.1 Corn Production and Utilization

Corn (*Zea mays*) is a member of the *Poaceae* family, and is the largest crop grown worldwide (Canadian Grain Commission, 2013 & Statistic Canada, 2014). Corn ranks as the third most valuable crop in Canada, after canola and wheat (Statistic Canada, 2014). Corn is mostly produced in central Canada, where Ontario accounts for 61.7 % of the seeded area, followed by Quebec (30.2%) and Manitoba (6.4%) (Statistic Canada, 2014). Corn produced in Canada is mainly used as grain, silage and sweet corn.

2.2.2 Composition and Nutritional Profile of Corn

The nutritional values of white sweet corn are summarized in Table 2.8.

Table 2.8. Nutritional Values of Raw White Sweet Corns (Adapted from the USDA National Nutrient Database for Standard Reference, Release 25, 2012)

Nutrient	Units	Value per 100 g
Proximate Analysis		
Water	g	75.96
Energy	kcal	86
Protein	g	3.22
Total lipids (fat)	g	1.18
Carbohydrate, by difference	g	19.02
Fiber, total dietary	g	2.7
Sugars, total	g	3.22
Mineral	mg	414
Calcium, Ca	mg	2
Iron, Fe	mg	0.52
Magnesium, Mg	mg	37
Phosphorus, P	mg	89
Potassium, K	mg	270
Sodium, Na	mg	15
Zinc, Zn	mg	0.45

Accessed in 2014

2.2.3 Corn Tortillas Processing

There are basically two ways to produce table tortillas. The traditional home-made corn tortillas are prepared by cooking corn kernels in a calcium hydroxide solution followed by stone-grinding to produce *masa*, a Hispanic term for dough (Serna-Saldivar, 2012; Qarooni, 1996). Then the dough is divided into small pieces, and flattened to the desired diameter and thickness using a tortilla press, followed by baking on hot clay or metal plate. In industrial production, the *masa* is sheeted and cut into thin discs and then baked (Serna-Saldivar, 2012). Most commercial tortillas are baked on a three-tier gas-fired oven at temperatures ranging from 280°C to 302°C (536°F to 575°F) for 45s to 60s (Serna-Saldivar, 2012). Fresh *masa* is used in production of corn tortillas by these traditional methods.

The other popular method to produce table tortilla is to use the dry *masa* flour. The dry *masa* production is described and explained in next section. Dry *masa* flour is preferred to make nixtamalized foods such as corn tortillas in the United States and in other parts of the world due to the convenience (Serna-Saldivar, 2012). However, corn tortillas made from dry *masa* flour can be less flavorful (flavor and aroma) than those made from fresh *masa.* Additionally, dry *masa* flour can be easily blended with other dry ingredients such as other flours, hydrocolloids or preservatives to produce corn tortillas with improved nutritional values and quality, as well as longer shelf life.

2.2.4 Dry Masa Flour Production

Dry *masa* flour production includes alkaline (lime) cooking, washing and grinding the nixtamal to produce *masa*, followed by drying, sieving, regrinding coarse particles, resieving, classifying, and blending to form different types of dry *masa* flour to meet certain requirements (Serna-Saldivar, 2010). The drying process is generally performed in large tunnels or drying towers with warm air flowing counter-current to the *masa*, and with final moisture content of about 8-10% (Serna-Saldivar et al., 1990; Serna-Saldivar, 2010). The final dry *masa* flour contains various particle sizes with optimum particle size distribution for different applications (Serna-Saldivar et al., 1990). Currently, different types of dry *masa* flour are available in the market with various colours, pH, particle-size distribution, water absorption and viscosity for different food applications (Serna-Saldivar, 2010). The composition of white corn masa flour is shown in Table 2.9.

Table 2.9. Nutritional Values of White Corn Masa Flour (Adapted from the USDA National Nutrient Database for Standard Reference, Release 25, 2012)

Nutrient	**Units**	**Value per 100 g**
Proximate Analysis		
Water	g	9.04
Energy	kcal	365
Protein	g	9.28
Total lipids (fat)	g	3.86
Carbohydrate, by difference	g	76.29
Fiber, total dietary	g	6.4
Sugars, total	g	16.1
Mineral		714
Calcium, Ca	mg	136
Iron, Fe	mg	1.47
Magnesium, Mg	mg	93
Phosphorus, P	mg	214
Potassium, K	mg	263
Sodium, Na	mg	5
Zinc, Zn	mg	1.80

Accessed in 2014.

2.2.5 Factors Affecting Corn Tortilla Quality

Tortilla quality and colour mainly depend on the characteristics of the corn kernel (raw material), lime cooking, amount of water addition, pH and particle distribution of *masa*, baking and cooling. The kernel and cob colour, and the amount of lime used during cooking all have a great influence on the tortilla colour (Serna-Saldivar et al., 1990). *Masa* used for table tortilla production generally requires high moisture content (~60%) and fine particle size distribution, in order produce soft products (Serna-Saldivar et al., 1990; Serna-Saldivar, 2010). The pH of *masa* can also affect the flavor, texture and colour of the tortilla (Serna-Saldivar, 2012). During the baking processing, starch gelatinizes and protein denatures. Tortillas also develop the colour and flavor due to Maillard reactions during baking (Serna-Saldivar, 2012). The baking and cooling processing can also affect the moisture content of the final tortilla products; over-cooking might lead to excessive moisture loss, resulting in tortillas with poor textural attributes such as rollability.

2.2.6 Improving the Nutritional Properties of Corn Tortillas

As mentioned previously, tortillas are the major source of carbohydrates and calcium in the diet of Mexico (Feria-Morales & Pangborn, 1983; Serna-Saldivar, 2012). Typically, corn tortillas contain approximately 45% carbohydrates, 6% protein, and have a moisture content of 44-46% (Qarooni, 1996) (Table 2.10). Assume 5 corn tortillas (30 g per tortilla) were consumed in the everyday diet of Mexico, the amount of protein and carbohydrate intake from corn tortillas would be 8.55 g and 66.96 g, respectively. These values account for 17.1% and 22.32% of daily values (DV) for protein and total carbohydrate based on a caloric intake of 2000 calories, respectively (FDA, 2014). The fortification of corn tortilla with other flours or micronutrients to improve the nutritional properties has been studied since the 1950s.

Table 2.10. Nutritional Values of Ready-to-bake Corn Tortillas (Adapted from the USDA National Nutrient Database for Standard Reference, Release 25, 2012)

Nutrient	Unit	Value per 100.0 g
Water	g	45.89
Energy	kcal	218
Protein	g	5.70
Total lipid (fat)	g	2.85
Carbohydrate, by difference	g	44.64
Fiber, total dietary	g	6.3
Sugar, total	g	0.88
Minerals, total	mg	700

2.2.6.1 Glycemic Index

The term Glycemic Index (GI), was first introduced by Jenkins et al. in 1981, and is defined as the area under the blood glucose response curve for food products, and then expressed as a percentage of the area after taking the same amount of carbohydrate using a reference food (glucose or white bread) (Jenkins, et al., 1981; Goni et al., 1997). GI is used to classify foods based on their postprandial blood glucose response (Goni et al., 1997). Thus, the lower the GI of a food, the less it affects the blood glucose and insulin levels in the human body. There is evidence showing that carbohydrates in foods such as legumes, pasta and whole grain cereals are slowly digested and absorbed, and are favorable in the dietary management of metabolic disorders (e.g. diabetes and hyperlipidemia) (Goni et al., 1997). Typically, corn tortillas have GI value of 49 ± 6 (glucose as reference), whereas soybeans have much lower GI value. (Atkinson, Foster-Powell, & Brand-Miller, 2008). The average GI for soybeans using glucose as reference food is 16 ± 1 (Atkinson et al., 2008).

2.2.6.2 Amino Acid Profile

Due to the deficiency of lysine and tryptophan in corn (Table 2.11), studies have been conducted since the 1950s to incorporate other ingredients (e.g. milk solids, soybeans/proteins, oilseed flour, sorghum) into corn tortillas to improve the amino acid balance (Feria-Morales & Pangborn, 1983). Soybean has been preferred among all the oilseeds to be used to fortify corn tortillas due to its favorable amino acid composition, which complements the amino acid profile of cereals (Anton, 2008).

Table 2.11. Essential Amino Acid Composition of Nixtamalized Corn Flour (g/100g g Protein) (Adapted from McPherson & Ou, 1976)

Essential Amino Acid	**Plain Corn Tortillas**
Isoleucine	2.96
Leucine	12.48
Lysine	2.91
Methionine	1.69
Phenylalanine	5.20
Tyrosine	3.70
Threonine	3.98
Tryptophan	0.56
Valine	4.17

In the 1970s, several studies were conducted where corn tortillas were fortified with soy to improve the protein quality and nutritive value. Bressani, Murillo and Elias (1974), Del Valle (1974), Franz (1975), and Green et al. (1977) studied the direct use of whole soybeans (15%, 16%, 20% and 18%, respectively) in the alkaline cooking of corn kernels during the preparation of fresh *masa*, and Collins and Sanchez (1980) studied the incorporation of hammer-milled cooked full fat soybean flour into corn tortilla (up to 30%). The resulting corn-soy tortillas all showed superior protein quality compared with tortillas prepared with corn alone, and still had good consumer acceptability. Waliszewski, Pardio, & Carreon (2002) conducted research on the physicochemical and sensory properties of corn tortillas made from nixtamalized corn flour fortified with spent soymilk residue (okara), and the result showed that a maximum of 10% of dry okara could be supplemented without causing any change in 6 tortilla attributes in sensory evaluation. The 10% fortification of soy could effectively improve the lysine requirement to 93%, tryptophan requirement to 92% compared to the FAO requirements, and covered totally the other 2 limiting amino acids (threonine and isoleucine) in tortillas (Waliszewski et al., 2002).

2.2.7 Determining Tortilla Quality

The evaluation of tortilla quality is of importance to compare soy fortified corn tortillas with commercial corn tortillas.

2.2.7.1 Physical Characteristics

The physical characteristics, including weight, diameter, thickness and colour, rollability and texture, are considered among the most important attributes that affect the acceptability of a corn tortilla. Generally, corn tortillas are 1 – 2.5 mm thick and 10-15 cm in diameter (Reyes-Vega et al., 1998). Although the flavor and aroma of the product are important as well, the purchasing decisions are usually made based on the appearance and texture, since they can be observed and felt while purchasing. The various components of the physical properties can be estimated by conducting sensory evaluation. However, instrumental measurements are of value since they are easier to standardize and to reproduce.

2.2.7.1.1 Colour

Although subjective visual assessment and the use of visual colour standards are still widely used in the food industry, the objective measurement of colour is desired for both research and industrial applications due to the stability and ruggedness of today's instruments (Wrolstad & Smith, 2010). Various systems and scales have been developed to describe and specify colour. Among all the scales, the CIE Lab (L*a*b*) scale is a straightforward system, and therefore is commonly used in research and industry. In CIE Lab scale, L^* indicates the lightness (0 to 100, 0 is black and 100 is white), a^* is for red (+) and green (-), and b^* is for yellow (+) and blue (-) (Wrolstad & Smith, 2010). The limits for a^* and b^* are from -80 to +80 (Wrolstad & Smith, 2010).

The colours of the cereal grains and their products are influenced by several factors, which include the colour of the grains, milled fractions and natural pigments (e.g. phenolics, carotenoids) (Serna-Saldivar, 2012). The colour evaluation for tortillas can be used to determine the uniformity of the samples, and to evaluate the effects of the addition of soy press cake/defatted soy flours.

2.2.7.1.2 Rollability

A good tortilla should be soft and can be rolled into a "taco" form without any damage (Rendon-Villalobos et al., 2006). Thus, rollability of tortillas is a good indication of quality. The rollability of tortillas can be tested subjectively and objectively. The subjective assessment is generally done by rolling a tortilla over a dowel, and then evaluating the extent of cracking by a trained human judge (Serna-

Saldivar, 2012), while, the objective assessments can be made with the use of a texture analyzer and appropriate attachments.

2.2.7.1.3 Texture

The texture of tortillas is of important to manufacturers and consumers when tortillas are handled.

Cohesiveness of tortillas is measured by calculating the area under the curve (work = force × time) using a puncture test with a TA.XT 2 plus Texture Analyzer (Texture Technologies Crop., Scarsdale, NY & Stable Micro Systems, Godalming, Surrey, UK). Hardness or firmness of a tortilla sample can be assessed through the use of a puncture test with the same instrument. The peak force is measured as the resistance to puncture, and can be defined as the firmness of the tortilla (Anton et al., 2009).

The substitution of corn flour with other flours, or the addition of hydrocolloids, or changes in processing procedures may change the firmness and the cohesiveness of tortillas. The nixtamalization process changes the outer layers of the grain (Cortez-Comez et al., 2005), as well as the proportion of amorphous and crystalline regions in the starch and results in a higher gelatinization temperature (Clubbs et al., 2008). These changes directly affect the texture of tortillas. Cortes-Comez et al. (2005) reported better tortilla cohesiveness when using a higher concentration of calcium hydroxide and 30 min of nixtamalization.

2.2.7.2 Shelf-life

The shelf life of a corn tortilla greatly depends on the effectiveness of the cooling procedure (Serna-Saldivar, 2012). High moisture content (38-46%) and rapid staling are the major problems affecting the shelf life of commercial corn tortillas (Serna-Saldivar et al., 1990), and result in microbial spoilage and increased firmness and brittleness (Clubbs et al., 2008). During staling, the molecules in the tortillas are realigning themselves into a more ordered crystalline structure and as a result, tortillas easily crack when rolled and folded (Weber, 2000). There are various reactions that occur during staling, and these include gelatinization of starch granules, retrogradation of starch, moisture loss over time, as well as interactions between other ingredients in the tortilla (Weber, 2000). Weber (2000) also stated that the textural changes in tortillas due to staling process can be reversed by heating.

Various hydrocolloids have been studied to delay staling. Friend, Waniska, & Rooney (1993) studied the effects of hydrocolloids on the qualities of wheat tortillas. They found that among the natural gums, xanthan gum (at 0.2% level) significantly improved tortillas rollability over time, and shelf stability was significantly extended with 0.5% arabic, guar or xanthan gum (Friend, Waniska, & Rooney, 1993). It was also reported that tortillas containing modified cellulose (carboxymethyl cellulose - CMC) or cellulose-based commercial blends improved rollability during storage compared to the control, and tortilla with higher levels of hydrocolloids retained their rollability longer during storage (Friend et al., 1993).

Clubbs et al. (2008) studied the addition of glycerol/salt in standard corn tortilla production to increase the softness and pliability of corn tortilla by reducing the rate of staling. They reported that the addition of CMC and glycerol/salt inhibited mold growth beyond 14 days at 25 °C compared to the control, where mold growth was observed by day 8 in the control tortillas.

2.2.7.3 Sensory

The various components of the physical properties of food products can be estimated by conducting sensory evaluation, and sensory evaluation has proven to be an effective and important tool in designing or improving food products.

2.2.7.3.1 Consumer Acceptance Test

Typically, a consumer test involves 100 to 500 target consumers, and the reasons for conducting consumer tests include: (1) Product maintenance; (2) Product improvement/optimization; (3) Development of new products; and (4) Assessment of market potential (Meilgaard, Civille, & Carr, 1987). Consumer affective tests can be categorized into two types, the preference test and the acceptance test. The preference test forces consumer to make a choice (pick one product directly against another), and is commonly used in situations such as product improvement or competition (Meilgaard, Civille, & Carr, 1987). In contrast, an acceptance test gives the information about how well the product is liked by consumers, and the product is generally compared to a well-liked company product (Meilgaard et al., 1987). A hedonic scale is used in consumer acceptance test with balanced or unbalanced points (5 to 9 points).

Improper use of sensory evaluation techniques might lead to unexpected bias. For example, the order of presentation of samples or time of day the sensory session is conducted, are all the potential causes of bias. The samples placed near the centre tend to be preferred over those placed at the end, and the first sample is abnormally preferred or rejected (Meilgaard et al., 1987). Sensory evaluation conducted just after meals or coffee breaks may also introduce bias (Meilgaard et al., 1987). These potential biases can be minimized by serving samples in a randomized and balanced order, scheduling the evaluation at the time of the day the product is normally consumed, and avoiding to conducting sensory sessions just after meals or coffee breaks (Meilgaard et al., 1987).

2.2.8 Tortillas Made with Composite Flours

As mentioned previously, studies have been conducted on fortifying corn or wheat tortillas with other ingredients for the purpose of improving nutritional properties since the 1950s. In the 1970s, several studies were conducted where corn tortilla was fortified with soy to improve the protein quality and nutritive value. Bressani et al. (1974), Del Valle (1974), Franz (1975), and Green et al. (1977) studied the direct use of whole soybeans (15%, 16%, 20% and 18% respectively) in the alkaline cooking of corn kernels during the preparation of fresh *masa*. Feria-Morales and Pangborn (1983) found tortillas to be harder and less yellow when pinto bean was directly used during corn tortilla processing. Waliszewski et al. (2002) conducted research on the physicochemical and sensory properties of corn tortillas made from nixtamalized corn flour fortified with spent soymilk residue (okara).

The use of legume or other cereal flours to fortify corn or wheat tortillas to improve nutritional properties have been of interest due to the convenience and ease of use. McPherson and Ou (1976) found that fortifying corn tortillas with cottonseed flour at 10, 15, 20 and 25% improved the lysine and tryptophan contents and increased the growth rate of rats. They also reported that 10 and 15% cottonseed flour fortified corn tortillas had similar acceptability compared to plain corn tortillas in sensory testing (McPherson & Ou, 1976). Collins and Sanchez (1980) studied the incorporation of hammer-milled cooked full fat soybean flour into corn tortilla (up to 30%). Tortillas fortified with soybeans showed increased firmness, lighter, less red and more yellow colour, and showed similar flavour score to tortillas containing 0 to 30% soy (Collins & Sanchez, 1980). Gonzalez-Agramon and Serna-Saldivar (1988) reported increased water absorption and decreased dough elasticity when wheat tortillas were fortified with 11.1% defatted soybean meal. They also found that tortillas containing 11.1% defatted soybean meal had 30% more protein and twice as much lysine as 100% wheat tortillas (Gonzalez-Agramon & Serna-Saldivar, 1988).

Effects of common bean enrichment on nutritional quality of wheat tortillas were studied by Mora-Aviles et al. (2007), who reported improvement in protein content, as well as tryptophan and lysine contents. Scazzina et al. (2008) studied the fortification of wheat tortillas with whole soy flour and whole meal kamut, and better overall acceptability was reported for tortillas containing these additives in sensory testing, compared to wheat standard tortillas. Anton et al. (2008) studied the physical and nutritional properties of wheat tortillas substituted with 15, 25 and 35% of small red, black, pinto or navy bean flours. They found that the dough rheology, firmness, cohesiveness and rollability were negatively affected by the levels of substitution, but tortillas made with composite flours had significantly higher levels of crude protein and total phenols (Anton et al., 2008). Composite pea and wheat flour with 5 different combinations of pea flour, pea hull and wheat flour were studied by Maskus (2008), who reported that tortillas made with pea flours were acceptable to consumers in terms of appearance, flavour, texture and overall acceptability.

2.3 Pizza Crust

Pizza is one of the most famous Italian foods in the world. Due to the convenience, taste and nutritional value, pizza is now one of the most popular foods in Europe and North America. Pizza has been produced and consumed since the 18^{th} century in Napoli, Italy, and was introduced to the Americas at the end of the 19^{th} century (Serna-Saldivar, 2010). Pizza is a round oven-baked flat bread covered with tomato sauce, mozzarella cheese and a variety of other ingredients (Singh &Goyal, 2011), in which approximately 55% of the pizza weight is the baked pizza dough base (crust) (Serna-Saldivar, 2010). Thus, most pizzas are high in nutritional value. Most pizzas contain around 10-14% of protein, less than 10% of fat, and are high in complex carbohydrates, mainly from starch (Singh & Goyal, 2011). Due to developments in the food industry and increasing consumer needs, different pizza dough products including partially baked frozen pizza dough and raw frozen pizza dough (bake to rise) have been introduced into the market.

2.3.1 Pizza Dough Ingredients and Functionality

Pizza dough is made from simple ingredients, which basically include flour water, yeast/chemical leaven agent, shortening/vegetable oil, and salt. A typical formula of pizza dough is shown in Table 2.12. Optional ingredients might include sugar (1.0-5.5%), calcium propionate (0.1-0.3%), L-cysteine or sodium metabisulfite (45-95 ppm), a protease enzyme, vinegar (0.5-1.0%), vital wheat gluten (1.0-2.0%), or sodium stearoyl-2-lactylate (SSL) (0.25-0.50%) (Qarooni, 1996). The functional role of the essential ingredients will be further discussed.

Table 2.12. Typical Formulations for Pizza Doughs (Adapted from Serna-Saldivar, 2010)

Ingredients	Thick Crust (%)	Thin Crust (%)
Wheat flour	100	100
Water	60-65	55-60
Yeast	5-6	4-5
Shortening	1.5-5	5-10
Salt	1-2	1-2

2.3.1.1 Wheat Flour

Flour is the basic ingredient in the production of bakery goods. In wheat flour, gluten has been found to be responsible for the quality of bread, pasta and other bakery goods. Both water and mechanical mixing are important in developing proper gluten network (Serna-Saldivar, 2010). Gluten contains four fractions, albumin, globulin, prolamins or gliadins, and glutenins (MacRitchie, Du Cros, & Wrigley, 1990). A relatively high protein content (11-14%) from hard wheat flour is recommended in frozen dough production, due to the higher resistance to freeze-thaw circles of the strong gluten network (Serna-Saldivar, 2010; Singh & Goyal, 2011). The amino acid composition of wheat gluten is shown in Table 2.13. Among all the essential amino acids, wheat like other cereal crops, is limited in lysine. Therefore, combination of wheat flours with legume flours to improve the amino balance and protein quality has been getting lots of attention recently. Soybean has been preferred among all the oilseeds to fortify corn tortillas due to its favorable amino acid composition, which complements the amino acid profile of cereals (Anton, 2008).

Table 2.13 Amino Acid Composition of Wheat Gluten Protein (mg/g protein) (Adapted from Day, 2011 & FAO, 2014)

Amino Acid	Gluten	FAO Requirements (Estimates) [1]
Essential Amino Acids		
Histidine	21	15
Isoleucine	38	30
Leucine	67	59
Lysine	16	45
Methionine	14	16
Cysteine	25	6
Methionine + Cysteine	39	22
Phenylalanine + Tyrosine	83	38
Threonine	25	23
Tryptophan	11	6
Valine	39	39
Total indispensable amino acids	339	277
Arginine	36	-
Alanine	23	-
Asparagine/Aspartic acid	31	-
Glutamine/Glutamic acid	375	-
Glycine	28	-
Proline	120	-
Serine	47	-

[1] Mean nitrogen requirement of 105 mg nitrogen/kg per day (0.66 g protein/kg per day)

2.3.1.2 Water

Water is also an essential ingredient. Water is the medium for the solublilization of ingredients (salts and sugars). (Singh & Goyal, 2011). It hydrates and swells starch granules, and activates yeasts (Serna-Saldivar, 2010). Water absorption mainly depends on the type and quality of flour used. Generally, lower levels of water absorption are desired in frozen dough production. This is because free water will damage the gluten and cell viability of yeast during freezing and thawing (Serna-Saldivar, 2010; Singh & Goyal, 2011). Flours with higher protein contents generally have better water absorption rates. Increased water absorption was observed when cowpea, lupin, soybean, triticale and common beans were added into wheat flour (Deshpande et al., 1983; Gonzalez-Agramon & Serna-Saldivar, 1988; Abdel-Kader, 2000; Doxastakis et al., 2002; Hallen et al., 2004; Anton et al., 2008). Deshpande et al. (1983) also stated that approximate 70 – 90% of dry bean proteins were water-soluble whereas 80 – 90% of wheat proteins were water insoluble. Therefore, this increased water absorption was probably due to better water holding capacity of legume proteins.

2.3.1.3 Yeast/Chemical Leavening Agents

Yeast is a unicellular microorganism that reproduces by budding, and is known as a fermenting agent (Serna-Saldivar, 2010). It produces CO_2 during fermentation, and expands the dough to a desire volume. Yeast ferments simple sugar, and provides flavor and aroma through the production of complex chemical compounds such as aldehydes and ketones during the fermentation process (Serna-Saldivar, 2010; Singh & Goyal, 2011). The level of yeast used in pizza dough production is about 4-6% (Table 2.12), and the concentration will be doubled in frozen dough production, due to the potential cell damage in freeze-thaw circle (Serna-Saldivar, 2010).

2.3.1.4 Shortening/Vegetable Oil

Shortening or vegetable oil is used in pizza dough production as a lubricant to improve the dough texture. The addition of shortening can soften the dough, and lower the dough sickness (Serna-Saldivar, 2010). Due to the health concern of using shortening (trans fat), vegetable oil such as olive oil has been used in pizza dough production as a replacement for shortening.

2.3.1.5 Salts

Apart from giving the salty taste, salt also plays other important roles in breading making. Generally, 1-2% of salt is added during bread/pizza dough production (Table 2.12). Salt can strengthen the gluten network through ionic protein modification, by increasing the dough mixing time, enhancing the flavor of final products, and stabilizing and controlling yeast fermentation by decreasing the gas production rate (Serna-Saldivar, 2010). In addition, salt can lower water activity, and therefore extend the shelf life.

2.3.2 Pizza Crust Production

There are basically two types of pizza dough base: (1) thin (crispy) crust or cracker type and (2) thick crust or deep dish style (Qarooni, 1996; Serna-Saldivar, 2010). Pizza dough production is similar to bread making, except the pizza dough is pressed/cut/rolled into a round flat bread shape and holes are made to enable the release of CO_2 during baking. Pizza dough is commonly baked for 5 to 8 min at 232 °C (450 °F) (Qarooni, 1996). There are two methods for industrial production of fresh pizza dough, the pressing (stamping) method and the sheeting and die-cut method. In the former method, the mixed dough is transferred to a dividing and rounding machine, and dough is divided into proper size (allow 10 to 15 min proofing prior to dividing), followed by pressing (intermediate proofing after dividing and pressing) (Qarooni, 1996). In the latter method, dough is allowed to proof after mixing, is then sheeted into a desired thickness, followed by the die-cut step (Qarooni, 1996). In frozen dough production, yeast and salt are added at the end when other ingredients are well mixed, to prevent yeast activation (Serna-Saldivar, 2010). The resulting dough is divided and formed immediately in a refrigerated room, and then blast frozen to achieve a quick-freezing procedure (Serna-Saldivar, 2010).

2.3.3 Determine Pizza Crust Quality

In the past, pizza was simply covered with tomato sauce, mozzarella cheese and other ingredients (Singh & Goyal, 2011). Nowadays, pizza can be covered with various savoury ingredients including different sauces, and toppings. Regardless, there is no doubt that pizza quality is largely dependent on pizza dough quality. The appearance, texture and taste of pizza crust can affect the consumer acceptability of pizza (Limongi, Simoes, & Demiate, 2012). Thus, the evaluation of pizza crust quality is of importance.

2.3.2.1 Factors Affecting Pizza Crust Quality

It is important to know that there is no standard method for pizza dough production, and also a lack of identity for parameters as final products appearance, texture and other important quality aspects (Limongi et al., 2012).

Generally, wheat flour is known as the structural component of bakery product. Gluten, the major protein in wheat flour, is the dough forming protein that support the baking performance of leavened products due to its extensity and elasticity to retain carbon dioxide (Day, 2011; Limongi, 2012). Thus, the protein content of flour, as well as the microbial composition of starter culture could affect the texture of the final pizza crust (Coppola, Pepe, & Mauriello, 1998).

Other factors such as pizza dough formulation, leaven time, as well as baking condition such as oven type, baking time and temperature could also affect the appearance (colour on the edge), texture, aroma, and taste of pizza crust.

2.3.2.2 Physical Properties

The physical properties of pizza crust such as colour (edge and center), and texture, which includes softness of the dough and crispiness of the edge, are considered important attributes that affect the acceptability of a pizza crust. The evaluation of colour and texture has been discussed in Section 2.2.5.

Though the flavour and aroma of pizza crust is important, pizza is always served with a topping, which could complement and cover the taste of pizza crust. Montesano, Duffrin and Heidal (2006) reported that consumers could not detected differences in crust made with high gluten flour and crust made with high gluten flour and flaxseeds when topping was added. Therefore, the taste of pizza crust is less critical compared to its texture.

2.3.2.3 Shelf Life of Frozen Pizza Dough

Frozen dough started being popular in the1970s, due to its long-shelf, convenience, ease of use and consistency (Asghar et al., 2007). The sales of frozen bakery foods have increased from $514 million to $6.5 billion from 1971 to 1995 in the United States (Asghar et al., 2007). Research has been conducted on the use of hydrophilic gum such as Arabic gum and CMC to improve frozen pizza dough quality and to extend its shelf life by retaining moisture and retarding staling (Asghar et al., 2007; Anton, 2008).

2.3.4 The Use of Composite Flours in Wheat-based Leaven Bakery Products

The use of legume or other cereal flours to fortify wheat-based leavened bakery product to improve nutritional properties has been of interest. However, due to desired structure of leavened bakery products, the addition of non-gluten ingredients could have a critical impact on the texture (Anton, 2008). Doxastakis et al. (2002) reported that the volume of bread decreased with the increased level of lupin and soy substitution, and in the case of substitution with triticale, the volume increased with increased levels of substitution. Brewer et al. (1992) reported there was a negative correlation between levels of SPI and acceptability of flavour and texture. They also found that muffins containing SPI had a darker, redder and less yellow colour compared to those made with wheat flour only (Brewer et al., 1992). Changes in colour parameters of soy fortified pizza dough could be related to more intense Maillard reaction due to the relatively higher levels of lysine (Duodu & Minnaar, 2011). Abdel-Kader (2001) reported an increase of 36% in protein, 18% in fat, 123% in calcium, 52% in phosphorus and 40% in iron contents when 0 to 20% decorticated cracked broad bean flours was used to replace wheat flour in the Egyptian “Balady” bread. Gomez et al. (2008) found that the volume of layer and sponge cake decreased with increased levels of substitution of chickpea flour, and an increase in firmness of cake with 50% or 100% chickpea flour.

3. INFLUENCE OF ADDED SOY PRESSCAKE AND SOY FLOUR ON NUTRITIONAL PROPERTIES OF CORN TORTILLAS

3.1. Abstract

Corn tortillas fortified with soybean presscake (SP) and defatted soy flour (SF) were studied and compared. Corn tortillas fortified with SF showed higher ash and protein contents than those made with SP, but higher fat contents were found in corn tortillas fortified with SP. Ash and protein contents of corn tortillas fortified with soy were significantly higher than control tortillas that contained only white corn masa flour. Protein content in tortillas containing 10% SP or SF was increased by 44% and 51% compared to the control, and was increased by 170% at fortification levels of 40 and 35% for SP and SF, respectively. Protein and ash contents for samples fortified with both SF and SP increased with the increased levels of soy products. A decrease in fat content was found with the increased levels of SF. Trypsin inhibitor activity increased significantly in soy fortified tortillas in comparison with control. Phytic acid levels in tortillas made with SF were slightly higher than those made with SP, and were significantly higher compared to the control. Thus, the addition of SP and SF into corn tortillas had a critical impact on tortilla composition and selected anti-nutritional factors.

3.2. Introduction

Corn tortilla, is the traditional Mexican and Central American flat bread made from an alkaline (e.g. lime stone) cooking of corn, known as nixtamalization (Herrera-Corredor et al., 2007). The average consumption of corn tortillas is more than 80 kg per Mexican annually, and thus corn tortillas are the major sources of carbohydrates and calcium (from alkali cooking) for Mexicans (Feria-Morales & Pangborn, 1983; Serna-Saldivar, 2012). The tortilla industry is reported to be the fastest growing sector in the U. S. baking industry; the increase in tortilla sales was up 3.5% while the consumption of fresh bread was increased only 0.3% in 2005 compared to the previous year (Sparks Companies, 2003 & Kuk, 2006).

Corn tortillas are traditionally made from fresh masa (wet dough), but there has been a shift to the use of dry masa flour over last several decades due to the ease of use and increased efficiency. The levels of dry masa flour used in Mexico's tortilla industry increased from 21% to 50% from 1991 to 1997 (Sparks Companies, 2003). Additionally, dry *masa* flour can be easily blended with other dry ingredients such as other flours, hydrocolloids or preservatives to produce corn tortillas with improved nutritional values and quality, as well as longer shelf life.

Soybean is known as one of the most nutritious and economical agricultural commodities in the world due to its unique chemical composition and nutritional profile. Among all the cereals and legumes, soybean has the highest protein content (~40%). In addition to the high protein content, the protein nutritional quality of soybeans has also been shown to be equal to the protein quality of milk, meat, fish and eggs (Riaz, 2006; Endres, 2001). Soy protein provides a complete range of all the essential amino acids that are needed for human growth, and health maintenance

(Hoogenkamp, 2005; Endres, 2001). The United States Food and Drug Administration acknowledged that soy protein could lower total cholesterol and low-density lipoprotein (LDL) cholesterol, allowing food products to carry a health claim when they have met the specifications (Clayton, 2001). Research has shown that soy nutrients such as soy protein and isoflavones, have functions in prevention and treatment of some cancers, cardiovascular disease, bone health problems and other chronic diseases (Riaz, 2006 & Clayton, 2001).

Soybeans produced in Canada are high-yield, high-quality food grade beans, and therefore approximately 35 percent of Canadian produced soybeans is exported to premium markets such as Japan and Europe (Canadian Soybean Council, 2012). Soybean presscake, also known as soybean cake or meal, is the by-product obtained after oil extraction from soybeans (Ramachandran et al., 2006). Oil cake production had an annual growth rate of 2.3% over the decade from 2000 to 2010 (Ramachandran et al., 2006). Soybean meal production accounted for 67.5% of world total major protein meals, followed by rapeseed meal (13.7%) and sunflower seed meal (9.0%) (USDA, 2014). The composition of soybean press cake varies due to different soybean varieties, growing conditions and processing (extraction) methods. The soybean presscake is now commonly used as animal feed since it contains a high level of protein. However, the consumption or the use of soybean presscake as a food ingredient is still not common.

There is a rising demand for consumers in North America to see more varieties of products that are convenient, nutritionally enhanced and safe, with high quality (Sparks Companies, 2003). Nutritionally, corn based tortillas are rich in carbohydrates (approx. 45%) and low in protein content. Due to the deficiency of lysine and tryptophan in corn, studies have been conducted since the 1950s to incorporate other ingredients (e.g. milk solids, soybeans/proteins, oilseed flour, sorghum) into corn tortillas to improve the amino acid balance (Feria-Morales & Pangborn, 1983). Soybean has been preferred among all the oilseeds to be used to fortify corn tortillas due to its favorable amino acid composition which complements the amino acid profile of cereals (Anton, 2008). Bressani et al. (1974), Del Valle (1974), Franz (1975), and Green et al. (1977) studied the directly use of whole soybeans (15%, 16%, 20% and 18% respectively) in the alkaline cooking of corn kernels during the preparation of fresh *masa*, and Collins and Sanchez (1980) studied the incorporation of hammer-milled cooked full fat soybean flour into corn tortilla (up to 30%). The resulting corn-soy tortillas all showed superior protein quality compared with tortillas prepared with corn alone, and still had good consumer acceptability. Waliszewski et al. (2002) conducted research on the physicochemical properties of corn tortillas made from nixtamalized corn flour fortified with spent soymilk residue (okara), and the result showed that a maximum of 10% of dry okara in tortillas could effectively improve the lysine and tryptophan requirement from 56 and 70% of FAO profile to 93% and 92%, respectively (Waliszewsk et al., 2002). Effects of common bean enrichment on nutritional quality of wheat tortillas were studied by Mora-Aviles et al. (2007); they reported improvement in protein content, as well as tryptophan and lysine contents.

The purpose of current study was to investigate the nutritional properties of soy fortified corn tortillas, as well as to compare the properties among control corn tortillas, and tortillas made with soy presscake and defatted soy flour. Parameters measured include moisture, fat, protein and ash contents, as well as anti-nutritional factors including trypsin inhibitor activity and phytic acid content.

3.3. Material and Methods

3.3.1. General

Soy presscake, a by-product of cold press soy oil production, was purchased from Pristine Gourmet (Waterford, On, Canada). Soy presscake was sieved to pass a 590 μm sieve (30 mesh US standard Sieve Series) in the Department of Food Science when received, packaged in Ziploc bag and stored at 4°C. White masa flour was produced by Azteca Milling, L.P. (Irving, TX, USA), purchased from Sunny Day Products (Winkler, MB, Canada) and stored in the cold room (4°C). Defatted soy flour (Bulk Barn, Winnipeg, MB, Canada) and salt (Sifto, Mississauga, ON) were purchased from local grocery stores. Xanthan gum was provided by Tic Gums (White Marsh, MD, USA). The specification sheet for the gum can be found in Appendix 1.

3.3.2. Corn Tortilla Formulations

Corn tortillas were made using the method described by Serna-Saldivar (2012), with modifications based on preliminary research. The incorporation levels of soy were varied from 10 to 40% for presscake and 10 to 35% for defatted flour. Water addition for control corn tortillas (100% masa) was calculated using the following equation:

$$\left(\frac{100-\%moisture\ of\ dry\ masa\ flour}{100-desired\ masa\ moisture\ content}-1\right)\times amount\ of\ dry\ masa\ flour$$

This was used to prepare masa that contained 60% moisture (Serna-Saldivar, Gomez, & Rooney, 1990; Serna-Saldivar, 2012). The water addition for soy fortified corn tortillas was calculated using the equation above for the amount of dry masa flour plus 5% of the weight of soy ingredients based on preliminary research. The amount of additional water and other ingredients are shown in Table 3.1.

Table 3.1. Formulations of Soy Fortified Corn Tortillas*

	Flours (g)		Salt (g)	Gum (g)	Water (g)
	Dry Masa	Soy	0.50%	1%	
Control	100.00	0.00	0.50	1.00	119.08
10% SP	90.00	10.00	0.50	1.00	107.67
20% SP	80.00	20.00	0.50	1.00	96.26
25% SP	75.00	25.00	0.50	1.00	90.56
30% SP	70.00	30.00	0.50	1.00	84.85
35% SP	65.00	35.00	0.50	1.00	79.15
40% SP	60.00	40.00	0.50	1.00	73.45
10% SF	90.00	10.00	0.50	1.00	107.67
20% SF	80.00	20.00	0.50	1.00	96.26
25% SF	75.00	25.00	0.50	1.00	90.56
30% SF	70.00	30.00	0.50	1.00	84.85
35% SF	65.00	35.00	0.50	1.00	79.15

*Formulations were described on 100g total flour weight basis.

3.3.3. Corn Tortilla Preparation

Three hundred grams of flour (either masa or composites) was mixed with 1.5 g salt and 3 g of Xanthan gum for 30 s at a low speed (Stir) using a Stand Mixer (Professional 600, Kitchenaid, Michigan, USA) with a flat beater. Water was then weighed, added to the dry ingredients mix, and mixed for 1 min at speed 2. Dough was divided into 35 g pieces after mixing. These pieces were rounded and placed in containers covered with plastic wraps to prevent moisture loss. Each 35 g ball was then pressed on a tortilla press for 20 s at the smallest thickness setting (Doughpro, Stearns Product Development Corporation, CA, USA). Thereafter, tortillas were transferred to an electric hot plate preheated at 205°C and cooked for 15 s on the first side, flipped and cooked for 20 s on the second side, then flipped one more time and cooked the first side for another 5 s. Cooked tortillas were cooled on a rack for 3 min and packed in open polyethylene plastic bags. These bags were sealed after 3 h, and left at 25°C for 1 h prior to further analysis.

3.3.4. Chemical Analysis

Moisture content of fresh tortillas was determined the same day of production by drying 2 g of crumbed tortilla pieces at 100 °C in an air oven for 16 h (overnight). Chemical analysis were determined after tortillas were frozen for 16 h at – 40°C and then freeze dried at -50°C, 5 Pa, for 48 h in a Virtis Genesis Freeze Dryer (Gardiner, NY, USA). Freeze dried samples were ground with mortar and pestle.

Fat and nitrogen contents were measured by Soxhlet and Dumas methods, respectively. The nitrogen content was multiplied by a factor of 6.25 for raw flours and tortillas to estimate protein content (FAO, 2002). Ash content was determined by AOAC method 923.03 with modifications. Approximate 2 g of freeze-dried ground tortilla was weighed and pre-ashed before putting into a furnace (Blue M Electric Company, Thermal Product Solutions, White Deer, PA, USA) at 550°C for approximately 16 h.

The AACC International Method 22-40.01 (1999) was followed for measurement of trypsin inhibitor activity of soy products with minor modifications. In this method, α-N-benzoyl-DL-arginine-p-nitroanilidehydrochloride (BAPNA) (Sigma-Aldrich) was used as the substrate for trypsin. Five hundred milligrams of sample ground to pass a 105 μm sieve (140 mesh US standard Sieve Series) was extracted with 25 mL of 0.01N sodium hydroxide (Fisher Scientific) for 3 h at room temperature in a rotatory shaker. The extract was centrifuged at 14,190 g (Sorvall RC

6 Plus Centrifuge, Thermo Fisher Scientific Inc., NC, USA) at 4°C for 10 min, and the supernatant was collected. Thereafter, the pH of supernatant was adjusted to between 8.4 and 10.0 with 0.5N HCl (Fisher Scientific) and 0.1N HCl. The supernatant was then diluted to the point when 1 mL of sample produce trypsin inhibition of 40% to 60%. Dilution factors were between no dilution to 1: 5 (sample : distilled water, v/v) for white corn masa flour and tortilla samples, 1:16 for defatted soy flour and 1: 20 for soy presscake. Five portions of diluted extracts (0, 0.6, 1.0, 1.4, and 1.8 mL) were pipetted into test tubes and the final volume was adjusted to 2 mL with distilled water for both sample measurement and corresponding reagent blank. For sample measurements, 2 mL of trypsin solution (0.02 mg/mL in 0.001M HCl, Sigma-Aldrich T-1426) was added and the tubes were placed in the water bath at 37 °C for 5 min, followed by addition of 5 mL of pre-warmed substrate solution (40 mg BAPNA in 1 mL dimethyl sulfoxide and diluted to 100 with Tris buffer 0.05M, pH 8.2). After exactly 10 min, the reaction was stopped by adding 1 mL of 30% acetic acid (Sigma-Aldrich) to each test tube. The reagent blank was prepared by adding 5 mL BAPNA substrate solution, then incubating at 37°C for 10 min, and adding 1 mL of 30% acetic acid followed by addition of 2 mL of trypsin solution. Both the sample and blank were then filtered through Whatman No. 2 paper (GE Healthcare UK Limited, Buckinghamshire, UK). The absorbance was read at 410 nm with an Ultrospec 1100 Pro spectrophotometer (Amersham Pharmacia, NJ, USA). One trypsin unit was arbitrarily defined as increase of 0.01 absorbance unit at 410 nm per 10 mL of reaction mixture under conditions used herein as described in the AACC (1999) method. Trypsin inhibitor activity (TIA) is expressed in terms of trypsin inhibitor units (TIU).

Phytic acid levels were determined in flours and tortillas by the method of Latta and Eskin (1980) with minor modifications. This is an anion exchange method done with a chromaflex column (Kimble Chase, Vineland, NJ, USA) containing 0.5 g of an anion-exchange resin (200 – 400 mesh, chloride form; AG 1-X8, Bio-Rad Co.). Flour and freeze-dried and ground tortilla samples (0.5 g) were extracted with 10 mL of 2.4% HCl, and then centrifuged at 14,190 g for 10 min. The supernatant was collected and diluted (20 fold for SF sample and 10 fold for all other samples). Columns were packed with glass wool at the bottom, and rinsed with distilled water, followed by addition of a slurry of the anionic resin to form a resin bed. Thereafter, columns were washed with 20 mL of distilled water, 10 mL of diluted sample extract and followed by 15 mL of 0.1 M of sodium chloride. Once the 0.1 M sodium chloride passed through the column, 15 mL of 0.7 M sodium chloride was added, and eluent was collected. The Wade reagent (1 mL, 0.03% $FeCl_3 \bullet 6H_2O$ and 0.3% sulfosalicylic acid in distilled water) was added into 3 mL of the eluent or sodium phytate standards (10, 20, 30 & 40 μg/mL), and vortexed for 30 s. The absorbance of the supernatant was measured at 500 nm with a UV-Vis spectrophotometer (Ultrospec 1100 Pro spectrophotometer, Amersham Pharmacia, NJ, USA). The reaction between ferric ion and sulfosalicylic acid from the Wade regent results in a pink colour when phytate is present; if the iron binds to the phosphate ester, and is no longer available to react with sulfosalicylic acid, there is a decrease in pink colour intensity (Latta & Eskin, 1980).

The soy presscake, defatted soy flour and white corn masa flour, as well as freeze-dried tortilla samples, were analyzed for their moisture content by AACC Moisture – Air-oven Method (44-15.02) with modifications (AACC, 1999). Approximately 2 g of ground samples were weighed into pre-dried aluminum dishes, and dried in an air-oven for 16 hrs. Due to the different moisture content of samples, all results were reported on a dry matter basis.

3.3.5. Statistical Analysis

All data were reported as means ± standard deviation (sd), and analyzed by Statistical Analysis Software (SAS, version 9.3, SAS Institute Inc., Gary, NC, USA) using ANOVA followed by Tukey test. All characteristics were considered to be significantly different when $p < 0.05$. Triplicate analysis was conducted for all chemical analysis except for protein content and trypsin inhibitor activity, for which only duplicate analysis were carried out.

3.4. Results and Discussion

3.4.1. Effect of Added Soy Presscake and Soy Flour on the Composition of Corn Tortillas

The effect of added soy presscake and soy flour on the composition of corn tortillas, as well as the compositional analysis of soy presscake, defatted soy flour and white corn masa flour are summarized in Table 3.2. As mentioned in Chapter 2, corn tortillas contain approximately 44 to 46% moisture (Qarooni, 1996 & USDA, 2012). Therefore, 25 and 30% SP fortified tortillas, as well as 30 and 35% SF fortified tortillas, were within the range. Moisture contents of all other tortillas were all between 42 – 53%, close to this range, with the highest level for the control corn tortilla (100% dry masa). Moisture content was decreased by addition of increasing amounts of soy presscake and soy flour. This could be caused by the decreased water addition during the formulation of tortillas, as described above. The moisture contents appeared to be slightly higher in tortillas made with SF compared to tortillas made with SP at the same substitution level, though the differences were not significant except at 25% substitution level where the moisture contents of SF fortified tortillas were significantly higher than SP fortified tortillas. This could be due to the higher protein and lower moisture content of SF, compared to SP, since the water addition was the same during tortilla preparation. Based on the tortilla formulations, the percent water addition for 10, 20, 25, 30, 35 and 40% soy fortified corn tortillas compared to the water addition to the control corn tortilla were 90.4, 80.8, 76.0, 71.4, 66.5 and 61.7%, respectively. However, the moisture contents of soy fortified tortillas compared to the moisture content of control corn tortillas, described as percentage, were 93.9, 90.0, 86.8, 85.2, 82.9, and 79.9% for 10 – 40% SP fortified tortillas, and 95.5, 90.0, 89.0, 85.9, and 85.4% for 10 – 35% SF fortified tortillas. The better water retention in soy fortified corn tortillas was probably due to better water absorption of soy proteins. This may also be explained by the differences in size and thickness of tortillas, which contributed to different moisture losses during cooking. Differences in tortilla size and thickness will be further discussed in Chapter 4.

Table 3.2. The Effect of Added Soy Presscake and Soy Flour on the Composition of Corn Tortillas and Compositional Analysis of Flours

Flour	%	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Carbohydrates (%)
Masa	N/A	12.37 ± 0.07 g	9.35 ± 0.11 k	4.35 ± 0.03 d,e	1.39 ± 0.02 k	72.54*
SP	N/A	7.69 ± 0.07 h	49.06 ± 0.19 b	14.54 ± 0.16 a	4.75 ± 0.11 b	23.96*
SF	N/A	6.40 ± 0.02 i	54.31 ± 0.04 a	1.21 ± 0.05 j	6.10 ± 0.04 a	31.98*
Masa	100	52.59 ± 0.18 a	9.23 ± 0.05 k	3.08 ± 0.08 g	1.86 ± 0.02 j	33.24*
SP	10	49.37 ± 0.40 b	13.28 ± 0.04 j	4.09 ± 0.12 e,f	2.23 ± 0.04 i	31.03*
	20	47.35 ± 0.30 c	16.83 ± 0.07 h	4.49 ± 0.08 d	2.62 ± 0.04 g	28.71*
	25	45.65 ± 0.440 d	19.30 ± 0.05 f	3.93 ± 0.03 f	2.93 ± 0.02 f	28.19*
	30	44.79 ± 0.22 d,e	20.66 ± 0.08 e	5.50 ± 0.12 c	2.97 ± 0.01 f	26.08*
	35	43.62 ± 0.20 e	22.53 ± 0.23 d	5.67 ± 0.20 c	3.15 ± 0.01 e	25.03*
	40	42.03 ± 0.20 f	24.65 ± 0.01 c	6.28 ± 0.24 b	3.21 ± 0.13 e	23.83*
SF	10	50.20 ± 0.49 b	13.95 ± 0.02 i	3.19 ± 0.09 g	2.41 ± 0.01 h	30.25*
	20	47.32 ± 0.37 c	18.59 ± 0.34 g	2.98 ± 0.11 g,h	2.93 ± 0.01 f	28.18*
	25	46.77 ± 0.38 c	20.18 ± 0.02 e	2.90 ± 0.09 g,h	3.08 ± 0.05 e,f	27.07*
	30	45.15 ± 0.11 d	22.92 ± 0.05 d	2.68 ± 0.10 h	3.44 ± 0.03 d	25.81*
	35	44.93 ± 0.44 d,e	24.94 ± 0.11 c	2.30 ± 0.06 i	3.68 ± 0.03 c	24.15*

SP: Soy Presscake; SF: Soy Flour
Results listed are Mean ± Standard Deviation of three replications for moisture, fat and ash contents, and two replications for protein content.
Results are reported as dry matter basis.
*Data listed are calculated values, carbohydrate contents were calculated by difference to 100.
Values followed by the same letter in the same column are not significantly different ($p>0.05$).

Protein contents were increased by the addition of SP and SF and increased further with higher levels of substitution, as shown in Table 3.2. Tortillas fortified with SF had significantly higher protein contents than those made with SP at the same substitution level. This is logical since SF had higher protein content than SP, which could be due to the different soybean varieties or crop years, as well as a lower fat content of SF. Compared to the control corn tortillas, protein contents of tortillas fortified with SP and SF are significantly higher. Protein content in tortillas containing 10% SP or SF was increased by 44% and 51% compared to the control, and was increased by 170% at fortification levels of 40 and 35% for SP and SF.

Compared to the control, fat contents were increased by the addition of SP, and except for 25% SP, higher levels of substitution resulted in higher fat contents; there was less fat in the 25% SP tortilla than the 20% SP tortilla (Table 3.2). Fat levels decreased upon addition of SF at 20, 25, 30 and 35%; fat contents of tortillas fortified with SF at levels of 10, 20 and 25% were not significantly different from the control. At substitution levels of 30% or more, the fat content of SF tortillas were significantly lower than the control. Overall, at all substitution levels, fat content of SP tortillas was significantly higher than those made with white corn masa flour, with tortillas made using soy flour having the lowest fat content. The differences in fat contents of flours could explain these results.

Ash contents in tortillas made with SP and SF were higher than in the control, and increased with increased levels of substitution, as summarized in Table 3.2. This maybe a consequence of higher mineral content in soy than corn (USDA, 2012). Since SF had a higher ash content than SP due to its lower fat content and different processing, tortillas fortified with SF had significantly higher ash contents than those made with SP at the same fortification level (except for 25%).

The compositions of fresh (uncooked) corn tortillas were calculated based on the compositional analysis in Table 3.2, and are reported on an “as is” basis in Table 3.3. Compared to the commercial ready-to-bake corn tortillas, the control tortilla had lower protein content (1.31 g V.S. 1.71 g), similar fat content (0.84 g V.S. 0.86 g) and higher ash content (mineral content) (0.54 g V.S. 0.21 g). This could be caused by higher moisture content of our control tortilla, as well as the use of different ingredients that were used to formulate the tortillas. However, it is worthwhile to mention that, the protein contents of soy fortified tortilla have increased 20% at 10% fortification level, and 150% for SP at 40% fortification level, compared to literature value (1.71 g).

Table 3.3. The Effect of Added Soy Presscake and Soy Flour on Nutritional Values of Fresh Corn Tortillas per 1 piece (30 g).

Flour	%	Protein (g)	Fat (g)	Ash (g)
Ready-to-bake Corn Tortilla[1]	N/A	1.71*	0.86*	0.21*
Masa	100	1.31*	0.84*	0.54*
SP	10	2.02*	1.07*	0.64*
	20	2.66*	1.12*	0.75*
	25	3.15*	0.95*	0.85*
	30	3.42*	1.31*	0.84*
	35	3.81*	1.32*	0.89*
	40	4.29*	1.42*	0.90*
SF	10	2.09*	0.82*	0.70*
	20	2.94*	0.73*	0.86*
	25	3.22*	0.69*	0.90*
	30	3.77*	0.62*	1.01*
	35	4.12*	0.52*	1.08*

SP: Soy Presscake; SF: Soy Flour
*Results listed are calculated values.
[1]Values in this row were calculated using data from the USDA National Nutrient Database for Standard Reference, Release 25, 2012.

3.4.2. Effect of Added Soy Presscake and Soy Flour on Anti-nutritional Factors of Corn Tortillas

Changes in selected anti-nutritional factors of corn tortillas are summarized in Table 3.4. The anti-nutritional factors in soybeans have limited its use for human and animal consumption. The phytic acid contents of soy fortified tortillas varied from 11.72 to 14.59 mg/g for SP, and 14.06 to 15.59 mg/g for SF. The phytic acid contents were increased with increased levels of soy substitution, except for 30% SP. Since phytic acid is known to be associated with protein bodies (Jaffe, 1981; Liu, 1997), the increased phytic acid levels was probably due to the higher protein contents in soy fortified tortillas. SF tortillas had slightly higher phytic acid contents than SP tortillas since the fat content in SP was higher. Compared to control tortillas, tortillas fortified with SF (10 – 35%) and SP (25, 35 & 40%) had significantly higher phytic acid contents.

Phytic acid levels in flour composite were calculated based on tortilla formulations, so the effect of tortillas preparation could be evaluated. The calculated values were only slightly higher or lower than those measured values in cooked tortillas. Phytic acid has been found to be thermally stable, but controversial results had been reported. Wang et al. (2010) reported no significant reduction of phytic acid contents for beans and chickpeas after soaking and cooking. Yang, Hsu and Yang (2014) reported reductions in phytic acid levels of 16.2, 18.7, 25.7 and 24.9% with roasting (180 °C), microwaving, and boiling for 30 min and 60 min, respectively, for yellow soybeans. Similar results have been reported to show reductions in phytic acid levels in legumes processed under various conditions, including soaking, roasting, boiling and autoclaving (Yang et al., 2014; Rehman & Shah, 2005; Abd El-Hady & Habiba; 2003). However, it is difficult to make a direct comparison of our results to the literature, since the phytic acid contents of the raw material were only calculated and the reactions involved in tortilla making were different from the heat treatment of whole beans. Furthermore, considering the relatively short cooking time for the soy fortified tortillas, it is logical to expect no or slight reductions in phytic acid levels.

Table 3.4. Effect of Added Soy Presscake and Soy Flour on Selected Anti-nutritional Factors of Flours and Fortified Tortillas

Flour	%	Trypsin Inhibitor Activity (TIU/mg)		Phytic Acid (mg/g)	
		Raw Flour	Tortilla	Raw Flour	Tortilla
SP	N/A	37.90 ± 0.34 a	N/A	16.34 ± 0.45 b	N/A
SF	N/A	30.75 ± 0.70 b	N/A	24.98 ± 0.24 a	N/A
Dry Masa	100	n/d	n/d	11.94 ± 0.50 f	12.17 ± 0.47 f
SP	10	3.79*	2.32 ± 0.01 i,j	12.38*	12.80 ± 0.68 e,f
	20	7.58*	3.96 ± 0.02 g,h	12.82*	12.62 ± 0.47 f
	25	9.48*	4.93 ± 0.14 f,g	13.04*	14.47 ± 0.55 c,d
	30	11.37*	5.15 ± 0.08 e,f	13.26*	11.72 ± 0.43 f
	35	12.22*	6.63 ± 0.36 c,d	13.48*	14.55 ± 0.37 c,d
	40	15.16*	7.44 ± 0.24 c	13.70*	14.59 ± 0.18 c,d
SF	10	3.08*	1.43 ± 0.09 j	13.24*	14.06 ± 0.39 d,e
	20	6.15*	3.19 ± 0.02 h,i	14.55*	14.68 ± 0.24 c,d
	25	7.69*	4.92 ± 0.06 f,g	15.20*	14.73 ± 0.35 c,d
	30	9.23*	5.44 ± 0.21 e,f	15.85*	15.17 ± 0.64 b,c,d
	35	10.76*	6.18 ± 0.08 d,e	16.50*	15.59 ± 0.49 b,c

SP: Soy Presscake; SF: Soy Flour; TIU: Trypsin Inhibitor Unit
Results listed are Mean ± Standard Deviation of three replications for phytic acid contents, and two replications for trypsin inhibitor activity.
Results are reported as dry matter basis.
Values followed by the same superscript letter in the same column are not significantly different ($p>0.05$).
*Results are calculated values.

The presence of trypsin inhibitor in legumes could result in decreasing protein digestibility and inducing pancreatic hypertrophy (Yuan et al., 2008). The TIA of soy fortified tortillas varied from 2.32 to 7.44 TIU/mg for SP, and 1.43 to 6.18 TIU mg for SF. The TIA had the same trend as phytic acid contents, such that trypsin inhibitor activity increased with increased levels of soy substitution. At comparable levels of substitution, tortillas containing SP and SF had similar levels of trypsin inhibitor activity.

Trypsin inhibitors are generally thermo-labile, and can be eliminated or inactivated through different methods. Compared with the calculated trypsin inhibitor activity values in raw flour composites, trypsin inhibitors in tortillas were reduced ranging by 37 to 55% (considering only the flour portion in tortilla formulation, which is 98.5% of the dry materials in the tortilla). These reductions are similar to the findings of Anton et al. (2008), who reported 50 – 66% reduction of trypsin inhibitor activity in wheat tortillas with added bean flours. Radha, Kumar and Prakash (2008) found that trypsin inhibitor activities were reduced 13% after 30 min dry roasting, and reduced by 34 to 98% after autoclaving 2 to 30 min for soy flours. Various reductions of TIA has been reported for soybeans (whole bean or flours) using different treatments including thermal treatment, chemical modification, enzymatic hydrolysis, and gamma irradiation (Yuan et al., 2008; Faris et al., 2008; Dia et al., 2012; Dixit et al., 2011; Yang et al., 2014).

The levels of TIA and phytic acid have been converted to an "as is" basis for each tortilla (approx. 30 g). The results are shown in Table 3.5. For soy fortified corn tortillas, every tortilla contained 0.21 – 1.30 TIU of trypsin inhibitor, and 1.94 – 2.58 mg of phytic acid. Though trypsin inhibitor and phytic acid are known as anti-nutritional factors, studies have also been shown beneficial effects on human health, including strong anticarcinogenic activity, delayed postprandial glucose absorption and reduced the bioavailability of toxic heavy metal (Kennedy, 1993; Campos-Vega, Loarca-Pina, Oomah, 2010). Liener et al. (1988) found that Bowman-Birk Inhibitor (BBI) was associated with increased secretion of trypsin, chymotrypsin and elastase in human duodenum. However, Kennedy (1993) observed no adverse side effect in long-term animal studies in which, animals such as mice, rats and hamsters were exposed to high doses of BBI concentrate (containing 100 units of chymotrypsin inhibitor and as much as 40 units of trypsin inhibitor). Doell, Ebden and Smith (1981) estimated that the daily intake of TIA for average British diet was 330 mg per person per day, and they reported a TIA value of 18.7 mg/g raw whole soybeans in an "as is" basis. Thus, if the TIA for average British diet all came from soybeans, they would consume 17.65 g of raw whole soybean per day. Despite the degree of inactivation reached and considering the soy fortification levels in tortillas (max. 12 g SP per tortilla), the consumption of soy fortified corn tortillas could be consider as safe with respect to TIA.

Table 3.5. Contents of Selected Anti-nutritional Factors in Fresh Soy Fortified Tortillas (Serving Size 30 g per Tortilla)*

Flour	%	Trypsin Inhibitor Activity (TIU/30g)[1]	Phytic Acid (mg/30g)[1]
Masa	100	n/d	1.73
SP	10	0.35	1.95
	20	0.63	1.99
	25	0.80	2.36
	30	0.85	1.94
	35	1.12	2.46
	40	1.30	2.54
SF	10	0.21	2.10
	20	0.51	2.32
	25	0.79	2.35
	30	0.90	2.50
	35	1.02	2.58

SP: Soy Presscake; SF: Soy Flour; TIU: Trypsin Inhibitor Unit
*Values are shown in "as is" basis and are reported based on per tortilla (30 g).

3.5. Conclusions

The addition of SP and SF into corn tortillas caused significant changes in tortilla composition and the contents of selected antinutritional factors, in comparison to corn control tortillas. Exceptions were the fat content in tortillas containing 25% or less SF and phytic acid levels in tortillas containing 10, 20 or 30% SP. Protein content in tortillas containing 10% SP or SF was increased by 44% and 51% compared to the control, and was increased by 170% at fortification levels of 40 and 35% for SP and SF. The trypsin inhibitor activity was increased significantly in tortillas. Nevertheless, based on the TIA reduction during cooking and considering the soy fortification levels in tortillas (max. 12 g SP per tortilla), the consumption of soy fortified corn tortillas should consider to be safe regarding to TIA. Therefore, all soy fortified (SP & SF) corn tortillas showed improved nutritional profile in comparison to the corn tortilla control with levels of antinutritional factors that were considered to be safe.

Physical and sensory properties should be carried out to evaluate the consumer acceptability of corn tortillas fortified with soy.

4. INFLUENCE OF ADDED SOY PRESSCAKE AND SOY FLOUR ON SOME PHYSICAL AND SENSORY PROPERTIES OF CORN TORTILLAS

4.1. Abstract

Corn tortillas fortified with soybean presscake (SP) and defatted soy flour (SF) were studied and compared. Texture including firmness and cohesiveness, as well as colour using a CIE *L*a*b** were determined instrumentally. Physical properties such as size, thickness and rollability, were also investigated. A consumer acceptance test was conducted to evaluate the acceptance of tortillas at high levels of soy fortification (35% SF & 40% SP). Tortillas fortified with soy were found to be smaller and thicker with increased firmness and cohesiveness. Tortillas made with SF showed the poorest rollability, and were almost unrollable at high SF fortification levels (30% & 35%). Soy fortified tortillas were redder and more yellow than control corn tortillas. In the consumer acceptance test, 40% SP and 35% SF had significantly higher overall acceptability scores than the commercial corn tortillas for the total population (n=76). In addition, overall flavour and texture of both soy fortified corn tortillas had significantly higher scores than commercial corn tortillas. Thus, the fortification with SP and SF had significant effects on tortilla size, thickness, firmness, cohesiveness, rollability, as well as colour, and our results appeared to show that these changes were acceptable to consumers.

4.2. Introduction

The average annual consumption of corn tortillas made from nixtamalized (cook in lime stone) corn, is more than 80 kg per Mexican. These corn tortillas provide significant levels of carbohydrates and calcium in Mexican diets (Feria-Morales and Pangborn, 1983; Serna-Saldivar, 2012). Total sales of tortillas have also been increasing outside of Central America; tortillas are the fastest growing segment of the U.S. baking industry where sales were up by 3.5% compared to an increase of only 0.3% for fresh bread in 2005 compared to the previous year (Sparks Companies, 2003; Kuk, 2006).

Soybean is known to be a nutritious and economical agricultural commodity worldwide due to its unique chemical composition and nutritional profile. Soybean has the highest protein content (~40%) among all the cereals and legumes. Soy protein also provides a complete range of all the essential amino acids that are needed for human growth, and health maintenance (Hoogenkamp, 2005; Endres, 2001). Research has shown that soy protein has an effect on lowering serum levels of total cholesterol and low-density lipoprotein (LDL) cholesterol, and serum triacylglycerol (Urade, 2011). Therefore, in 1999, the U.S. Food and Drug Administration approved the following health claim for soy protein: "25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease". (FDA, 2014). FDA also stated that the "food product must contain at least 6.25 g of soy protein per reference amount customarily consumed of the food product", and the food must "meet the nutrient content requirements for a "low saturated fat" and "low cholesterol"", as well as meeting "the nutrient content requirement for a "low fat" food unless it consists of or is derived from whole soybeans and contains no fat in addition to the fat inherently present in the whole soybeans it contains or from which it is derived" could bear the health claim (FDA, 2014). Research has shown that soy nutrients have functions in prevention and treatment of some cancers, cardiovascular disease, bone health problems and other chronic diseases (Riaz, 2006; Clayton, 2001).

There is a rising demand for consumers in North America to see a greater variety of products that are convenient, nutritionally enhanced and safe, with high quality (Sparks Companies, 2003). Nutritionally, corn based tortillas are rich in carbohydrates (approx. 45%) and low in protein content. Due to the deficiency of lysine and tryptophan in corn, studies have been conducted since the 1950s to incorporate other ingredients (e.g. milk solids, soybeans/proteins, oilseed flour, sorghum) into corn tortillas to improve the amino acid balance (Feria-Morales & Pangborn, 1983). Soybean has been preferred among all the oilseeds to be used to fortify corn tortillas due to its favorable amino acid composition which complements the amino acid profile of cereals (Anton, 2008). However, incorporating soybean into foods is not yet well accepted due to the beany taste, which is primarily caused by soy lipoxygenases oxidizing linoleic acid, especially at high levels of fortification (Urade, 2011). Therefore, consumer acceptance is important to evaluate the acceptability of the product.

Bressani et al. (1974), Del Valle (1974), Franz (1975), and Green et al. (1977) studied the direct use of whole soybeans (15%, 16%, 20% and 18% respectively) in the alkaline cooking of corn kernels during the preparation of fresh *masa*, and Collins and Sanchez (1980) studied the incorporation of hammer-milled cooked full fat soybean flour into corn tortilla (up to 30%). The resulting corn-soy tortillas all showed superior protein quality compared with tortillas prepared with corn alone, and still had good consumer acceptability. Waliszewski et al. (2002) conducted research on the physicochemical and sensory properties of corn tortillas made from nixtamalized corn flour fortified with spent soymilk residue (okara), and the result showed that a maximum of 10% dry okara could be supplemented without causing change in 6 tortilla attributes including aroma, flavour, after taste, appearance, manual texture and oral texture in sensory evaluation. Anton et al. (2009) also found that wheat tortillas fortified with common beans had better overall acceptability, flavour and texture scores than control wheat tortillas.

In the previous chapter, we showed that corn tortillas fortified with SP and SF had improved nutritional profiles in comparison to the corn tortilla control, as well as levels of antinutritional factors that were considered to be safe. The protein content was increased by 170% at fortification levels of 40 and 35% for SP and SF. Therefore, it is important to study the physical properties of soy fortified tortillas including appearance (size, thickness & colour) and texture, as well as sensory properties at high levels of fortification (35% & 40%).

The purpose of this study was to examine the effect of added soybean presscake and defatted soy flour on some physical properties and to evaluate the consumer acceptance of tortillas at high fortification levels (35% SF & 40% SP). The physical properties such as size of raw and cooked tortillas, thickness, firmness, cohesiveness, rollability and colour were determined. Sensory properties including overall acceptability, overall appearance, overall flavour, overall texture and the intent to purchase were investigated by 76 untrained panelists.

4.3. Material and Methods

4.3.1. General

Soy presscake, a by-product of cold press soy oil production, was purchased from Pristine Gourmet (Waterford, On, Canada). Soy presscake was sieved to pass a 590 μm sieve (30 mesh US standard Sieve Series) in the Department of Food Science when received, packaged in Ziploc bag and stored at 4 °C. White masa flour was produced by Azteca Milling, L.P. (Irving, TX, USA), purchased from Sunny Day Products (Winkler, MB, Canada) and stored in the cold room (4 °C). Defatted soy flour (Bulk Barn, Winnipeg, MB, Canada) and salt (Sifto, Mississauga, ON) were purchased from local grocery stores. Xanthan gum was provided by Tic Gums (White Marsh, MD, USA). A specification sheet of the gum can be found in Appendix 1. The reference samples used in sensory test were Manny's Corn Tortilla (Manny's Corn Tortilla, Mexican Accent, New Berlin, Wisconsin, USA) purchased from Safeway.

4.3.2. Corn Tortilla Formulations and Preparation

The formulations of corn tortillas were discussed in Chapter 3.3.2; 300 g of flour (either dry masa or composite) was mixed with 1.5 g salt and 3 g of Xanthan gum for 30 s at a low speed using a Stand Mixer (Professional (Stir). Water was then weighed and added into the dry ingredients mix, and mixed for 1 min at speed 2. All mixing was done by using a Stand Mixer (Professional 600, Kitchenaid, Michigan, USA) with a flat beater at different speeds. Dough was divided into pieces of 35 g followed by mixing. These pieces were rounded and placed in containers covered with plastic wrap to prevent moisture loss. Each 35 g ball was then pressed on a tortilla press for 20 s at the smallest thickness setting (Doughpro, Stearns Product Development Corporation, CA, USA). Thereafter, the diameters of tortillas were measured and transferred to an electric hot plate preheated at 205°C and cooked for 15 s on the first side, flipped and cooked for 20 s on the second side, then flipped one more time and cooked the first side for another 5 s. Cooked tortillas were cooled on a rack for 3 min and packed in open polyethylene plastic bags. These bags were sealed after 3 h, and left at 25°C for 1 h prior to measuring the diameters of cooked tortillas. Colour, thickness and other physical properties such as rollability, firmness and cohesiveness were determined 4 h after production.

4.3.3. Physical Properties

Tortilla diameters were measured using a ruler and thickness with a caliper at three different places for each tortilla. The mean was calculated for each tortilla, and considered as one value. Six tortillas from the same batch were measured for diameter and thickness for each tortilla formulation at different fortification levels. Diameters were measured for both raw and cooked tortillas.

Rollability was evaluated using a subjective dowel test (Serna-Saldivar, 2012; Anton et al., 2009). The tortillas were rolled over a dowel (1.0 cm diameter), and the cracking and breakage was rated in a scale of 1 to 6, where 1 = no indication of cracking (best), 2 = edge cracking only, 3 = edge cracking and/or cracking in the centre, 4 = cracking and breaking on one side, 5 = cracking and breaking on both side (clean break) but still rollable, 6 = unrollable.

Tortilla firmness and cohesiveness were determined using a puncture text with the TA.XT 2 Plus Texture Analyzer (Texture Technologies Crop., Scarsdale, NY & Stable Micro Systems, Godalming, Surrey, UK). A tortilla fixture with a rounded edge cylindrical probe (TA-108, 18 mm diameter) with a force of 20 g was used. Tortillas were placed first-cooked side up on the tortilla fixture, and firmness was measured as the maximum force needed to rupture the tortillas. Cohesiveness, expressed as the area under the curve, was calculated as the work during compression.

Colour measurements were performed using a Minolta CM-3500 model spectrophotometer. The CIE $L^*a^*b^*$ colour scale, in which L^* stands for lightness, a^* (+) for redness, a^*(-) for greenness, b^*(+) for yellowness, and b^*(-) for blueness was used. Samples () were cut from the center of the tortilla for colour measurement, and three measurements were performed for each tortilla. The mean was then calculated for each tortilla, and considered as one value. Six tortillas were measured for each tortilla formulation at different fortification levels.

4.3.4 Sensory Evaluation

The sensory evaluation was approved by the Research Ethics and Compliance Board at the University of Manitoba (Protocol#J2013:095), which can be found in Appendix 2a. Consumer acceptance tests were conducted using 80 untrained panelists consisting of Faculty of Human Ecology and Faculty of Agriculture and Food Sciences staff and students in the sensory panel room (Rm. 221) in the Ellis building at the University of Manitoba. From all 80 panelists, the ballots of 4 panelists were incomplete. Therefore, results of only 76 panelists were collected and reported. In this test, tortillas containing 40% soybean presscake and 35% soy flour, as well as one reference sample (Manny's Corn Tortilla, Mexican Accent, New Berlin, Wisconsin, USA) that was purchased commercially from grocery store were evaluated.

Tortillas were evaluated using a nine point hedonic scale, where 1 represented dislike extremely, 5 represented neither like or dislike, and 9 represented like extremely. Panelists were asked to indicate the acceptability of overall appearance, overall flavor, overall texture and overall acceptability of the three samples coded with a random 3-digit number. Panelists were also asked to indicate how often they consume corn tortillas (at least once a week, at least once a month, at least six times a year, at least three times a year or other) in order to understand their familiarity with corn tortillas (Table 4.1). Based on this, all 76 panelists were categorized into two groups: those who were not familiar with corn tortillas (never had corn tortillas before or only once) (UF group), and those who were familiar with corn tortillas (consumed corn tortillas at least 3 times a year or more frequently) (F group). As a result, 9 panelists formed the group that was not familiar with corn tortillas, and 67 panelists formed the other group that is familiar with corn tortillas.

Table 4.1. Distribution of Panelists Regarding the Familiarity with Corn Tortillas

Familiarity	Number of Panelists
At least once a week	10
At least once a month	21
At least six times a year	22
At least three times a year	14
Unfamiliar	9

All sample tortillas were prepared 24 hours prior the sensory tests, and stored at 4°C in a refrigerator until cut. Samples were removed from refrigerator 15 min prior to evaluation, reheated in a microwave for 15-20 sec and served warm. Each panelist was given 3 types of tortillas, which were all presented as one-third of a full sized tortilla. Microbial tests including total plate count, yeast and mold, as well as coliform were conducted on cooked tortillas before sensory test to ensure the safety of products.

All the documents related to the sensory evaluation including recruitment letter (2b) and poster (2c), a questionnaire (2d), consent form (2e), sensory instruction (2f) and ballot (2g) and can be found in Appendix 2.

4.3.5. Statistical Analysis

All data were reported as means ± sd, and analyzed by Statistical Analysis Software (SAS, version 9.3, SAS Institute Inc., Gary, NC, USA) using ANOVA followed by a Tukey test. All characteristics were considered to be significantly different when $p < 0.05$. Six replicates were conducted for size, thickness, rollability, firmness, cohesiveness, and colour measurement of tortillas. Triplicate measurements were conducted for colour of flour samples. For sensory test, there were in total 76 panelists with complete ballots.

4.4. Results and Discussion

4.4.1. Effect of Added Soy Presscake and Soy Flour on Some Physical Properties of Corn Tortillas

The effect of added soy presscake and soy flour on tortilla size and thickness are summarized in Table 4.2. All the soy fortified tortilla samples except 10% SP were significantly smaller than the corn control tortilla, which was made with 100% corn masa flour. The diameters of raw and cooked tortillas decreased slightly accompanied by increased thickness when the levels of soy substitution increased.

This could be due to the decreased moisture content when higher levels of soy were incorporated as indicated in the previous chapter (Table 3.2). Alternatively, Chen and Rasper reported that incorporating SPI into wheat flour dough for breads would increase the resistance to extension (Chen & Rasper, 1982). This could also explain why diameters of tortillas would decrease with increased levels of soy substitution since tortillas were made using a tortilla press. Tortillas fortified with SF were significantly smaller and thicker compared with tortillas made with SP at the same fortification levels. As discussed in the previous chapter, tortillas made with SF had significantly lower fat content than those made using SP (Table 3.2). The larger diameter could be caused by the higher fat contents in SP tortillas. In addition, thinner and larger tortillas would result in more moisture loss during cooking, which could explain the moisture content differences noted in the previous chapter.

Table 4.2. The Effect of Added Soy Presscake and Soy Flour on Tortilla Size and Thickness

Flour	%	Size (cm)		Thickness (mm)
		Raw	Cooked	
Masa	100	16.71 ± 0.37 a	14.94 ± 0.31 a	1.44 ± 0.06 e
SP	10	16.46 ± 0.17 a	14.23 ± 0.14 a	1.49 ± 0.04 e
	20	15.89 ± 0.21 b	13.67 ± 0.14 b	1.53 ± 0.09 e
	25	15.55 ± 0.16 b,c	14.03 ± 0.15 b,c	1.83 ± 0.07 d
	30	15.18 ± 0.16 c,d	13.40 ± 0.17 c,d	1.75 ± 0.06 d
	35	14.75 ± 0.41 d,e	13.36 ± 0.46 d,e	2.18 ± 0.21 b,c
	40	15.22 ± 0.47 c,d	13.78 ± 0.43 c,d	1.81 ± 0.13 d
SF	10	15.53 ± 0.22 b,c	13.94 ± 0.07 b,c	1.78 ± 0.02 d
	20	14.66 ± 0.30 e,f	13.25 ± 0.21 e,f	2.07 ± 0.08 c
	25	14.30 ± 0.16 f,g	12.87 ± 0.17 f,g	2.27 ± 0.07 a,b
	30	14.17 ± 0.21 g	12.68 ± 0.14 g	2.33 ± 0.06 a
	35	13.97 ± 0.17 g	12.55 ± 0.22 g	2.37 ± 0.12 a

SP: Soy Presscake; SF: Soy Flour
Results listed are Mean ± Standard Deviation of six replications
Values followed by the same letter in the same column are not significantly different ($p>0.05$).

The effect of added soy presscake and soy flour on tortilla firmness and cohesiveness are shown in Table 4.3. Addition of soy ingredient significantly affected firmness and cohesiveness of corn tortillas. Firmness and cohesiveness increased significantly compared with corn control tortillas, except for 10% SP, which showed no significant differences. Firmness was increased by 45.8% to 120.8% in tortillas containing 20% to 40% of SP, and was increased by 33.3% to 130.2% in tortillas containing 10% to 35% of SF. These increases are probably due to lower water addition and lower moisture content in final tortilla samples. Collins and Sanchez (1980) reported decreased firmness when corn tortillas included soy at 10, 20 and 30% levels. However, water addition was increased when soy was added in their tortilla formulations, and they concluded that the increased water addition caused the decrease in tortilla firmness. Although tortillas containing SP and SF both had significantly increased firmness and cohesiveness compared to corn control tortillas, firmness of 10%, 30% and 35% SF tortillas were higher than SP tortillas at the same levels. Cohesiveness of all SF tortillas was also higher than SP tortillas at the same fortification levels. This could be caused by higher protein and lower fat content of SF tortillas compared to SP tortillas as discussed in Chapter 3 (Table 3.3). In addition, firmness and cohesiveness of SF and SP tortillas increased with increasing levels of soy substitution. This could be due to increased thickness of tortillas at higher levels of fortification, as well as the increased protein content and protein-protein interactions when soy was included (Brewer et al., 1992).

Table 4.3. The Effect of Added Soy Presscake and Soy Flour on Firmness, Cohesiveness and Rollability of Soy Fortified Corn Tortillas

Flour	%	Firmness (kg)	Cohesiveness (kg/s)	Rollability
Masa	100	0.24 ± 0.03 g	1.17 ± 0.18 h	1.0 b
SP	10	0.23 ± 0.03 g	1.19 ± 0.15 g,h	1.5 ± 1.2 b
	20	0.35 ± 0.02 e,f	1.97 ± 0.10 e,f	1.5 ± 1.2 b
	25	0.42 ± 0.02 c,d,e	2.42 ± 0.10 d,e	1.0 ± 0.0 b
	30	0.44 ± 0.03 c,d	2.66 ± 0.20 c,d	2.0 ± 1.5 b
	35	0.48 ± 0.05 a,b,c	3.23 ± 0.35 b	1.0 ± 0.0 b
	40	0.53 ± 0.06 a,b	3.48 ± 0.43 a,b	1.5 ± 1.2 b
SF	10	0.32 ± 0.03 f	1.65 ± 0.15 f,g	4.5 ± 1.4 a
	20	0.34 ± 0.03 f	2.13 ± 0.16 e	4.8 ± 0.4 a
	25	0.41 ± 0.04 d,e	2.65 ± 0.18 c,d	5.2 ± 0.8 a
	30	0.47 ± 0.02 b,c,d	3.06 ± 0.05 b,c	5.8 ± 0.4 a
	35	0.55 ± 0.04 a	3.77 ± 0.41 a	5.8 ± 0.4 a

SP: Soy Presscake; SF: Soy Flour
Results listed are Mean ± Standard Deviation of six replications
Values followed by the same letter in the same column are not significantly different ($p>0.05$).

The effect of added soy presscake and soy flour on tortilla rollability is also shown in Table 4.3. As mentioned previously, rollability was scored from 1 (best) to 6 (unrollable). Control corn tortillas, as well as tortillas made with 25 and 35% SP showed the best rollability, followed by tortillas made with 10, 20, 30 and 40% SP. Pflugfelder, Rooney, and Waniska (1988) concluded that the flexibility of tortillas was primarily dependent on gelatinization of free starch. Therefore, corn control tortillas would have the best rollability, which could be due to higher moisture content, as well as higher starch content. Tortillas made with SF showed worst rollability, and were almost unrollable at high SF fortification levels (30% & 35%). Higher fat contents in SP tortillas probably contributed to better rollability than SF tortillas since Pflugfelder et al. (1988) reported that free lipids could have a tenderizing effect on tortilla texture.

The colour of raw flour samples, as well as the effect of added soy presscake and soy flour on tortilla colour is summarized in Table 4.4 and Table 4.5, respectively. For lightness (*L**), tortillas made with SP were darker than corn control tortillas, while tortillas fortified with SF were lighter than corn control tortillas. Tortillas made with SF were, therefore, also lighter than tortillas made with SP. In addition, soy fortified tortillas showed more redness (*a**) and yellowness (*b**) than corn control tortillas. SF tortillas also showed more redness and yellowness than SP tortillas, even though the presscake (SP) was more red and yellow than the flour (SF). These results were in agreement with those of Green et al. (1977), and were partially in agreement with Rababah et al. (2012). Green et al. (1977) indicated that the addition of soybean into corn would result in darker corn tortilla products. Rababah et al. (2012) reported decreased lightness and yellowness, and increased redness when SPI were added into extruded corn chips. Studies also showed opposite results. Collins and Sanchez (1980) produced corn tortillas with lighter, less red and less yellow colour when soybean was added. However, direct comparison of values with literature values cannot be made since the corn and soy flour would come from different cultivars (e.g. yellow corn or white corn) and crop years, as well as different processing methods used for flours and different preparation and formulations for corn tortillas.

Table 4.4. Colour Measurement of Corn Masa, Soy Presscake and Defatted Soy Flours

Flour	%	*L**	*a**	*b**
Masa	N/A	88.69 ± 0.05 b	0.529 ± 0.038 b	14.08 ± 0.09 b
SP	N/A	80.95 ± 0.02 c	0.720 ± 0.006 a	33.81 ± 0.03 a
SF	N/A	92.56 ± 0.01 a	-0.775 ± 0.026 c	11.61 ± 0.02 c

SP: Soy Presscake; SF: Soy Flour
Results listed are Mean ± Standard Deviation of three replications for flour samples.
Values followed by the same letter in the same column are not significantly different ($p>0.05$).

Table 4.5. The Effect of Added Soy Presscake and Soy Flour on Tortilla Colour

Flour	%	*L**	*a**	*b**
Masa	100	70.8 ± 0.2 c,d	-0.64 ± 0.11 f	19.6 ± 0.2 h
SP	10	69.5 ± 0.4 e	-0.58 ± 0.07 f	21.6 ± 0.4 g
	20	69.3 ± 1.0 e	-0.13 ± 0.20 e	23.8 ± 0.5 e
	25	69.2 ± 0.5 e	0.16 ± 0.09 d,e	25.9 ± 0.3 c,d
	30	69.2 ± 0.6 e	0.39 ± 0.26 d	26.6 ± 0.4 c
	35	70.1 ± 0.5 d,e	0.77 ± 0.18 c	28.9 ± 0.5 a
	40	69.2 ± 0.5 e	0.88 ± 0.12 c	29.2 ± 0.3 a
SF	10	71.6 ± 0.3 a,b,c	0.39 ± 0.10 d	22.7 ± 0.3 f
	20	72.6 ± 0.8 a	1.32 ± 0.13 b	25.7 ± 0.5 d
	25	71.6 ± 0.7 a,b,c	1.57 ± 0.25 b	26.0 ± 0.7 c,d
	30	72.4 ± 0.7 a,b	2.32 ± 0.13 a	27.6 ± 0.5 b
	35	71.3 ± 0.6 b,c,d	2.61 ± 0.14 a	28.9 ± 0.4 a

SP: Soy Presscake; SF: Soy Flour
Results listed are Mean ± Standard Deviation of six replications for tortilla samples.
Values followed by the same letter in the same column are not significantly different ($p>0.05$).

4.4.2. Effect of Added Soy Presscake and Soy Flour on Sensory Properties of Corn Tortillas

Table 4.6 shows the sensory scores of tortillas made out of corn masa with 40% SP and 35% SF, as well as a commercial corn tortilla as reference. For the total population (76), 40% SP and 35% SF had significantly higher overall acceptability scores than the commercial corn tortilla. Overall acceptability scores remained the same for panelists who were familiar with corn tortillas when breaking the results into UF and Fgroups. There was no significant differences in overall acceptability, overall appearance, overall flavour and overall texture scores among three tortilla evaluated for panelists who were not familiar with corn tortillas.

Overall flavour and texture of both soy fortified corn tortillas had significantly higher scores than commercial corn tortillas. No significant differences were observed for overall appearances or any parameters evaluated by the UF group. Overall flavour produced the lowest acceptance by panelists among all four parameters. It has to be kept in mind that, the commercial corn tortillas also contained preservatives to extend its shelf-life, which may have influenced the scores, while soy fortified tortillas were freshly made with only basic ingredients. Tortillas fortified with SP also had higher scores than tortillas made with SF for overall appearance. The overall appearance of commercial corn tortilla was not significantly different from either of the soy fortified tortillas. This occurred despite the fact that commercial corn tortillas were made using a die-cut method, which produced smoother edge than soy fortified tortillas made in the lab. The increased in yellow colour for the tortillas fortified with soy may also have been appealing to consumers.

Table 4.6. Sensory Scores of Selected Tortillas

	Population (n)	Reference [1]	40% SP	35% SF
Overall Acceptability	Total (76)	5.09 ± 1.74 b	6.61 ± 1.29 a	6.04 ± 1.69 a
	F [2] (67)	5.10 ± 1.69 b	6.66 ± 1.32 a	6.16 ± 1.63 a
	UF [3] (9)	5.11 ± 2.15 a	6.22 ± 0.97 a	5.11 ± 1.96 a
Overall Appearance	Total	6.74 ± 1.20 a,b	7.14 ± 1.21 a	6.38 ± 1.44 b
	F	6.81 ± 1.20 a,b	7.25 ± 1.19 a	6.51 ± 1.49 b
	UF	6.22 ± 1.20 a	6.33 ± 1.12 a	5.33 ± 1.22 a
Overall Flavour	Total	4.63 ± 1.82 b	6.45 ± 1.47 a	6.07 ± 1.69 a
	F	4.64 ± 1.80 b	6.48 ± 1.49 a	6.18 ± 1.61 a
	UF	4.56 ± 2.13 a	6.22 ± 1.39 a	5.22 ± 2.11 a
Overall Texture	Total	5.39 ± 1.70 b	6.37 ± 1.54 a	5.73 ± 1.83 a,b
	F	5.39 ± 1.67 b	6.45 ± 1.59 a	5.79 ± 1.80 a,b
	UF	5.44 ± 2.01 a	5.78 ± 0.97 a	5.33 ± 2.12 a

[1] Commercial corn tortilla (Manny’s Corn Tortilla, Mexican Accent, New Berlin, Wisconsin, USA) purchased from Safeway.
[2] F: Panelists were familiar with corn tortillas, who declared to usually consume corn tortillas at least three times a year
[3] UF: Panelists were familiar with corn tortillas, who declared they never consumed corn tortillas before, or only consumed corn tortillas once
SP: Soy Presscake; SF: Soy Flour
Results listed are Mean ± Standard Deviation
Values followed by the same letter in the same row are not significantly different ($p>0.05$).

Green et al. (1977), and Collin and Sanchez (1980) fortified corn tortillas with whole soybean meals, and reported lower overall acceptance, and lower mouthfeel and flavour scores, respectively. Scazzina et al. (2008) reported higher overall acceptability scores and lower texture and flavour scores when soybean was added into wheat tortillas. Anton et al. (2009) also found that wheat tortillas fortified with bean flours had better overall acceptability, flavour and texture scores. This is logical since with the development of food industry and increased demand of healthy food, consumers are more likely to accept a new nutritious food product with a different texture and flavour.

Panelists were also asked to indicate their intent to purchase, and there results are summarized in Table 4.7. Fifty-nine and 41 panelists out of total population showed their willingness to purchase corn tortillas fortified with 40% SP and 35% SF, respectively. Only 18 panelists would like to purchase the commercial corn tortillas. This has shown the potential of this soy fortified corn tortilla in the future market.

Table 4.7. Intention of Panelists to Purchase

Sample name	Yes	No
40% SP	59	17
35% SF	41	35
Commercial Reference	18	58

SP: Soy Presscake; SF: Soy Flour

4.5. Conclusion

Fortification with SP and SF had significant effects on physical and sensory properties of corn tortillas. Tortillas fortified with SF were all significantly smaller and thicker than corn control tortillas; changes in size were also significant at high levels of SP fortification (25% - 40%). These changes were more noticeable at higher soy fortification levels. Due to decreased water addition, firmness was increased by 45.8% to 120.8% in tortillas containing 20% to 40% of SP, and was increased by 33.3% to 130.2% in tortillas containing 10% to 35% of SF. Cohesiveness was also significantly higher in tortillas with soy addition except for the tortilla made with 10% SP. Compared to tortillas made with SP, increased firmness was also found in 10%, 30% and 35% SF tortillas; cohesiveness of all SF tortillas was also higher than the SP tortillas at the same fortification levels. Corn control tortillas showed the best rollability, followed by tortillas made with SP. Tortillas made with SF showed worst rollability, and were almost unrollable at high SF fortification levels (30% & 35%). For colour measurements, tortillas made with SP were darker than corn control tortillas, while tortillas fortified with SF were lighter. Increased redness and yellowness could also be found for tortillas fortified with soy. In the consumer acceptance test, 40% SP and 35% SF had significantly higher overall acceptability scores than the commercial corn tortillas for total population (n = 76). In addition, overall flavour and texture of both soy fortified corn tortillas had higher scores than commercial corn tortillas. More importantly, 59 out of 76 panelists stated that they would like to purchase tortillas with 40% SP substitution. Though the fortification of SP and SF had significant effect on tortilla size, thickness, firmness, cohesiveness, rollability, as well as colour, our results appeared to show that these changes were still acceptable by consumers. Therefore, tortillas fortified with SP and SF would have potential in a healthy food market.

5. INFLUENCE OF ADDED SOY PRESSCAKE ON *IN VITRO* STARCH HYDROLYSIS AND *IN VITRO* GLYCEMIC RESPONSES OF CORN TORTILLAS

5.1. Abstract

The influence of added soy presscake (SP) on *in vitro* starch hydrolysis and *in vivo* glycemic responses of corn tortillas was studied. Starch hydrolysis over time (120 min) was determined by *in vitro* method with α-amylase for the *in vivo* glycemic response. Ten eligible healthy individuals (5 female & 5 male) were recruited and 4 test meals containing 25 g available carbohydrate were consumed: corn control tortilla, 10%, 25% and 40% SP corn tortilla. Blood samples were collected over 120 min after consumption of meals to evaluate the glycemic response and to determine the glycemic index (GI). GI was calculated using the incremental AUC method and glucose solution was used as reference. Corn tortillas containing SP decreased the rate and extent of starch hydrolysis *in vitro*. The GI values determined by the *in vivo* method decreased from 52.62 for corn control tortillas to 34.9 with 40% SP fortification. A high correlation (r = 0.9920) was found between the GI values from *in vivo* analysis and the AUC of *in vitro* starch hydrolysis. The best correlation between degree of hydrolysis (DH) and GI was found at 60 min of *in vitro* starch hydrolysis. The incorporation of SP in corn based tortillas evidently provides a food with lower glycemic responses. In addition, *in vitro* starch hydrolysis could be used to predict and estimate the GI values of food products due to its good correlation with *in vivo* results.

5.2. Introduction

Corn tortilla, produced by the alkaline cooking of corn (nixtamilization), is a flat bread traditionally consumed in Mexico and Central America (Herrera-Corredor et al., 2007). Nutritionally, corn based tortillas are rich in carbohydrates (approx. 45%) and low in protein content. As the average annual consumption of corn tortillas in Mexico is more than 80 kg, corn tortillas provide rich sources of carbohydrates and calcium (Feria-Morales & Pangborn, 1983; Serna-Saldivar, 2012).

Soybean is a nutritious and economically viable agricultural commodity due to its unique chemical composition and nutritional profile. Among all the cereals and legumes, soybean has the highest protein content (~40%). Moreover, soybean and its by-product have been shown to have effect on insulin secretion and action (Latorraca et al., 2011). Legumes are known to affect blood glucose responses more than other high fiber foods due to the intrinsically low enzyme susceptibility of legume starches (Thorne, Thompsom, & Jenkins, 1983; Goni & Valentin-Gamazo, 2003). In addition, the presence of other health-promoting compounds such as isoflavones in soybean has been linked with improved glycemic control and insulin resistance for postmenopausal women with type 2 diabetes (Latorraca et al., 2011).

Glycemic index was first introduced by Jenkins et al. in 1981 and is defined as the area under the blood glucose response curve for food products. It is expressed as a percentage of the area after taking the same amount of carbohydrate as a reference food (glucose or white bread) (Jenkins, et al., 1981; Goni et al., 1997). GI is used to classify foods based on their postprandial blood glucose response (Goni et al., 1997). Thus, the lower the GI of a food, the less it affects the blood glucose and insulin levels in human body. There are evidences showing that carbohydrates in foods such as legumes, pasta and whole grain cereals are slowly digested and absorbed, and are favorable in the dietary management of metabolic disorders (e.g. diabetes and hyperlipidemia) (Goni et al., 1997). Corn tortilla made with white corn (Diego's brand) has been reported to have a GI value of 49 ± 6 (Mean ± SEM) using glucose as the reference, whereas Canadian boiled dry soybean has a much lower GI value of 15 ± 5 (Atkinson et al., 2008).

Low GI diets are associated with reduced risk of type 2 diabetes, obesity and CVD (Hall, Thomas, & Johnson, 2005; Burton, 2011). Therefore, research has been conducted to lower GI of food products by incorporating legumes into traditional starch based foods. Goni and Valentin-Gamazo (2003) incorporated chickpea flours into wheat pasta. This resulted in the GI values being significantly lowered from 73 ± 5 to 59 ± 6 using white bread as reference (Goni & Valentin-Gamazo, 2003). Hall et al. (2005) also studied the effect of added Australian sweet lupin flour into a white bread breakfast on GI. The GI was reduced to 74.0 ± 9.6 (mean ± SEM) compared to the GI of white bread at 100 (Hall et al., 2005).

Evaluating GI using *in vivo* method is relatively costly and time consuming. Therefore, using *in vitro* starch hydrolysis to mimic human digestion of food products has been used to estimate or predict GI values of food products. Holm et al. (1985) reported that good agreement was found in between *in vivo* and *in vitro* starch availability after flaking, steam-cooking and popping of wheat. Goni et al. (1997) also reported the best correlation (r = 0.909) between GI obtained from *in vivo* and percentage of starch hydrolysis from *in vitro* at 90 min time point of the 120 min hydrolysis.

Therefore, the purpose of this study was to evaluate the *in vitro* starch digestibility and *in vivo* glycemic responses of tortilla fortified with SP at 10%, 25%

and 40%, as well as to compare those results with control corn tortillas. The correlation between *in vitro* and *in vivo* data was also analyzed. Tortillas fortified with soy flour had poor rollability, therefore, only tortillas fortified with soy presscake were selected for the evaluation of *in vitro* starch digestibility and *in vivo* glycemic responses.

5.3. Material and Methods

5.3.1. General

Soy presscake, a by-product of cold press soy oil production, was purchased from Pristine Gourmet (Waterford, On, Canada). Soy presscake was sieved to pass a 590 μm sieve (30 mesh US standard Sieve Series) in the Department of Food Science when received, packaged in Ziploc bag and stored at 4 °C. White masa flour was produced by Azteca Milling, L.P. (Irving, TX, USA), was purchased from Sunny Day Products (Winkler, MB, Canada) and stored in a cold room (4 °C). Defatted soy flour (bulk barn) and salt (Sifto, Mississauga, ON) were purchased from local grocery stores. Xanthan gum was provided by Tic Gums (White Marsh, MD, USA). The specification sheet for the gum can be found in Appendix 1.

5.3.2. Tortilla Preparation for in vitro Starch Hydrolysis and in vivo Glycemic Responses Analysis

The formulation and preparation of corn tortillas fortified with SP has been discussed in previous chapters (3.3.2, 3.3.3 and 4.3.2). Four tortilla formulations were selected to be evaluated using both *in vitro* and *in vivo* techniques. These four formulations were control corn tortilla, corn tortillas with 10%, 25% and 40% substitution with SP. The contents of available carbohydrates (mainly starch) were calculated by difference for SP and Corn masa flour, and are summarized in Table 5.1. Resistant Oligosaccharides (mainly raffinose and stachyose) were also quantified and subtracted in the calculation of available carbohydrates since soybeans and other legumes commonly contain a high level of oligosaccharides (Dixit et al., 2010 & Goni & Valentin-Gamazo, 2003).

Moisture contents were analyzed using AACC Moisture – Air-oven Method (44-15.02) with modifications (AACC, 1999). Approximately 2 g of ground samples were weighed into pre-dried aluminum dishes, and dried in an air-oven for 16 h.

Fat and nitrogen contents were measured by Soxhlet and Dumas methods, respectively. The nitrogen content was multiplied by a factor of 5.71 for SP and 6.25 for corn masa flour to estimate protein content (FAO, 2002). A factor of 5.71 for soy presscake was used instead of 6.25 could give a more accurate results, which is critical in the calculation of available carbohydrates. Ash content was determined by AOAC method 923.03 with modifications. Approximate 2 g of freeze-dried ground tortilla was weighed and pre-ashed before putting into a furnace (Blue M Electric Company, Thermal Product Solutions, White Deer, PA, USA) at 550 °C for approximately 16 h. Total dietary fiber and resistant oligosaccharides were analyzed by Medallion Lab, using AOAC 2001.03 and AOAC 991.43 methods with modifications, respectively. The final report from Medallion Labs can be found in Appendix 3.

The total dough weight was calculated and percentage of ingredients in different tortillas was calculated based on the tortilla formulation reported in Chapter

3 (Table 3.1). Percentages of ingredients in dough were calculated using the weight of the ingredient divided by total dough weight. Results are shown in Table 5.2 and Table 5.3. The number of tortillas needed to provide 25 g and 250 mg equivalent available carbohydrates were calculated and are reported in Table 5.4. For the *in vivo* estimation of glycemic index, tortillas were weighed and cut to the exact weight if less than one full tortilla was needed. For instance, 2.389 pieces of corn control tortilla were prepared using 2 full tortillas, and 0.389 piece of one full tortilla (0.389 × weight of one piece of corn control tortilla). For *in vitro* starch hydrolysis, one piece of tortilla was weighed and broken into pieces. An amount that contained 250 mg available carbohydrate (mainly starch) equivalent of sample was then weighed for further analysis.

Table 5.1. Content of Available Carbohydrates in Soy Presscake and Corn Masa Flour

Sample	MC (%)	Protein (%)	Fat (%)	Ash (%)	Total Dietary Fiber (%)	Resistant Oligosaccharides (%)	Available CHO (%)
SP	7.69	44.95	14.54	4.75	16.10	4.80	7.17 *
Masa	12.37	9.35	4.35	1.39	6.00	0.60	65.94 *

SP: Soy Presscake.
*Results are calculated values

Table 5.2. Total Dough Weight for Selected Tortilla Formulations*

	Flours (g)		Salt (g)	Gum (g)	Water (g)	Total Dough Weight (g)
	Masa	Soy	0.50%	1%		
Control	100	0	0.5	1	119.08	220.58
10% SP	90	10	0.5	1	107.67	209.17
25% SP	75	25	0.5	1	90.56	192.06
40% SP	60	40	0.5	1	73.45	174.95

SP: Soy Presscake.
*Formulations were described on 100g total flour weight basis.

Table 5.3. Percentage of Ingredient in Selected Tortilla Formulations

Samples	Flour (%)	Salt (%)	Gum (%)	Water (%)
Control	45.34	0.23	0.45	53.98
10% SP	47.81	0.24	0.48	51.47
25% SP	52.07	0.26	0.52	47.15
40% SP	57.16	0.29	0.57	41.98

SP: Soy Presscake

Table 5.4. Tortillas Containing 25 g and 250 mg Equivalent Available Carbohydrates

Sample	Flour (%)	Dough wt. (g)	Flour weight (g)			Available CHO (g)			Number of Tortillas	
			Total	Masa	Soy	Masa (65.94%)	Soy (7.17%)	Total	25 g CHO	250 mg CHO
Control	45.336	35	15.868	15.868	0.000	10.463	0.000	10.463	2.389	0.024
10% SP	47.809	35	16.733	15.060	1.673	9.930	0.120	10.050	2.487	0.025
25% SP	52.068	35	18.224	13.668	4.556	9.013	0.327	9.339	2.677	0.027
40% SP	57.161	35	20.006	12.004	8.003	7.915	0.574	8.489	2.945	0.029

SP: Soy Presscake.

5.3.3. in vitro Hydrolysis of Starch

For *in vitro* starch hydrolysis, all tortilla samples were prepared 24 h prior to the experiment, and stored at 4°C in sealed plastic packaging. Samples were prepared as described above.

The methods described by Brennan et al. (1996) and Goni et al. (1997) with modifications were followed for the starch hydrolysis. Duplicate analysis was conducted for each sample. Samples were weighed into 50 mL screw cap tubes and wet-homogenized (Ultra-Turrax T18 basic, IKA® Works Inc., Wilmington, USA) for 60 s with 30 mL of distilled water. 1M aqueous HCl (0.5 mL) was then added and the pH adjusted to 2.5. Samples were held at 37°C for 10 min in a shaking water bath at 150 rpm (SW22, Julabo, Seelbach, Germany), and 1 mL of pepsin (10% solution in 0.05 M aqueous HCl, P7000, Sigma-Aldrich) was then added and samples were incubated at 37°C for 30 min. After addition of 1 mL of 1 M aqueous solution of $NaHCO_3$ to adjust the pH to 6, the total volume was then adjusted to 50 mL. Amyloglucosidase (0.5 mL, A7095, Sigma-Aldrich) was added into samples to prevent end product inhibition of pancreatic amylase, followed by addition of 4.5 ml of α-amylase (360 units/mg in a pH 6 Na maleate buffer). Samples were then continuously incubated at 37°C with slow constant mixing (150 rpm) and 1 mL aliquots were withdrawn at 0, 15, 30, 45, 60, 90 and 120 min. The withdrawn aliquots were inactivated in 100 °C boiling water for 10 min, and kept at 4°C until the end of hydrolysis. When the hydrolysis was stopped after 120 min, all samples were diluted 50 times, and the glucose contents of the duplicate aliquots of measured using Glucose (GO) Assay Kit (Sigma-Aldrich). The degree of hydrolysis was calculated as the potion of starch hydrolyzed into glucose; the area under curve (AUC) was also calculated to compare with *in vivo* GI results.

5.3.4. in vivo Estimation of Glycemic Index

Tortilla samples were prepared, sealed in plastic bags and stored at -18°C for maximum of 6 weeks. All samples were labeled with randomized 3-digit codes (Appendix 4a). Tortillas samples were packed separately piece by piece based on the amount required to deliver 25 g of available CHO, and samples with the same code were packed again into larger Ziploc bags. Tortillas were reheated piece by piece with a microwave for 30 sec if frozen, and 15 to 20 sec if defrosted before serving.

The *in vivo* GI estimation was conducted in the St. Boniface Hospital – Asper Clinical Research Institute. This was a single site, double-blind and randomized controlled study. Ten eligible healthy individuals (5 female & 5 male) aged 24.78 ± 6.36 years with normal body mass indices (BMI: 23.40 ± 2.89 kg/m^2) were recruited into the study. Each participant visited the research clinic on 7 separate visits for the glycemic testing consisting of one screening visit and 6 study visits. At each visit, one of the following products was consumed after an overnight fast: i) 296 ml (~1 cup) of oral glucose solution; ii) 296 ml (~1 cup) of oral glucose solution for a duplicate assessment as per standard glycemic index testing protocols; iii) corn control tortillas which without substitution of SP; iv) corn tortillas with 10% SP; v) corn tortillas with 25% SP; and vi) corn tortillas with 40% SP. For tortillas, the amount of samples consumed contained 25 g available carbohydrates. Blood was sampled via capillary finger prick at time-point 0 (before the product was consumed), and 15, 30, 45, 60, 90 and 120 minutes after the first bite of the tortillas or first sip of the glucose solution to determine blood glucose concentrations using a glucometer. The method used to calculate GI of samples was described by Brouns et al. (2005). The incremental AUC method was used, which accounted for the area over the baseline (blood glucose concentration at 0 min) under the curve, ignoring area beneath the baseline. GI was calculated using the equation below:

$$\text{GI} = \frac{\text{AUC of the food}}{\text{AUC for an equal amount of carbohydrate as glucose solution}} \times 100$$

The GI was calculated for each participant, and means of individual ratios were then calculated and reported as the GI of tortilla samples.

The recruitment poster can be found in Appendix 4b.

5.3.5. Statistical Analysis

All data was analyzed by Statistical Analysis Software (SAS, version 9.3, SAS Institute Inc., Gary, NC, USA). One-way analysis of variance (ANOVA) followed by a Tukey test was carried out for *in vitro* starch hydrolysis, and a paired two-tail t-test was applied for *in vivo* estimation of glycemic index. All characteristics were considered to be significantly different when $p < 0.05$. Duplicate analysis was carried out for *in vitro* starch hydrolysis, and 10 participants were recruited for *in vivo* estimation of glycemic index. Pearson's correlation coefficient (r) between AUC of *in vitro* starch hydrolysis curve and *in vivo* GI values was also analyzed using mean values (n = 2 for AUC in *in vitro*, and n = 10 for GI in *in vivo*).

5.4. Results and Discussion

5.4.1. The Effect of Added Soy Presscake on in vitro Starch Hydrolysis of Selected Corn Tortillas

The results of *in vitro* starch hydrolysis are shown in Table 5.5 to show statistical differences and Figure 5.1 to show the change over time. Corn control tortillas and tortillas substituted with 10% SP showed no significant differences in terms of degree of starch hydrolysis (DH) over 120 min; higher DH were seen for these two samples than the samples with 25 and 40% SP at hydrolysis times between 45 and 90 min. At the 15 min time point, no significant differences were found for DH among all four tortillas. At 30 min time point, 40% SP tortilla showed a significantly lower DH than control and 10% SP tortillas. At the end of the 120 min digestion, the DH values were not significantly different. This is not unexpected since the amount of tortilla samples used for hydrolysis contained equal amount of available carbohydrates (250 mg), and at some point, they should all be hydrolyzed. However, we can still conclude that corn tortillas containing SP at levels of 25 and 40% could slow down the rates of starch hydrolysis, especially at the 40% SP fortification levels.

The results were in agreement with those of Goni & Valentin-Gamazo (2003), Hardacre et al. (2006), Utrilla-Coello, Osorio-Diaz, & Bello-Oerez (2007), Gallegos-Infante et al. (2010), Chillo et al. (2010), and Grajales-Garcia et al. (2012), where the *in vitro* starch hydrolysis rates and extent were lowered when legumes including common beans, soybeans, lentil and chickpeas were added into traditional starch based food products such as bread, tortillas, wafers and pasta.

Table 5.5. Degree of Starch Hydrolysis of Selected Tortillas over 120 min

	Control (%)	10% SP (%)	25% SP (%)	40% SP (%)
15	27.17 ± 4.07 a	34.31 ± 1.06 a	26.99 ± 2.31 a	25.84 ± 2.95 a
30	40.31 ± 2.15 a,b	44.78 ± 1.06 a	37.92 ± 1.82 b,c	33.28 ± 0.62 c
45	48.89 ± 2.29 a	49.66 ± 1.47 a	41.47 ± 1.63 b	39.00 ± 1.09 b
60	52.96 ± 2.53 a	53.03 ± 1.77 a	45.30 ± 0.25 b	42.74 ± 2.00 b
90	57.86 ± 4.59 a	57.91 ± 1.47 a	50.49 ± 0.89 a,b	45.56 ± 1.62 b
120	62.69 ± 4.21 a	58.91 ± 4.01 a	57.39 ± 0.30 a	50.04 ± 2.33 a

SP: Soy Presscake

Results listed are Mean ± Standard Deviation, n = 2
Values followed by the same letter in the same row are not significantly different ($p>0.05$).

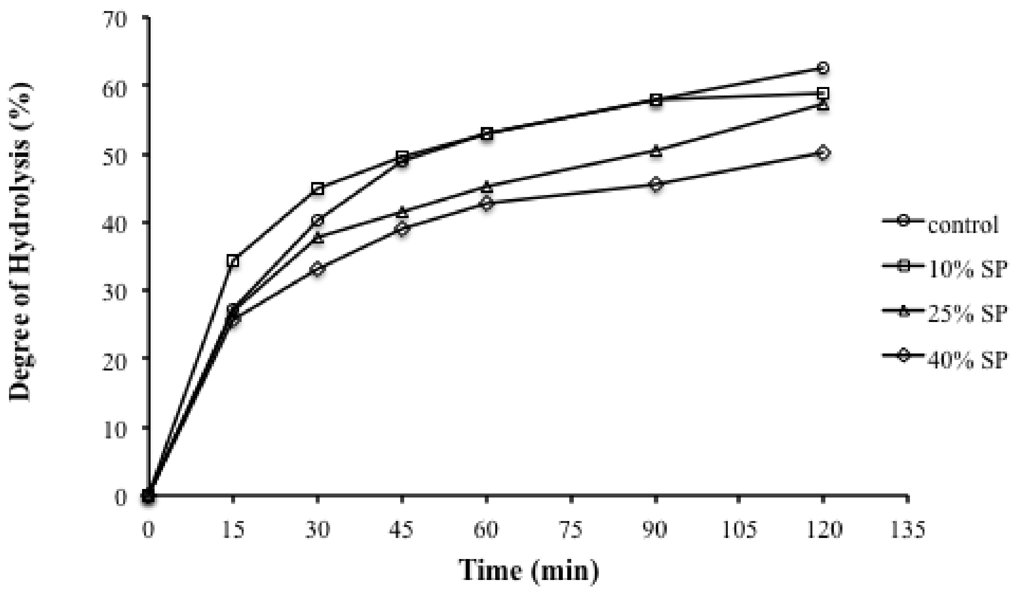

Figure 5.1. *In vitro* Starch Hydrolysis of Selected Corn Tortillas

5.4.2. The Effect of Added Soy Presscake on in vivo Glycemic Responses of Corn Tortillas

Mean blood glucose concentrations of 10 participants after taking in the oral glucose solution and four selected soy fortified corn tortillas are summarized in Figure 5.2. The mean blood glucose concentrations after intake of oral glucose solution were significantly higher than those of all tortillas during the entire experiment. Among all four tortillas at all the time points, significant difference could only be found at 120 min. At 120 min, the mean blood glucose concentration after eating tortillas containing 25% and 40% of SP were significantly lower than the mean blood glucose concentration of corn control tortillas and tortillas fortified with 10% SP. In addition, compared to the glucose solution and corn control tortillas, delays in the time needed to reach peak blood glucose concentration were found for tortillas fortified with soy at 10%, 25% and 40% levels. Mean blood glucose concentrations reached the peak at 30 min time point for glucose solution and corn control tortillas, while the maximum mean blood glucose concentrations were reached at 45 min time point for tortillas fortified with soy.

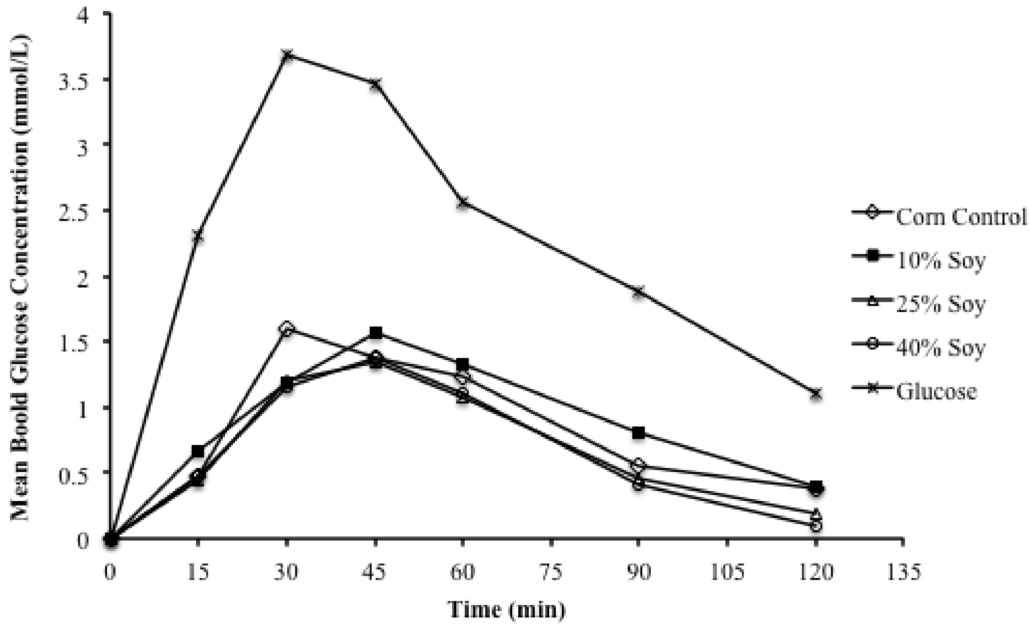

Figure 5.2. Mean Blood Glucose Concentrations in Healthy Subjects after Intake of Oral Glucose Solution and Selected Corn Tortillas, Adjusted with Baselines

The GI values of four selected tortillas were calculated and are reported in Table 5.6. The GI value of corn control tortilla were in agreement with Atkinson et al. (2008) who calculated that corn tortillas made with white corn have a GI value of 49 ± 6 (Mean ± SEM) when glucose was used as reference. The GI values appeared to be lower when corn tortillas were fortified with 25% (GI = 39.47) and 40% SP (GI = 34.93), but the differences were not significant due to the high deviations. Therefore, blood glucose concentration in all 10 participants consuming 40% SP tortillas were adjusted based on their fast blood glucose concentrations, and are shown in Figure 5.3. When looking at the individual blood glucose concentration curves, the 10 participants could be categorized into two groups, those who had high glycemic responses to soy and those who had low glycemic responses (peak blood glucose concentration < 1.5 mmol/L after intake 40% SP tortilla) as shown in Figure 5.3. This became more distinct when tortillas with higher levels of SP were consumed. The different responses to soy of 10 participants could explain the very high deviations of final GI values. The blood glucose concentration of individual participants adjusted based on their fast blood glucose level for other samples can be found in Appendix 4d, 4e, 4f, 4g.

Table 5.6. The Glycemic Index of Four Selected Corn Tortillas

Sample Name	Flour	%	GI	p
Corn Control	Masa	100	52.62 ± 54.24	
10% SP	SP	10	53.16 ± 43.83 *	0.9481
25% SP	SP	25	39.47 ± 23.62 *	0.2610
40% SP	SP	40	34.93 ± 17.00 *	0.2196

SP: Soy Presscake, GI: Glycemic Index
Results listed are Mean ± Standard Deviation, n = 10.
*Values are not significantly different comparing to corn control using a paired two-tail t-test (p>0.05).

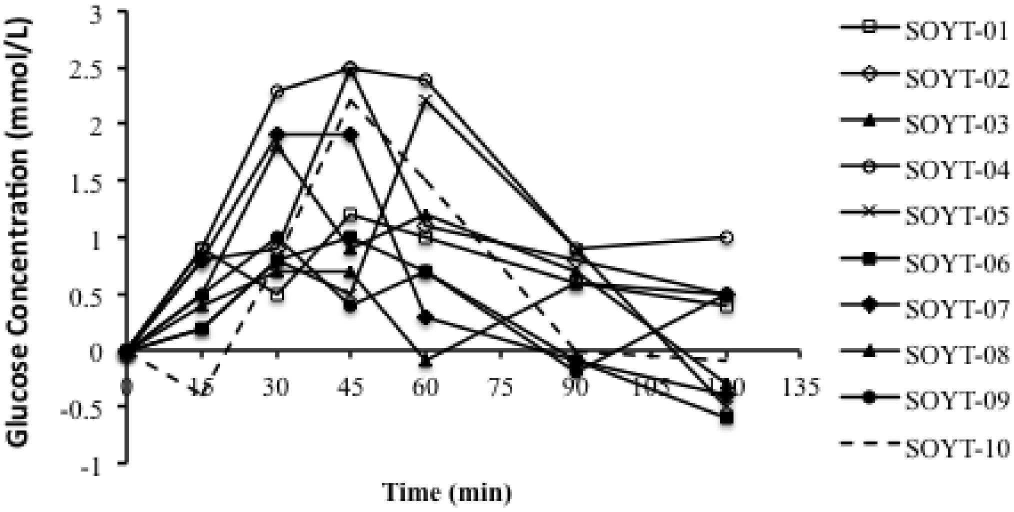

Figure 5.3. Individual Blood Glucose Concentrations for 10 Healthy Subjects after Intake 40% SP Corn Tortillas, Adjusted with Baseline.

The effect of added SP on *in vivo* glycemic responses is rather complex to explain. The amount of dietary fiber in food products was the focus of the hypotheses in 1980s (Thorne & Jenkins, 1983). However, dietary fiber has been excluded when calculating available carbohydrates for *in vivo* glycemic response studies in recent research. Nevertheless, we should still consider the indigestible fraction in food products as one of the factors that contribute to *in vivo* glycemic responses as it helps explain why, legumes and food products fortified with legumes would show different glycemic responses. The term indigestible fraction could include compounds that are resistant to the digestive enzymes such as dietary fibre, resistant starch, resistant protein, lignin, as well as oligosaccharides (Goni & Valentin-Gamazo, 2003). These compounds are not commonly quantified and excluded when calculating the amount of available carbohydrates. Other factors such as the nature of starch, protein-starch interactions, anti-nutrients, physical forms of food product, as well as cooking methods, are also contributors to the different glycemic responses of legume and foods containing legumes. As mentioned in Chapter 2, legumes generally contain higher amylose content than cereals and other foods (Thorne & Jenkins, 1983; Guillon & Champ, 2002). Thorne and Jenkins (1983) concluded that amylose has a much smaller surface area than amylopectin, and the glucose units are bound to each other by hydrogen bonds. In addition, carbohydrates in legumes are commonly bound to the protein matrix (Thorne & Jenkins, 1983). Therefore, the high amylose content and protein-starch interaction in legumes could cause differences in *in vivo* glycemic responses. Some presence of anti-nutrients such as amylase inhibitors, lectins or phytic acid were reported to decrease the rate of starch digestion or produce hypoglycemia in animal studies. In addition, cooking time and method and particle size can also affect the glycemic response of legumes or food containing legumes (Thorne & Jenkins, 1983).

5.4.3. Correlation of in vitro Starch Hydrolysis and in vivo Glycemic Responses

GI from *in vivo* results and the AUC of *in vitro* starch hydrolysis curve were analyzed to see if they were related. Mean values were used for the data analysis since the replications were different for *in vivo* (n=10) and *in vitro* (n=2). A very good correlation (r = 0.9920) was found between the GI values from *in vivo* analysis and the AUC of *in vitro* starch hydrolysis when considering all time points. Therefore, *in vitro* starch hydrolysis could be used to estimate or predict the GI values of food products to a certain extent.

The correlation between the DH and the GI from *in vivo* results at different time points are shown in Table 5.7. The correlation was significant at 45 min, 60 min and 90 min, and the best correlation between the DH and GI was found at 60 min. Bornet et al. (1989) reported that DH was well correlated with *in vivo* data within 30 min, while Goni et al. (1997) found that the best correlation between DH and GI from literature was at 90 min. Though at 90 min, the correlation was not the best in this research, the correlation was still very high (r = 0.9903).

Table 5.7. The Correlation Coefficient (r) and Probability Levels (p) between the Degree of Starch Hydrolysis at Different Time Points and the Glycemic Index from *in vivo* results[1].

Time Point (min)	r	p
15	0.6749	0.3251
30	0.9071	0.0929
45	0.9993	0.0007
60	0.9998	0.0002
90	0.9903	0.0097
120	0.8622	0.1378

r: Correlation Coefficient; p: Probability Levels
[1] Mean value were used to analyze the correlation

5.5. Conclusion

Corn tortillas containing SP could decrease the rate of starch hydrolysis *in vitro*. Corn control tortillas and tortillas substituted with 10% SP showed no significant differences in DH over 120 min. Significantly lower DH were found at 45 and 60 min for tortilla fortified with 25% SP, and at 30, 45, 60 and 90 min for tortilla fortified with 40% SP. The GI values determined by *in vivo* method appeared to decrease with the increasing levels of SP fortification; however, the decreases in GI were not significant. A good correlation (r = 0.9920) was found between the GI values from *in vivo* analysis and the AUC of *in vitro* starch hydrolysis. The best correlation between DH and GI was found at 60 min of *in vitro* starch hydrolysis. The results showed that incorporating legumes into traditional starch based food products would lower the GI. In addition, *in vitro* starch hydrolysis could be used to predict and estimate GI of food products.

6. EFFECT OF ADDED SOY PRESSCAKE AND SOY FLOUR ON SOME PHYSICAL PROPERTIES OF WHEAT-BASED PIZZA DOUGH

6.1. Abstract

Wheat based pizza crust fortified with soybean presscake (SP) and defatted soy flour (SF) at 15% and 35% levels were studied and compared. Textural properties evaluated included firmness and cohesiveness. Colour, using a CIE *L*a*b** values, was determined instrumentally. The firmness and cohesiveness were increased by the addition of SP and SF, and pizza crust made with SF showed increased firmness and cohesiveness compared to those made with SP at the same fortification levels. The addition of SP and SF also resulted in decreased lightness (*L**), and increased redness (*a**) and yellowness (*b**) on the top crust, bottom crust and middle of the pizza crust. Based on this, soy fortification of pizza crust resulted in significant changes on texture and colour. Acceptability of the changes on texture and colour should be further analyzed through a sensory test.

6.2. Introduction

Pizza is one of the most well-known Italian foods in the world. Due to the convenience, taste and nutritional value, pizza is now one of the most popular foods in European and North America. Growth in the sales of pizza has been reported in big chain and independent restaurants, as well as sales of frozen/chilled pizza (Rhodes et al., 2014).

Pizza is a round oven-baked flat bread covered with tomato sauce, mozzarella cheese and a variety of other ingredients (Singh & Goyal, 2011), in which approximately 55% of the pizza weight is the baked pizza dough base (crust) (Serna-Saldivar, 2010). Most pizzas crusts contain around 10-14% protein, less than 10% fat, and are high in complex carbohydrates, mainly from starch (Singh & Goyal, 2011). According to Rhodes et al. (2014), about 1 in 8 Americans consume pizza on any given day; and pizza provides approximately 27% of total energy on the day it is consumed. Thus, pizza plays an important role in North American diets.

The unique chemical composition and nutritional profile make it a well know nutritious commodity. Soybean has the highest protein content (~40%) or the cereals and legumes. In fact, the protein nutritional quality of soybeans has been shown to be equal to the protein quality of milk, meat, fish and eggs (Riaz, 2006; Endres, 2001). Soy protein provides a complete range of all the essential amino acids that are needed for human growth, and health maintenance (Hoogenkamp, 2005; Endres, 2001). Research has shown that soy protein has an effect on lowering serum levels of total cholesterol and low-density lipoprotein (LDL) cholesterol, and serum triacylglycerol (Urade, 2011). Therefore, in 1999, the U.S. Food and Drug Administration approved the following health claim for soy protein: “25 grams of soy protein a day, as part of a diet low in saturated fat and cholesterol may reduce the risk of heart disease" (FDA, 2014). Soy nutrients have also been associated with prevention and treatment of some cancers, cardiovascular disease, bone health problems and other chronic diseases (Riaz, 2006; Clayton, 2001).

Soybeans produced in Canada are high-yield, high-quality food grade beans, and therefore approximately 35 percent of Canadian produced soybeans are exported to premium markets such as Japan and Europe (Canadian Soybean Council, 2012). Soybean presscake, also known as soybean cake or meal, is the by-product obtained after oil extraction from soybeans (Ramachandran et al., 2006). Oil cake production has an annual growth rate of 2.3% over the decade from 2000 to 2010 (Ramachandran et al., 2006). Soybean meal production accounted for 67.5% of world total major protein meals, followed by rapeseed meal (13.7%) and sunflower seed meal (9.0%) (USDA, 2014). The composition of soybean press cake varies due to different soybean varieties, growing conditions and processing (extraction) methods. The soybean presscake is now commonly used as animal feed since it contains a high level of protein. However, the consumption or the use of soybean presscake as a food ingredient is still not common.

Wheat flour is the basic ingredient in the production of pizza dough (pizza crust). White unenriched all-purpose flour usually contains about 10.33% protein (USDA, 2012). However, like most of the cereal grains, wheat flour is low in lysine. Therefore, the addition of soybeans or other legumes would complement the deficiency of lysine in wheat based food products. Though pizza is commonly nutritious due to various toppings, a pizza crust of high protein quality and balanced essential amino acids would be of significant for consumers who are vegetarians or those who are allergic to dairy and meat products.

Legumes, including soybeans, when incorporated into starch based food products such as bread, tortillas (flour/corn), pasta, wafer and pretzels are able to improve the nutritional profiles. Recently studies on the effect of added legumes into food products have covered various aspects, including physical, chemical, nutritional, textural and sensory properties. However, very limited literature could be found on addition of legumes into pizza crust. This is probably due to the nature of pizza, which is commonly consumed with nutritious toppings.

Therefore, the purpose of current study was to investigate some physical properties of wheat based pizza crust fortified with 15% and 35% soy. Characteristics of final product examined included firmness, cohesiveness and colour.

6.3. Material and Methods

6.3.1. General

Soy presscake, a by-product of cold press soy oil production, was purchased from Pristine Gourmet (Waterford, On, Canada). Soy presscake was sieved to pass a 590 μm sieve (30 mesh US standard Sieve Series) in the Department of Food Science when received, packaged in Ziploc bag and stored at 4°C. Original wheat flour (Robin Hood, Smucker Foods of Canada Corp., Markham, ON), defatted soy flour (Bulk Barn, Winnipeg, MB, Canada), salt (Sifto, Mississauga, ON), Sugar (Rogers Sugar, Vancouver, BC), yeast (Fleischmann's, Associated British Foods, London, UK), Canola Oil (No Name, Loblaw Companies Limited, Toronto, ON), and vinegar (Heinz, Pittsburgh, PA, USA) were purchased from local grocery stores.

6.3.2. Pizza Crust Formulation and Preparation

The formulation of soy fortified pizza dough is shown in Table 6.1. SP pizza dough was made using the following method. Yeast and sugar were added into 120 g of warm water (37°C to 40°C), and set for 8 min for the yeast to activate. All dry ingredients were stirred for 1 min using a Standard Mixer at a low speed (1) (Professional 600, Kitchenaid. Michigan, USA) with a flat beater. Activated yeast, oil, and vinegar were added into mixed dry ingredients, and mixed for 4 min at speed 2. The mixed dough was allowed to rise for 1 h at room temperature while covered with plastic wrap. The dough was then separated into 150 g samples, and pressed on a hot press (Doughpro, Stearns Product Development Corporation, CA, USA) previously heated up to 150 °F (top platen) for 20 s at maximum thickness setting. A dough docker was used to poke holes in the dough before baking. The pizza dough was baked at 425°C for 6 min in a Moffat convection oven (Model ECO-3, Deltarex Canada Inc., Toronto, ON), and cooled for 2 h prior to further analysis.

Table 6.1.The Formulation of Soy Fortified Pizza Dough

	Flours (g)		Oil (g)	Sugar (g)	Salt (g)	Vinegar (g)	Yeast (g)	Water (g)
	Wheat	Soy	5%	2.5%	1.5%	0.5%	1.5%	
Control	200	0	10	5	3	1	3	120
15% SP	170	30	10	5	3	1	3	120
35% SP	130	70	10	5	3	1	3	120
15% SF	170	30	10	5	3	1	3	120
35% SF	130	70	10	5	3	1	3	120

SP: Soy Presscake; SF: Soy Flour

6.3.3 Physical Properties

Pizza crust firmness and cohesiveness was determined using a puncture test with a TA.XT 2 Plus Texture Analyzer (Texture Technologies Crop., Scarsdale, NY & Stable Micro Systems, Godalming, Surrey, UK), and a ½ inch ball probe. The pizza crust was placed topside down to reduce the variations caused by uneven crust, and firmness was measured as the peak force at 50% strain. Cohesiveness was calculated as the work (area under the curve) during compression. For each pizza dough, four measurements were taken for both edge and middle for each tortilla formulation.

Colour measurements were performed using a Minolta CM-3500 model spectrophotometer. CIE $L^*a^*b^*$ was used, in which L^* stands for lightness, a^* (+) for redness, a^*(-) for greenness, b^*(+) for yellowness, and b^*(-) for blueness. Samples were cut from the center of the pizza dough for colour measurement, and the top crust, bottom crust and center were measured for colour separately. Three measurements were performed for each occasion for each tortilla formulation.

6.4. Results and Discussion

6.4.1. Effect of Added Soy Presscake and Soy Flour on Some Physical Properties of Wheat Pizza Crust

The effect of added soy presscake and soy flour on pizza crust firmness and cohesiveness are shown in Table 6.2. Addition of soy ingredient affected firmness and cohesiveness of pizza crust. Firmness and cohesiveness of pizza crust was significantly higher at a substitution level of 35% SP in the center. Pizza crust with 15%

SP substitution showed no significant differences in cohesiveness on either the edge or the center compared to the wheat control, but the firmness of 15% SP substituted pizza crust was significantly higher than the wheat control at the center. Thus, when 35% SP was included in the pizza crust, significantly increased firmness and cohesiveness could only be found when the measurements were taken in the center. Pizza dough made with 15% and 35% SF showed significantly increased firmness and cohesiveness on both edge and center, compared to wheat control. Pizza crust made with SF showed higher firmness and cohesiveness compared to those made with SP at the same fortification level. The lower fat content in SF compared to SP could contribute to the higher firmness and cohesiveness for pizza dough made with SF. The increase in firmness and cohesiveness could be caused by the reduction of gluten network, which could result in a denser and more compact structure (Duodu & Minnaar, 2011). The results are in agreement with those of Brewer et al. (1992), who reported that inclusion of soy protein increased the force needed to compress. Urade et al. (2003) reported an increased force value at failure point when SPI was added into wheat dough. In addition, Urade et al. (2003) also reported an increase in water absorption in wheat dough as assessed by Farinograph due to the water holding capacity of soy protein. The increase in water absorption could decrease the amount of free water in dough, which could further increase the firmness and cohesiveness of the baked pizza dough. Though the proximate analysis was not carried out for pizza crust samples, we could still expect that pizza crust made with SP to have the highest fat content (fat content of soy presscake: 14.5%; Soy flour: 1.2%; Wheat flour: 1.1%, all on dry basis (USDA, 2012)), which would support the loss of gluten network while producing a pizza crust for which the texture was closer to wheat control than those made with SF.

Table 6.2. The Effect of Added Soy Presscake and Soy Flour on Firmness and Cohesiveness Soy Fortified Pizza Dough

Flour	%	Center		Edge	
		Firmness (g)	Cohesiveness (g/s)	Firmness (g)	Cohesiveness (g/s)
Wheat	100	338.2 ± 30.8d	1129.9 ± 99.6c	533.3 ± 15.3c	1732.6 ± 124.5c
SP	15	427.5 ± 35.1c	1330.4 ± 139.7c	554.9 ± 48.3c	1727.9 ± 131.8c
	35	594.0 ± 37.4b	1876.5 ± 97.1b	683.1 ± 81.1b,c	2268.5 ± 439.6b,c
SF	15	560.8 ± 37.4b	1680.6 ± 126.6b	832.5 ± 109.1b	2590.9 ± 426.0b
	35	921.1 ± 53.4a	2324.9 ± 177.8a	1282.0 ± 193.9a	3507.2 ± 541.7a

SP: Soy Presscake; SF: Soy Flour

Results listed are Mean ± Standard Deviation of four measurements

Values followed by the same letter in the same column are not significantly different (p>0.05).

Table 6.3. The Effect of Added Soy Presscake and Soy Flour on Pizza Dough Colour

Flour	%	Top			Bottom			Middle		
		*L**	*a**	*b**	*L**	*a**	*b**	*L**	*a**	*b**
Wheat	100	76.5 ± 0.5a	3.72 ± 0.35c	23.8 ± 0.3d	78.7 ± 0.3a	-0.08 ± 0.01b	18.8 ± 0.1d	78.3 ± 0.5a	-0.50 ± 0.07c	14.4 ± 0.2d
SP	15	70.5 ± 0.1b	9.01 ± 0.06b	32.3 ± 0.3c	74.2 ± 0.2a	0.11 ± 0.11b	24.5 ± 0.4c	72.6 ± 0.7c	-1.06 ± 0.04d	18.6 ± 0.3c
	35	54.4 ± 0.8d	18.31 ± 0.39a	35.6 ± 0.2b	68.8 ± 3.5b	4.33 ± 4.35b	31.1 ± 4.1b	72.8 ± 1.0c	-1.39 ± 0.06e	22.0 ± 0.5a
SF	15	70.1 ± 0.8b	9.02 ± 1.45b	30.8 ± 1.4c	75.1 ± 0.5a	2.20 ± 0.15b	26.7 ± 0.4b,c	74.9 ± 0.3b	-0.16 ± 0.09b	20.1 ± 0.5b
	35	58.2 ± 1.3c	17.06 ± 0.73a	38.1 ± 0.1a	58.8 ± 2.4c	16.32 ± 2.73a	39.4 ± 0.7a	71.1 ± 0.5c	0.54 ± 0.10a	22.5 ± 0.3a

SP: Soy Presscake; SF: Soy Flour

Results listed are Mean ± Standard Deviation of three measurements

Values followed by the same superscript letter in the same column are not significantly different (p>0.05)

The effect of added soy presscake and soy flour on pizza dough colour is presented in Table 6.3. The addition of SP and SF resulted in significantly decreased lightness (L*), and increased redness (a*) and yellowness (b*) on top crust and middle of the pizza crust compared to wheat control. No significant differences could be found in the lightness (L*) and redness (a*) of SP and SF tortillas at 15% fortification level on the bottom crust. The lightness (L*) of the pizza crust is most likely being affected by the yellow colour of SP and SF, and the redness (a*) is most likely caused by the Maillard reaction occurred during baking. Therefore, when only 15% of SP or SF was added into the pizza crust, the effect on colour and lysine content would not be critical to further affect the lightness (L*) and redness (a*) of the pizza crust, especially when there is no direct exposure to heat for the bottom crust. Decreased lightness, and increased redness and yellowness were also noted with the increase in SP and SF fortification level from 15 to 35%, except for the redness (a*) of the bottom crust with SP due to the high variance and lightness (L*) of middle with SP. Colour of top crust and bottom crust can also be affected by baking conditions. Therefore, the colour of the middle may be a better parameter to compare the effects of added SP and SF on pizza dough colour.

Significantly decreased lightness (L*), and increased yellowness (b*) of pizza crust fortified with SP and SF at both 15 and 35% levels could be found in the middle in comparison to the control. However, no significant differences could be found in lightness (L*) between pizza crust fortified with 15 and 35% SP and 35% SF, whereas the pizza crust with 15% SF was significantly lighter than the other soy fortified pizza crusts. The yellowness (b*) of pizza crust in the middle was significantly increased with the increasing of soy fortification levels from 15 to 35%; however, the yellowness (b*) of pizza crust fortified with 35% SP and SF did not show any significantly difference in the middle. Redness/greenness (a*) values for the fortified tortillas were also significantly different from the control, but the value with SP were lower than the control (more green) and the values for SF were higher (more red). This trend was more evident at higher levels of substitution. The trend towards green for the SP tortillas may be a result of the higher oil level, whereas pigmentation of the SF flour may have been responsible for the redder tortillas.

Comparing the colour of pizza crust made with SF to those made with SP, the pizza crust made with SF tended to be lighter, more red and yellow, even though SP was more red and yellow than SF (Table 4.4). SF, however, was much lighter than SP (Table 4.4). The tendency of being more red and yellow when SF was used might be due to the higher protein content of SF (Table 3.2) that resulted in more intense Maillard reaction. The changes of colour in comparison to the wheat control were in agreement with our previous study on the effect of added SP and SF on corn tortilla colour, as well as Doxastakis et al. (2002), who reported that the crust colour was darker and crumb colour was more yellow when lupin and soy was added into breads. Brewer et al. (1992) also reported that muffins containing SPI had a darker, redder and less yellow colour compared to wheat bread. The changes in colour parameters, particularly on the outside of the soy fortified pizza crust could, in fact, be related to more intense Maillard reaction due to the relatively higher levels of lysine (Duodu & Minnaar, 2011).

6.5. Conclusions

The addition of SP and SF into wheat pizza crust caused significant changes in pizza dough texture and colour. Compared to the wheat control, pizza dough made with 15% and 35% SF had significantly increased firmness and cohesiveness on both the edge and center, whereas significant increases in firmness and cohesiveness for pizza crust fortified with 15% and 35% SP could only be found when measurements were taken in the center. Overall firmness and cohesiveness of pizza dough severally increased with the increasing levels of soy fortification, and pizza dough made with SF showed higher firmness and cohesiveness than those made with SP at the same fortification levels. The addition of SP and SF resulted in decreased lightness (L^*), and increased yellowness (b^*) on top crust and center of pizza crust. The changes in redness/greenness were the opposite for the SP and SF samples. Differences were not always the same on the bottom crust, presumably because of the higher degree of cooking on the bottom. Decreased lightness, and increased redness and yellowness were generally associated with increased SP and SF fortification. Therefore, soy fortified pizza dough showed significantly changed texture and colour, compared to a wheat control. Further research on nutritional profiles and sensory properties are required to better evaluate the perspective and potential of this product.

7. GENERAL DISCUSSION

The nutritional, physical and sensorial properties of corn tortillas fortified with SP and SF have been studied. The addition of SP and SF into corn tortillas caused important, yet expected, changes in tortilla composition and the contents of selected anti-nutritional factors. Protein content in tortillas containing 10% SP or SF was increased by 44% and 51%, respectively, compared to the control, and was increased by 170% at fortification levels of 40 and 35% for SP and SF. It was observed that tortillas fortified with SF (10 – 35%) and SP (25, 35 & 40%) had significantly higher phytic acid contents, compared to control tortillas. Since phytic acid is known to be associated with protein bodies (Jaffe, 1981; Liu, 1997), the increased phytic acid levels was explained to be linked to the higher protein contents in soy fortified tortillas. The trypsin inhibitor activity (TIA) had the same trend as phytic acid contents, such that trypsin inhibitor activity increased with increased levels of soy substitution.

In order to evaluate the effect of tortillas preparation, phytic acid levels and TIA in flour composite were calculated based on tortilla formulations in Chapter 3. The calculated phytic acid levels in flour composite were only slightly higher or lower than measured values in tortillas, due to the thermal stability of phytic acid. However, the TIA in tortillas were reduced by 37 to 55%, compared with the calculated TIA values in raw flour composites. This could be due to the fact that trypsin inhibitors are generally thermo-labile, and can be eliminated or inactivated through different methods.

Moisture contents of all other tortillas were between 42 – 53%, close to the moisture content of common corn tortillas (44 – 46%), with the highest level for the control corn tortilla (100% dry masa). The water absorption of dry corn masa flour and soy composite flour can be determined by calculating the percentage of water addition and the percentage of moisture content for soy fortified tortillas compared to the corn control. Based on the tortilla formulations, the percent water addition of 10, 20, 25, 30, 35 and 40% soy fortified corn tortillas compared to the water addition of control corn tortilla were 90.4, 80.8, 76.0, 71.4, 66.5 and 61.7%, respectively. However, the moisture contents of soy fortified tortillas compared to the moisture content of control corn tortillas, described as percentage, were 93.9, 90.0, 86.8, 85.2, 82.9, and 79.9% for 10 – 40% SP fortified tortillas, and 95.5, 90.0, 89.0, 85.9, and 85.4% for 10 – 35% SF fortified tortillas. Therefore, we can conclude that soy composite flour had better water retention and was probably due to better water absorption by soy proteins. The difference in moisture content could also be associated with the size and thickness of the tortillas, which were discussed in Chapter 4. The diameters of raw and cooked tortillas decreased slightly accompanied by increased thickness when the levels of soy substitution increased. The larger and thinner of the tortillas would result in more moisture loss during cooking. However, the size and thickness of tortillas were also affected by different amount of water addition when preparing the dough as discussed in Chapter 4.

The most important texture properties of soy fortified corn tortillas including firmness and cohesiveness, was measured instrumentally. The firmness and cohesiveness increased significantly compared with corn control tortillas, except for 10% SP, which showed no significant differences. This was attributed to the addition of less water and therefore lower moisture content of final products. In addition, firmness and cohesiveness of SF and SP tortillas increased with increasing levels of soy substitution. This could be due to increased thickness of tortillas at higher levels of fortification, as well as the increased protein content and protein-protein interactions when soy was incorporated (Brewer et al., 1992). Control corn tortillas, as well as tortillas made with 25 and 35% SP were observed to have the best rollability, followed by tortillas made with 10, 20, 30 and 40% SP, and tortillas made with SF showed the worst rollability. This is probably due to the high starch content in control corn tortillas compared with tortillas made with SP and SF, and higher fat contents in tortillas made with SP.

A darker colour was observed for tortillas made with SP in comparison to corn control tortillas, while tortillas fortified with SF were lighter. Increased redness (*a**) and yellowness (*b**) could also be found for tortillas fortified with soy. The differences in lightness (*L**) were mostly like affected by the colour of raw flours, where soy presscake was darker than dry masa flour, and soy flour was the lightest. The redness (*a**) and yellowness (*b**) could be associated with raw flour colour as well as a more intense Maillard reaction due to higher lysine content of soy fortified tortillas.

In the consumer acceptance test, 40% SP and 35% SF had significantly higher overall acceptability scores than the commercial corn tortillas for total population (76). In addition, overall flavour and texture of both soy fortified corn tortillas had higher scores than commercial corn tortillas. More importantly, 59 out of 76 panelists stated that they would like to purchase tortillas with 40% SP substitution. It has to be kept in mind that, the commercial corn tortillas also contained preservatives to extend its shelf-life, which may have influenced the scores, while soy fortified tortillas were freshly made with only basic ingredients.

In Chapter 5, a decrease in the *in vitro* starch hydrolysis rate was found for corn tortillas containing SP. The GI values determined by *in vivo* method appeared to decrease with the increasing levels of SP fortification; however, the decreases in GI were not significant due to the high variances. The decrease in *in vitro* starch hydrolysis rate could be due to the nature of legume starch as it generally contains higher amylose content than starches in cereal and other foods (Thorne & Jenkins, 1983; Guillon & Champ, 2002). Other than the nature of the legume starch, protein-starch interactions, anti-nutrients, physical forms of food product, as well as cooking methods could also contribute to the decreased GI values. However, it is a challenge to explain the complex behaviour leading to the decrease in GI value, as well as the high variances seed in the GI evaluation. The physical condition of the 10 participants including their unique insulin responses towards soy, how strict did they follow the diet restriction during the whole period of 6 visits, as well as fasting time were all the factors that could affect the GI results, and were also beyond our control. However, a good correlation (r = 0.9920) was still found between the GI values from *in vivo* analysis and the AUC of *in vitro* starch hydrolysis. The best correlation (r = 0.9998) between DH and GI was found at 60 min of *in vitro* starch hydrolysis. Thus, *in vitro* starch hydrolysis could be used to predict and estimate GI of food products. A reference food of white bread could also be included in the *in vitro* starch hydrolysis. The DH of a reference food could be used to calculate the hydrolysis index (HI), which may be a better parameter to use to compare with GI values (Goni et al., 1997). However, in that case, the reference food used in the *in vivo* test should also be white bread instead of oral glucose solution, to be comparable with *in vitro* starch hydrolysis.

Soybean presscake (SP) and defatted soy flour (SF) was added into wheat based pizza crust at 15% and 35% levels. The texture including firmness and cohesiveness, and colour were measured instrumentally, and results were discussed in

Chapter 6. Compared to wheat control, pizza crust made with 15% and 35% SF had significantly increased firmness and cohesiveness on both the edge and center, whereas significant increases in firmness and cohesiveness for pizza crust fortified with 15% and 35% SP could only be found when measurements were taken in the center. Overall firmness and cohesiveness of pizza dough increased with the increasing levels of soy fortification, and pizza dough made with SF showed higher firmness and cohesiveness than those made with SP at the same fortification levels. The increase in firmness and cohesiveness for SF could be caused by lower fat content, as well as the reduction of gluten network, which could result in a denser and more compact structure (Duodu & Minnaar, 2011). The addition of soy presscake and defatted soy flour in wheat-based pizza crust had important, yet expected effect on the colour of top and bottom crust, as well as the center, as discussed in Chapter 6. The factors that would affect the colour of soy fortified pizza crust are mainly the colour of the raw flour, the different degree of Maillard reaction due to higher lysine content of soy, as well as the baking condition (top crust were darker than bottom).

8. CONCLUSION AND FUTURE RESEARCH OPPORTUNITIES

8.1. Conclusions

Soybean presscake (SP) and defatted soy flour (SF) were able to be successfully incorporated into corn-based tortillas, as well as wheat-based pizza crust at relatively high levels. The fortification levels were 10 to 40% of SP, and 10 to 35% of SF for corn-based tortillas, and 15 and 35% of SP or SF for wheat-based pizza crust.

The effect of added soybean presscake and defatted soy flour on some nutritional and physical properties was more significant at the highest fortification levels (40% SP & 35% SF). Protein content was increased by 170% at fortification levels of 40 and 35% for SP and SF. The anti-nutritional factors in tortillas with 40% SP and 35% were significantly higher than the corn control, however, still considered to be safe for human consumption. In terms of size and thickness, tortillas made with 40% SP and 35% SF were significantly smaller and thicker than the corn control. Significantly lighter colour was observed for tortilla with 40% SP, but not 35% SF. However, both 40% SP and 35% SF tortillas were significantly redder and yellower than the control. The rollability of tortillas is a very important texture parameter, since most of the tortillas were consumed as a “taco”. Tortillas made with 40% SP had similar rollability compared to corn control, whereas, tortillas made with 35% SF had the worst rollability, and was almost unrollable. The firmness and cohesiveness of 40% SP and 35% SF tortillas were significantly increased, in comparison to corn control. In addition, tortillas fortified with SP showed decreased *in vitro* starch hydrolysis rate.

Further sensory studies and clinical trials were also carried out in order to evaluate the acceptability of tortillas fortified with soy at high levels (40% SP & 35%

SF), as well as to estimate the glycemic index of tortillas fortified with SP at levels of 10, 25 and 40% SP. The consumer acceptance test was conducted using 80 untrained panelists (4 of them did not complete the ballot sheets). 40% SP and 35% SF had significantly higher overall acceptability scores than the commercial corn tortillas for total population (n=76). In addition, overall flavour and texture of both soy fortified corn tortillas had significantly higher scores than commercial corn tortillas. Thus, the results appeared to indicate the changes in physical properties were acceptable by consumers. The clinical trial also showed decreased glycemic index values of tortillas fortified with SP, though the changes were not significant due to the high variances. A good correlation (r = 0.9920) was found between the GI values from *in vivo* analysis and the AUC of *in vitro* starch hydrolysis, which showed the possibility of using *in vitro* starch hydrolysis to predict and estimate the GI values of food products.

In terms of the texture of soy fortified pizza crust, the firmness and cohesiveness were increased significantly in the center for at the fortification level of 35% SP and SF. The addition of SP and SF also resulted in decreased lightness (L^*), and increased redness (a^*) and yellowness (b^*) on top crust, bottom crust and middle of the pizza crust.

In conclusion, incorporating soybean presscake and defatted soy flour into corn-based tortillas at high levels could improve the nutritional properties of the final product, however compromise the texture properties, but were still acceptable by consumers. Further research on nutritional profiles and sensory properties are required for soy fortified pizza crust to better evaluate the perspective and potential of this product.

8.2. Future Research Properties

From this study, the perspective and potential of incorporating soy ingredients, especially soybean presscake, into traditional starch based bakery food products can be seen. However, further research is still required in order to optimize the food products, and maximize the potential of using soybean presscake as a food ingredient. Dough properties should be further studied to optimize and standardize the amount of different ingredients, in order to produce bakery products with more preferable texture. The effect of particle sizes of soybean presscake on chemical and physical properties can also be studied. The amino acid profile should be further analyzed in order to evaluate the amino acid balance of soy fortified bakery products. It would also be beneficial to determine the shelf-life of the final products, as well as the effect of addition of hydrocolloids on improvement of texture properties, especially rollability. In addition, further research on nutritional profiles and sensory properties are required for soy fortified pizza crust to better evaluate the perspective and potential of this product.

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10. APPENDICES

Appendix 1.

Specification sheet of Ticaxan Xanthan Powder

Distributed by:

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ingredients for your success!™

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TIC GUMS — We're your Gum Guru™

Ticaxan® Xanthan Powder — PRODUCTDATA

Ticaxan® Xanthan Powder is a high molecular weight glucomannan gum which is derived from Xanthomonas campestris by a pure-culture fermentation process. Ticaxan Xanthan's unique physical properties are pseudoplasticity, heat and pH stability, and high viscosity. It is used by the food industry as an all-purpose stabilizer, thickener and processing aid.

Typical Usage Level	0.05% to 0.35%			
Solubility	Cold Water Soluble			
Suggested Uses	Xanthan Gum, suspension, stabilization, relish, salad dressings, sauces, pulp suspension in beverages			
Label Declaration	Xanthan Gum			
Country of Origin	Product of Austria and/or France and/or USA			
CFR #	21 CFR 172.695	Kosher Ⓤ	Y	(Y/N)
CAS #	11138-66-2	Kosher for Passover Ⓤ	N	(Y/N)
EU #	415			
HS Tarrif #	3913.90.2000			
Minimum Qty				
Standard Packing	55# cartons, 1,650# per pallet	All Natural	Y*	(Y/N)
Lead Time	Stock Product	Shelf-Life	3 years	
Storage & Handling	Each container is identified with the product name and lot number. Store in cool dry place for maximum shelf life.			

*A finished product derived from naturally occurring raw materials that were processed without adding or removing functional groups to the native structure

NUTRITIONAL INFORMATION

Calories (Total)	324 Kcal	Sodium	3180 mg	Insoluble Dietary Fiber	2.0 g
%Calories from Fat	0.00 %	Potassium	363 mg	Simple Carbohydrates	0 g
Calories from fat	0.00 Kcal	Calcium	28 mg	Complex Carbohydrates	0 g
Total Fat	0.00 g	Total Carbohydrates	78.00 g	Protein	5 g
Trans Fat	0.00 g	Soluble Dietary Fiber	76 g	Vitamins, Other Minerals	*ND
Cholesterol	0 mg				

(per 100 grams). This data is from analysis and calculation and should be considered "typical" and not a specification. Data is reported on an "as is" basis. Total fat and protein values are rounded to the nearest whole number.
* Calculated based on typical assay of component(s)
* N.D.; Not determined
**Total Calories are calculated in compliance with FDA Regulations requiring the inclusion of 4 Kcal/g for all soluble dietary fiber. However dietary fiber by definition consists of plant material that is resistant to hydrolysis by endogenous enzymes of the mammalian digestive tract. TIC GUMS has participated in discussions with the FDA as part of the Calorie Control Council to amend the FDA regulations to 2Kcal/g.
If all nutritional information is listed as "0" then these findings have yet to be evaluated.

SPECIFICATIONS

Bacteriological	Minimum	Maximum	
Aerobic Plate Count (Typical)	< 2000 cfu/g		-
E. coli (Typical)	Negative		-
S. aureus (BAM Typical)	Negative /10g		-
Salmonella (Typical)	Negative		-
Total Coliforms (Typical)	< 30 /g		-
Yeast and Mold (Typical)	< 200/g		-
Mesh	**Minimum**	**Maximum**	
USS#60 Mesh Through	90	100	%
USS#80 Mesh Through	85	100	%
Physical and Chemical	**Minimum**	**Maximum**	
Flavor (Typical)	Bland		-
Moisture (Infrared)	0	15	%
Odor (Typical)	Characteristic		-
pH (viscosity solution)	5.8	8.2	pH
Powder Color (Visual)	Off White		-
Texture (Qualitative)	Free Flowing Powder		-
Viscosity (1.0%,KCL,LV@60rpm,25C)	1200	1800	cps

This product, or ingredients used to make this product, has/have been demonstrated to conform with current Food Chemical Codex requirements

Version: 20120128.0005 TICA XANTHAN

Nealanders International Inc. (Corporate Headquarters)
6980 Creditview Rd
Mississauga, ON L5N 8E2
Tel: 905-812-7300
Fax: 905-812-7308

Nealanders International Inc.
1870 Boulevard St-Regis
Dorval, PQ H9P 1H6
Tel: 514-684-2120
Fax: 514-684-7424

Nealanders International Inc.
#201-7950 Huston Rd
Delta, BC V4G 1C2
Tel: 604-940-4181
Fax: 604-940-4180

Nealanders International Inc.
2425 Alft Lane
Elgin, IL 60124
Tel: 847-468-0001
Fax: 847-488-0007

Toll Free 1.800.263.1939
www.nealanders.com

Appendix 2a.

Approval Certificate of Consumer Acceptance Test

UNIVERSITY OF MANITOBA | Research Ethics and Compliance
Office of the Vice-President (Research and International)

Human Ethics
208-194 Dafoe Road
Winnipeg, MB
Canada R3T 2N2
Phone +204-474-7122
Fax +204-269-7173

APPROVAL CERTIFICATE

August 1, 2013

Manitoba Pulse Growers Association
39190

TO: **Mingjue Wu** (Advisor S. Arntfield)
Principal Investigator

FROM: **Susan Frohlick, Chair** [redacted]
Joint-Faculty Research Ethics Board (JFREB)

Re: **Protocol #J2013:095**
"Consumer acceptability of corn tortilla fortified with soy"

Please be advised that your above-referenced protocol has received human ethics approval by the **Joint-Faculty Research Ethics Board**, which is organized and operates according to the Tri-Council Policy Statement (2). **This approval is valid for one year only**.

Any significant changes of the protocol and/or informed consent form should be reported to the Human Ethics Secretariat in advance of implementation of such changes.

Please note:

- **If you have funds pending human ethics approval, please mail/e-mail/fax (261-0325) a copy of this Approval (identifying the related UM Project Number) to the Research Grants Officer in ORS in order to initiate fund setup. (How to find your UM Project Number: http://umanitoba.ca/research/ors/mrt-faq.html#pr0)**

- **if you have received multi-year funding for this research, responsibility lies with you to apply for and obtain Renewal Approval at the expiry of the initial one-year approval; otherwise the account will be locked.**

The Research Quality Management Office may request to review research documentation from this project to demonstrate compliance with this approved protocol and the University of Manitoba *Ethics of Research Involving Humans*.

The Research Ethics Board requests a final report for your study (available at: http://umanitoba.ca/research/orec/ethics/human_ethics_REB_forms_guidelines.html) **in order to be in compliance with Tri-Council Guidelines.**

umanitoba.ca/research

Appendix 2b.

Recruitment Letter for Consumer Acceptance Test

* Printed on Food Science letterhead

Recruitment Letter
The Department of Food Science

Oct 22, 2013

Dear Colleague,

We are recruiting volunteers to participate in a research study on the acceptability of corn tortillas which contain soy press cake or soy flour. You would have the opportunity to learn a research method for collecting data regarding consumer acceptability. The criterions are that you must be at least 18 years old, must be familiar with corn tortilla and consume them at least 3 times a year, and must have no allergies to soy and corn ingredients. This letter explains what your commitment would be. If you have any questions please call, Mingjue (Shirley) at XXX or e-mail XXX

Participants will be required to observe and taste samples and respond regarding how much they like/dislike the appearance, flavor, texture, overall acceptability as well as whether they would purchase the product. Other details regarding the commitment are provided in the attached consent form. A snack and drink will be offered following the participation.

Approximately 80 to 120 panelists will take part in the study. The one time session will last for approximately 15-20 minutes. Sessions will take place in the Food Science (Ellis) Building in room 221(Sensory Panel) at the University of Manitoba (Fort Garry Campus). Participants need to choose the date and time that is most convenient for you to attend the evaluation. Options for signing up for sessions are as follows:

Week 1 – November 5, 6, 7, 8 (Tuesday, Wednesday, Thursday, Friday)
Week 2 – November 12, 13, 14, 15 (Tuesday, Wednesday, Thursday, Friday)
Week 3 – November 19, 20, 21, 22 (Tuesday, Wednesday, Thursday, Friday)
Week 4 – November 26, 27, 28, 29 (Tuesday, Wednesday, Thursday, Friday)

Times to select from on all of the days are: 10:00, 10:30, 11:00, 11:30, 12:00, 12:30, 1:00, 1:30, 2:00 and 2:30

Allergy to one of the food ingredients may pose a risk to individuals involved in the study. A questionnaire regarding allergies completed by those who interested in participation in the study will screen and confirm for this potential risk. Information regarding the project objectives as well as results will be sent to participants within a month of the data collection.

If you are interested in participating in this research, notify me at XXX or email XXX to schedule date and time for the evaluation session. Please read and fill out the required consent form and the questionnaire attached to this letter and return it to me by XXX to confirm attendance.

We hope that you will be able to take part in this research and look forward to hearing from you. Alternatively, if you know of anyone else that might be interested in participating, we would appreciate it if you could forward this information to him or her.

Thank you for your time and assistance with this project.

Sincerely,

Mingjue (Shirley) Wu, Research Coordinator,
University of Manitoba
Department of Food Science
M. Sc. Student

Appendix 2c.

Recruitment Poster for Consumer Acceptance Test

Like Tortillas??

Great Tasting Opportunity!!

The Department of Food Science at the University of Manitoba is developing food products made partially with soy. A sensory analysis is being conducted to determine the consumer acceptability of corn tortillas, which include soybean press cake and defatted soy flour.

The study is open to people 18 years and older who are familiar with corn tortillas.

Commitment required for a one time session of approximately 15~20 minutes.
No experience is required.

The sensory test will be conducted in Room 221 Ellis building at the University of Manitoba starting on November 5th

Volunteers will be compensated for their participation

Please contact Mingjue (Shirley) Wu (principle investigator) at XXX for details.

Appendix 2d.

Questionnaire for Consumer Acceptance Test

Questionnaire

Consumer Acceptability of Soy Fortified Corn Tortillas

This information will be kept strictly confidential, and will only be viewed by the principal researcher and the supervisory professor.

1. Are you allergic to any food products? Yes______No ___ Unknown____

If yes, note them below.

____

____

2. Have you participated on sensory evaluation panels before? Yes_______ No___

(a) If yes, what product(s) did you evaluate?

____

____

(b)Was training part of the evaluation procedure? Yes________No________

If yes, indicate for which product(s).

____

____

3. Are there any foods specifically, or food flavors and textures generally, that you would prefer not to evaluate?

____

____

Thank you very much for completing this questionnaire!

Appendix 2e.

Consent Form for Consumer Acceptance Test

***Printed on Food Science Letterhead**

CONSENT FORM

Research Project Title: **Consumer Acceptability of Corn Tortilla Fortified with Soy**
Principal Investigator: Mingjue Wu, XXX
Research Supervisor: Susan Arntfield, XXX
Sponsor: Manitoba Pulse Growers Association

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 acceptability of corn tortilla made using a percentage of soy press cake (meal) and defatted soy flour. This research is being funded by the Manitoba Pulse Growers Association in order to increase the utilization and consumption of soybeans. **A potential risk would be allergic reactions to food products. Due to this risk, people with food allergies to corn and soy will not be allowed to participate. Completion by participants of the accompanying questionnaire will screen and confirm for this potential risk.**

The criterions necessary for each volunteer are that you must be at least 18 years old, must be familiar with corn tortilla and consume them at least 3 times a year, and must have no allergies to soy and corn ingredients. Participants will be requested to observe and taste 3 tortilla samples, and will be asked how much you like/dislike the appearance, flavor, texture and overall acceptability on a nine point scale, and whether you would like to purchase the products. The one time session will last for approximately 15-20 minutes.

You will receive a small snack and drink (pop or juice) following your participation as compensation. Information regarding the project will be sent to participants within a month of completion of the data collection. The study will take place in Room 221 (Sensory Panel) in the Ellis Building at the University of Manitoba.

All data will be recorded anonymously and therefore all participants will remain anonymous. Data published will be given as group means with no individual names given. All data collected relating to personal information and results obtained will be kept in a locked filling 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.

Your signature on this form indicates that you have understood to your satisfaction the information regarding participation in the research project and agree to participate as a subject. In no way does this waive your legal rights nor release researchers, sponsors, or involved institutions from their legal and professional responsibilities. You are free to withdraw from the study at any time, and/or refrain from answering any questions you prefer to omit, without prejudice or consequence. Your continued participation should be as informed as your initial consent, so you should feel free to ask for clarification or new information throughout your participation. This study is being conducted by Mingjue Wu (University of Manitoba Master's Student), cell phone: XXX, or email: XXX, under the supervision of Dr. Arntfield, telephone: XXX, or email: XXX.

The University of Manitoba may look at your research records to see that the research is being done in a safe and proper way.

This study 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 the above-named person or the Human Ethics Secretariat at 204-474-7122 or email XXX. A copy of this consent form will be given to you to keep for your records and reference.

____

Participant's Name (Please Print)

____

Participant's Signature Date

____________________ ________________________

Telephone Number E-mail Address

____

Researcher and/or Delegate's Signature Date

Appendix 2f.

Sensory Instruction for Consumer Acceptance Test

PLEASE READ BEFORE BEGINNING THE EVALUATION OF SAMPLES

Sensory Evaluation Instruction

The ingredients in the samples include:

Corn *masa* flour, soybean press cake (soybean meals), defatted soy flour, table salt, Xanthan gum and water

If you are allergic to any of the ingredients listed above, please stop the sensory evaluation immediately and leave the room quietly.

<u>If you develop any of the allergy symptoms within a few minutes to two hours after eating the sample foods, you should seek immediate medical attention!</u>

<u>The allergy symptoms could include:</u>

- <u>Tingling or itching in the mouth</u>
- <u>Hives, itching or eczema</u>
- <u>Swelling of the lips, face, tongue and throat, or other parts of the body</u>
- <u>Wheezing, nasal congestion or trouble breathing</u>
- <u>Abdominal pain diarrhea, nausea or vomiting</u>
- <u>Dizziness, lightheadedness or fainting</u>

If you would like to continue the session, please read this instruction carefully before beginning the evaluation

- Please cleanse your mouth with water provided before beginning you first sample, and in between samples
- Please evaluate each parameter
- If you do not like the sample, the expectoration cup is provided for you to expectorate the sample. Ingestion of samples is not mandatory
- When you are done, please leave quietly
- Don’t forget to take your treat and thank you for your participation!

Appendix 2g.

Ballot for Consumer Acceptance Test

Panelist No.________

Sensory Evaluation of Corn-based Tortilla

So that we know your familiarity with the commercial corn-based tortillas, please indicate how often you consume corn-cased tortillas

□ At least once a week
□ At least once a month
□ At least six times a year
□ At least three times a year
□ Other, please explain___________________________________

For the sample, according to the three digit code given_____ , please circle the number on the scale which best describes your opinion of the sample for each characteristic, in which 1 is dislike extremely and 9 is like extremely. Then indicate if you would purchase this sample at a comparable price, knowing that it contains additional nutritional benefits compared with the traditional corn tortillas.

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

On each of the scale: 1-Dislike extremely, 2-Dislike very much, 3-Dislike moderately, 4-Dislike slightly, 5-Neither like or dislike, 6-Like slightly, 7-Like moderately, 8-Like very much, 9-Like extremely.

Overall Appearance

1 2 3 4 5 6 7 8 9

Overall Flavor

1 2 3 4 5 6 7 8 9

Overall Texture

1 2 3 4 5 6 7 8 9

Overall Acceptability

1 2 3 4 5 6 7 8 9

Would you purchase this sample? □ Yes □ No

Appendix 3.

Final Report from Medallion Lab for Total Dietary Fiber and Resistant Oligosaccharides Analysis

MEDALLION LABS

9000 Plymouth Avenue North, Minneapolis MN 55427
1-800-245-5615 (763) 764-4453 Fax: (763) 764-4010

Final Report

Completion Date: July 16, 2013
Date Submitted: June 24, 2013
Medallion Company ID: U_MANITOBA03
Company Code: 15499

Susan Arntfield
University of Manitoba
Department of Food Science
250 Ellis Building
Winnipeg, MB R3T 2N2 Canada
Email: [redacted]

Library Number: 2013-MED-6561
PO Number: YL0893 - VISA
Fax:

Medallion Labs Sample ID: 2013-MED-6561-01 Soybean Press Cake
Customer Sample ID: SPC-F

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	06/24/13
² TDF and Resistant Oligosaccharides	Total Dietary Fiber	16.1 %	07/16/13
	Resistant Oligosaccharides	4.8 %	

Medallion Labs Sample ID: 2013-MED-6561-02 Soybean Press Cake
Customer Sample ID: SPC-S

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	06/24/13
² Total Starch	Total Starch	1.7 %	06/28/13

Medallion Labs Sample ID: 2013-MED-6561-03 MASA Corn Flour
Customer Sample ID: MCF-F

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	06/24/13
² TDF and Resistant Oligosaccharides	Total Dietary Fiber	6.0 %	07/16/13
	Resistant Oligosaccharides	0.6 %	

Medallion Labs Sample ID: 2013-MED-6561-04 MASA Corn Flour
Customer Sample ID: MCF-S

Assay Group	Test	Results	Test Date
Sample Handling Processing Level 1	Sample Process Fee	Sample Processed	06/24/13
² Total Starch	Total Starch	68.4 %	06/28/13

Results Approved By: Jagdish Gurav (Authorized Reviewer)

Method References:

Assay Group	Method Reference
TDF and Resistant Oligosaccharides	AOAC: 2001.03, 991.43*

* This method has been modified.

Medallion Labs maintains A2LA accreditation to ISO/IEC 17025 for the specific tests listed in A2LA Certificate # 2769.01.

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.

Unless otherwise noted above, samples were received in acceptable condition and analyzed as received.

Limits of Detection and Measurement Variability are available upon request.

² This test is not considered in-scope of our current A2LA accreditation. For a listing of in-scope tests, please visit www.medlabs.com.

Date Issued: July 16, 2013 Page 1 of 1

Appendix 4a.

Randomized Code for Sample Labeling Used in Clinical Trial

#1	3 147 control	1 593 Glc soln	4 745 10% soy	6 384 40% soy	5 286 25% soy	2 917 Glc soln
#2	6 442 40% soy	5 984 25% soy	2 248 Glc soln	4 257 10% soy	3 229 control	1 315 Glc soln
#3	4 712 10% soy	2 828 Glc soln	1 952 Glc soln	3 581 control	6 804 40% soy	5 790 25% soy
#4	5 175 25% soy	4 238 10% soy	3 487 control	1 143 Glc soln	2 572 Glc soln	6 832 40% soy
#5	5 705 25% soy	4 432 10% soy	6 122 40% soy	2 162 Glc soln	3 514 control	1 112 Glc soln
#6	1 191 Glc soln	3 318 control	6 465 40% soy	5 680 25% soy	2 271 Glc soln	4 607 10% soy
#7	2 743 Glc soln	1 225 Glc soln	3 818 control	4 437 10% soy	5 276 25% soy	6 555 40% soy
#8	1 353 Glc soln	3 858 control	5 236 25% soy	2 557 Glc soln	4 902 10% soy	6 340 40% soy
#9	2 243 Glc soln	4 953 10% soy	3 604 control	5 759 25% soy	6 769 40% soy	1 740 Glc soln
#10	1 826 Glc soln	2 569 Glc soln	6 449 40% soy	5 690 25% soy	4 201 10% soy	3 424 control

Appendix 4b.

Recruitment Poster for Clinical Trial

VOLUNTEERS NEEDED

We are looking for healthy individuals who are:

- Between the ages of 18 and 40;
- Are **not** taking any prescribed medications;
- Are **not** diagnosed with or being treated for a known disease;
- Not allergic to corn or soy products.

How do I get involved?

To be a part of this study, please call or email us at:

Phone: [redacted]
Email: [redacted]

You will receive a small honorarium for your participation.

What is the study about?

This study is investigating whether different levels of soy flour in corn **tortillas** affects the blood glucose response (or glycemic index).

Where?

Asper Clinical Research Institute at St. Boniface Hospital

What do I have to do?

- Attend a screening visit to determine eligibility.
- Those who are eligible will be scheduled for 6 study visits. Each study visit lasts 2½ to 3 hours.
- At each study visit, you will be asked to eat tortillas or drink a sugar solution. Blood samples will be collected before you consume the products and at various time points for 2 hours.

UNIVERSITY OF MANITOBA

Canadian Centre for Agri-Food Research in Health and Medicine
CCARM
Centre canadien de recherches agroalimentaires en santé et médecine

[redacted] Tortilla Study	[redacted] Tortilla Study	[redacted] Tortilla Study	[redacted] Tortilla Study	[redacted] Tortilla Study	[redacted] Tortilla Study	[redacted] Tortilla Study	[redacted] Tortilla Study	[redacted] Tortilla Study

Version 1.0 11-03-2013

Appendix 4c.

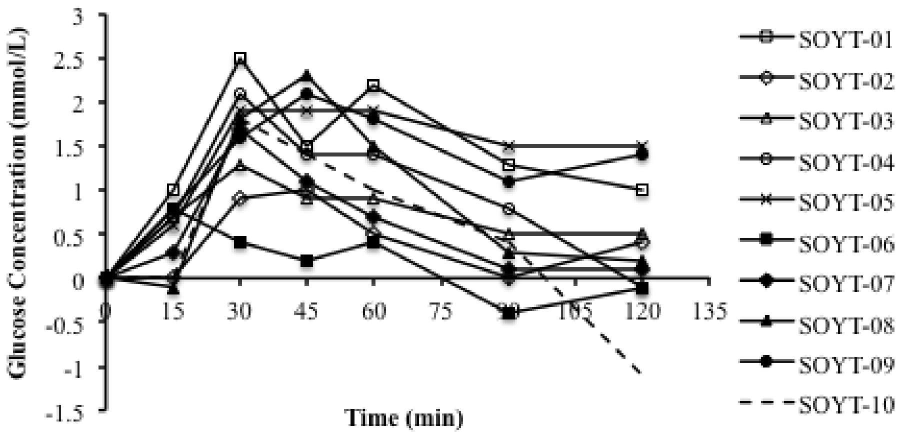
The Blood Glucose Concentration of Individual Participant After Intake of Oral Glucose Solution, Adjusted Based on their Fast Blood Glucose Level

Time (min)		0	15	30	45	60	90	120
SOYT-01	Glu_1	0	1.2	0.7	0.2	-0.3	0.6	0.5
	Glu_2	0	2.3	3.5	1.3	0.9	0.9	1.1
SOYT-02	Glu_1	0	2.9	4.7	5.1	4	3.1	0.5
	Glu_2	0	2.7	4.6	3.4	2.3	1.2	-0.8
SOYT-03	Glu_1	0	3.9	4.6	4.4	3.8	2	1
	Glu_2	0	2.7	4.3	4.3	3.7	2	1.3
SOYT-04	Glu_1	0	3.6	6.4	5.1	4.1	4.7	3.8
	Glu_2	0	2.6	4.5	6.1	5	2.8	1.1
SOYT-05	Glu_1	0	2.3	4	5.2	3.5	2.4	0
	Glu_2	0	1.2	4.7	6	3.2	2.3	1.7
SOYT-06	Glu_1	0	2.1	4.7	0.6	-0.4	1.2	2.7
	Glu_2	0	3.3	3.2	5.6	2.2	0	-2.1
SOYT-07	Glu_1	0	1.7	3.4	1.9	1.5	1.9	1.4
	Glu_2	0	2.3	2.1	1.8	3	1.8	2.1
SOYT-08	Glu_1	0	2.9	3.8	4	3.3	2.5	1.9
	Glu_2	0	1.7	4.5	6.4	4.8	4.3	1.7
SOYT-09	Glu_1	0	1.7	1.7	1.9	1.1	1.1	1.4
	Glu_2	0	0.5	1.8	-0.1	0.3	0.6	0.3
SOYT-10	Glu_1	0	2.1	3.3	2.3	1.8	1	1.1
	Glu_2	0	2.6	3.3	3.6	3.5	1.1	1.3

Appendix 4d.

The Blood Glucose Concentration of Individual Participant After Intake of Control Corn Tortillas, Adjusted Based on their Fast Blood Glucose Level

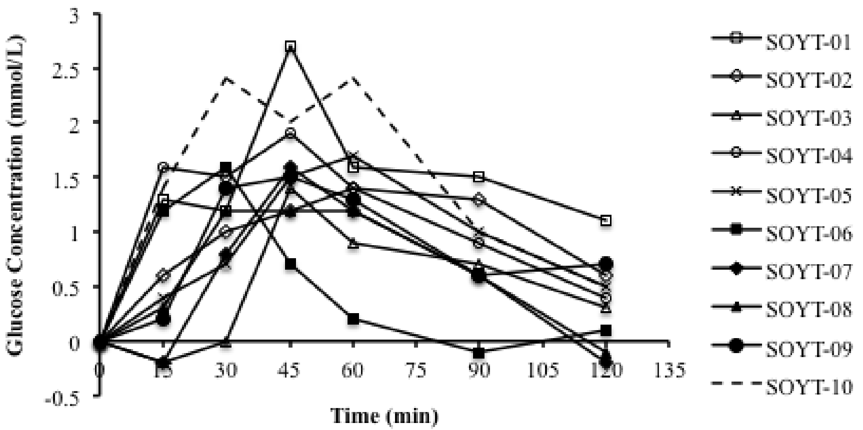
	Blood Glucose Concentration (mmol/L)									
Time (min)	SOYT-01	SOYT-02	SOYT-03	SOYT-04	SOYT-05	SOYT-06	SOYT-07	SOYT-08	SOYT-09	SOYT-10
0	0	0	0	0	0	0	0	0	0	0
15	1	0	0.7	0.7	0.6	0.8	0.3	-0.1	0.8	0
30	2.5	0.9	1.3	2.1	1.9	0.4	1.7	1.8	1.6	1.8
45	1.5	1	0.9	1.4	1.9	0.2	1.1	2.3	2.1	1.4
60	2.2	0.5	0.9	1.4	1.9	0.4	0.7	1.5	1.8	1
90	1.3	0	0.5	0.8	1.5	-0.4	0.1	0.3	1.1	0.4
120	1	0.4	0.5	-0.1	1.5	-0.1	0.1	0.2	1.4	-1.1



Appendix 4e.

The Blood Glucose Concentration of Individual Participant After Intake of 10% SP Corn Tortillas, Adjusted Based on their Fast Blood Glucose Level

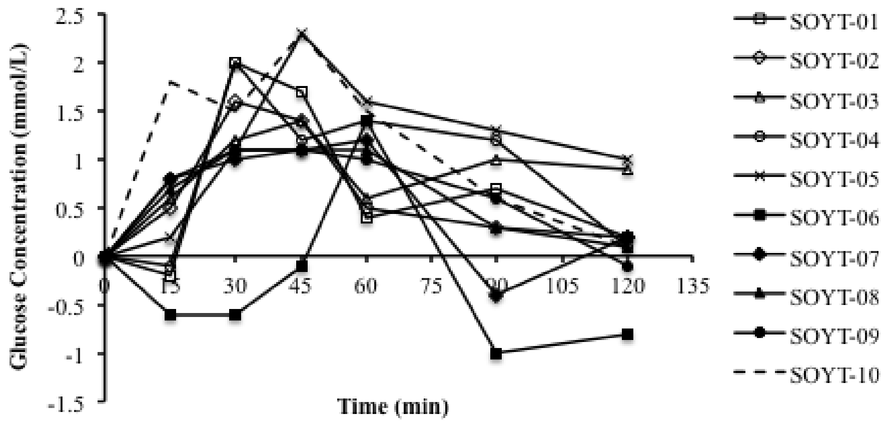
	Blood Glucose Concentration (mmol/L)									
Time (min)	SOYT-01	SOYT-02	SOYT-03	SOYT-04	SOYT-05	SOYT-06	SOYT-07	SOYT-08	SOYT-09	SOYT-10
0	0	0	0	0	0	0	0	0	0	0
15	1.3	0.6	-0.2	1.6	0.4	1.2	-0.2	0.3	0.2	1.4
30	1.2	1	0	1.5	0.7	1.6	0.8	1.2	1.4	2.4
45	2.7	1.2	1.4	1.9	1.5	0.7	1.6	1.2	1.5	2
60	1.6	1.4	0.9	1.4	1.7	0.2	1.2	1.2	1.3	2.4
90	1.5	1.3	0.7	0.9	1	-0.1	0.6	0.6	0.6	1
120	1.1	0.6	0.3	0.4	0.5	0.1	-0.2	-0.1	0.7	0.5



Appendix 4f.

The Blood Glucose Concentration of Individual Participant After Intake of 25% SP Corn Tortillas, Adjusted Based on their Fast Blood Glucose Level

	Blood Glucose Concentration (mmol/L)									
Time (min)	SOYT-01	SOYT-02	SOYT-03	SOYT-04	SOYT-05	SOYT-06	SOYT-07	SOYT-08	SOYT-09	SOYT-10
0	0	0	0	0	0	0	0	0	0	0
15	-0.2	0.5	0.6	-0.1	0.2	-0.6	0.8	0.7	0.8	1.8
30	2	1.6	1.2	2	1.1	-0.6	1	1.1	1.1	1.5
45	1.7	1.4	1.4	1.2	2.3	-0.1	1.1	1.1	1.1	2.3
60	0.4	0.5	0.6	1.4	1.6	1.4	1.2	1.1	1	1.5
90	0.7	0.3	1	1.2	1.3	-1	-0.4	0.3	0.6	0.6
120	0.2	0.2	0.9	0.1	1	-0.8	0.2	0.1	-0.1	0.1



Appendix 4g.

The Blood Glucose Concentration of Individual Participant After Intake of 40% SP Corn Tortillas, Adjusted Based on their Fast Blood Glucose Level

	Blood Glucose Concentration (mmol/L)									
Time	SOYT-01	SOYT-02	SOYT-03	SOYT-04	SOYT-05	SOYT-06	SOYT-07	SOYT-08	SOYT-09	SOYT-10
0	0	0	0	0	0	0	0	0	0	0
15	0.9	0.8	0.4	0.9	0.2	0.2	0.8	0.5	0.5	-0.4
30	0.5	0.9	0.7	2.3	0.8	0.8	1.9	1.8	1	0.8
45	1.2	2.5	0.7	2.5	0.5	1	1.9	0.9	0.4	2.2
60	1	1.1	-0.1	2.4	2.2	0.7	0.3	1.2	0.7	1.5
90	0.6	0.8	0.6	0.9	0.9	-0.1	-0.1	0.7	-0.2	0
120	0.4	0.5	0.5	1	-0.5	-0.6	-0.4	-0.3	0.5	-0.1