Soluble Fiber And Resistant Starch Components In Some Indian and Canadian Wheat Varieties And In A Wheat-Soy Product- *Chapati*

By

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Soluble Fiber And Resistant Starch Components In Some Indian and Canadian Wheat Varieties And In A Wheat-Soy Product- *Chapati*

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(Abstract)

This study aimed to quantify resistant starch (RS) beta-glucans (BG) and fructo-oligosaccharides (FOS) in Indian and Canadian wheat varieties and in *chapaties* made from these; and to assess the effects of soy flour on the levels of these components and its effects on the sensory and functional properties of the wheat-soy *chapaties*. Seven wheat varieties (Indian / Canadian) were milled into flour; supplemented with 0 % (control), 10 %, 20 % or 30 % defatted soy flour and made into *chapaties*. Flours and *chapaties* (freeze-dried, pulverized) were assayed for BG, FOS, RS and simple sugars (glucose / sucrose). Sensory evaluation was carried out by (9 point) hedonic rating of *chapaties* by 20 untrained Indian panelists. Flour water-holding capacity and water absorption indices (WAI) were determined. RS content of flours ranged from 7.1 g/100 g to 12.6 g/100g, but decreased when made into *chapaties*, (< 1 g/100 g), and decreased further with soy flour addition. BG content in flours ranged from 0.8 g/100 g to 1.4 g/100 g, while FOS content ranged from 1.3 g/100 g to 2.3 g/100 g. Minimal changes were observed in BG and FOS content when made into *chapaties*. Simple sugars were minimal in flours and *chapaties*. WAI of wheat flour was increased with addition of soy bean flour. Addition of up to 30 % soybean flour elevated the sensory acceptability of *chapaties*. While there is a decrease in RS with *chapati* making, the levels of BG and FOS are largely unchanged with processing.

DEDICATION

I would like to dedicate this thesis to my wonderful parents- my mother Mrs. Asha Vadnerkar and my father Mr. Anant Vadnerkar, for their unconditional, selfless love, support and understanding. Thank you for all your help.

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Contents

List of Appendices

LIST OF FIGURES

LIST OF TABLES

Chapter 1

Introduction and overview

Overview:

Carbohydrate foods form an essential part of a balanced diet. Carbohydrates are stored as food reserves by all plants, but by far the most widely distributed carbohydrate reserves are starches. The digestibility characteristics of starch based foods generally depend on the processing conditions to which they are subjected and the subsequent retrogradation steps (Siljestrom and others 1986). It is now well documented that during such processing treatments, the dietary starch partially undergoes physical modification leading to the formation of resistant starch (RS), that escapes digestion and absorption in the small intestine but later is fermented by the gut microflora (Berry 1986). Fermentation of undigested starch and soluble fibers is recognized to be a nutritionally beneficial phenomenon and is implied in preventive mechanisms against many intestinal disorders and other diseases like diabetes mellitus, cardiovascular diseases, and cancer (Tharanathan 2000; Gee and others 1992).

Wheat is one of the most commonly cultivated cereals in the world. In India, states such as Punjab, Uttar Pradesh and Gujrat grow wheat in large amounts. The distinctive pattern of Indian diets is that of high carbohydrate-fiber with cereals like wheat and rice forming the staple. Currently, average carbohydrate in Indian diets represents about 60-70% of calories of which 75% is starch derived mainly from cereals and pulses (Sharavathy and others 2001). *Chapaties*- which are unleavened flat breads made from whole wheat flour are served as the staple food in the Indian subcontinent.

Rationale and Justification:

Fermentation of resistant starch, that is the starch that has resisted hydrolysis by the amylolytic enzymes secreted by the healthy human (Champ and others 1999), and the soluble fiber components, produce short chain fatty acids (SCFA), especially butyric acid. These SCFA's have been reported to play important roles in the modulation of many disease conditions, like diabetes (Kumar and others 2002).

Resistant starch and the soluble fiber components, beta-glucans and fructo-oligosaccharides have been exploited for the development of resistant starch, beta-glucans and fructo-oligosaccharide based products as prebiotic, hence considered to be effective functional food ingredient by the food industry (Zeimer and Gibson 1998).

The objective of this study therefore was to quantify the resistant starch and soluble fiber content in various Indian wheat varieties with soy flour addition, by developing and/or formulating a wheat-soy composite product- *Chapati* (unleavened Indian flat bread). *Chapati* is one of the commonly consumed products in the Indian diet. Adding soybean flour to the whole-wheat flour would additionally improve the nutritional quality of the product. Identifying wheat flour varieties (in presence of soy flour) with optimal levels of resistant starch, β-glucans and fructo-oligosaccharides will thus be beneficial to consumers.

Objectives:

The specific objectives of the study were:

- To quantify the resistant starch and soluble fiber components (β-glucan and fructo-oligosaccharides) in various Indian wheat varieties;
- Assay the functional properties of various whole-wheat flour from different wheat varieties;
- To assess the effects of soy flour (added to whole wheat flour in 10%, 20% and 30% proportion respectively) on properties of wheat flour;
- To develop a wheat-soy composite product - *Chapati* (unleavened Indian flat bread) and assess the levels of soluble fiber components and resistant starch, and sensory quality.

Sample selection:

Various varieties of whole-wheat grains were purchased from India and the United States of America to obtain the Indian and Canadian varieties of wheat respectively (Table 1). Canadian wheat varieties were specifically used, since Indian immigrants residing in the United States preferably consume them. The whole-wheat samples once purchased were cleaned and milled in the mill and or the pulverizer to whole-wheat flour of the required particle size.

Defatted soy flour (JEM®) was obtained from Sonic Extractions Limited, Indore (Madhya Pradesh) India and was stored frozen at -20 ^{o}C.

These samples, their local names and the place of origin are shown in Table 1.1 (page 5). Pictorial representations of the wheat varieties used are shown in figures 1.1 - 1.7 (pages 6 - 13).

Table 1.1: Varieties of wheat used in chapatti preparation and analysis:

Wheat Variety	Local Name	Place of Cultivation/Origin
Wheat sample 1	Black Wheat	Canada
Wheat sample 2	Cracked wheat (Coarse variety	India.
Wheat sample 3	Cracked wheat (Finer Wheat)	India
Wheat sample 4	Halim (White Wheat)	Canada
Wheat sample 5	Lokvan	Gujrat, India.
Wheat sample 6	Punjab	Punjab, India
Wheat sample 7	Sehor	Madhya Pradesh, India

Figure 1.1: Blackwheat (Canada)

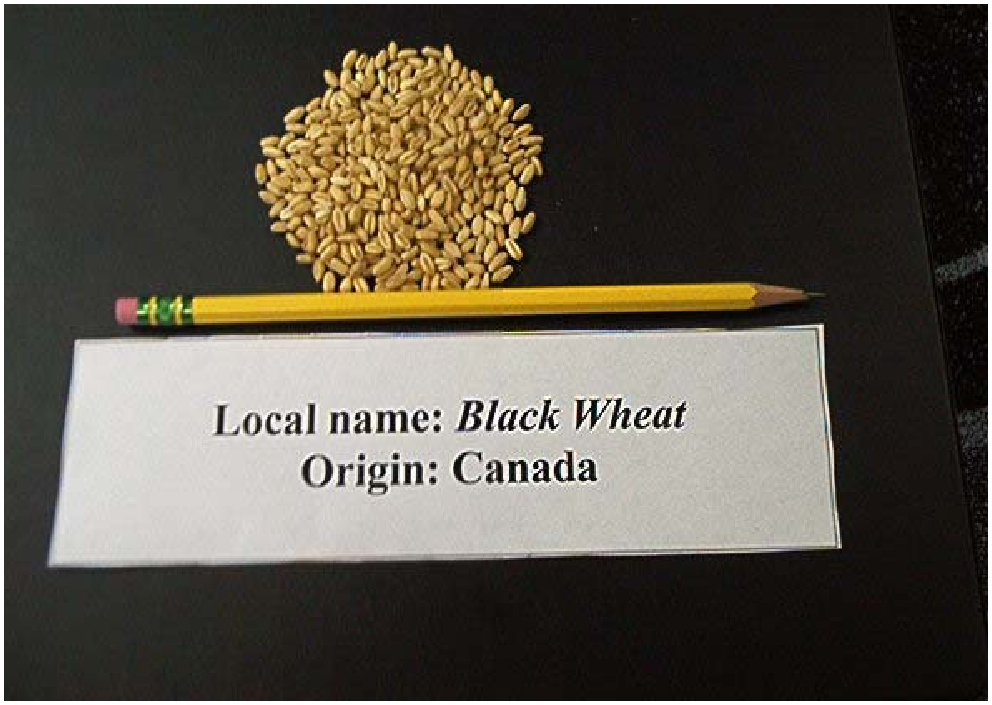


Figure 1.2: Cracked wheat (Coarse) (India):

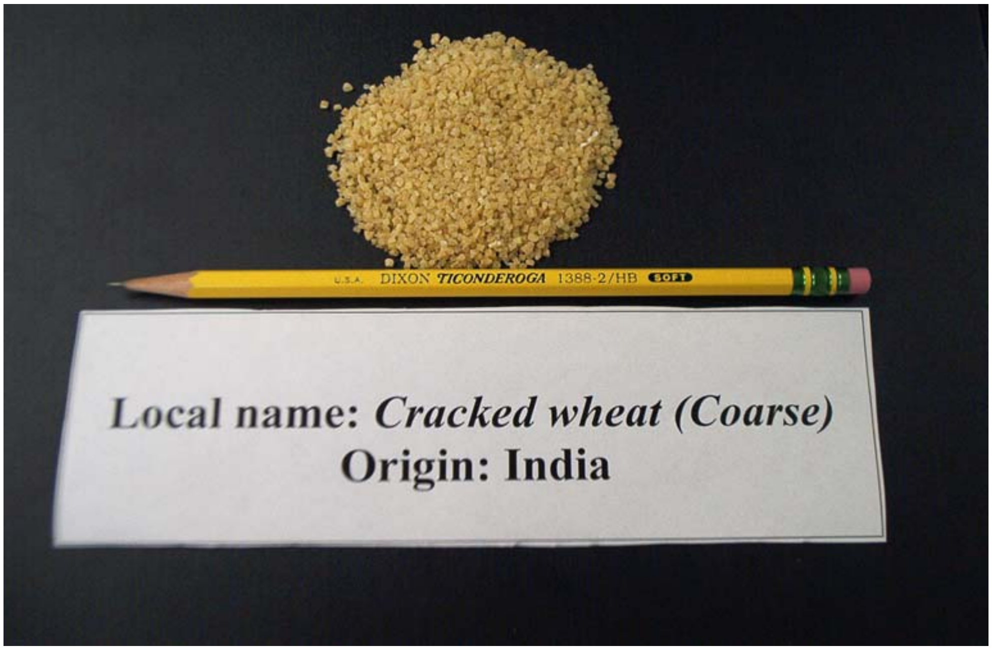


Figure 1.3: Fine Cracked wheat (India)

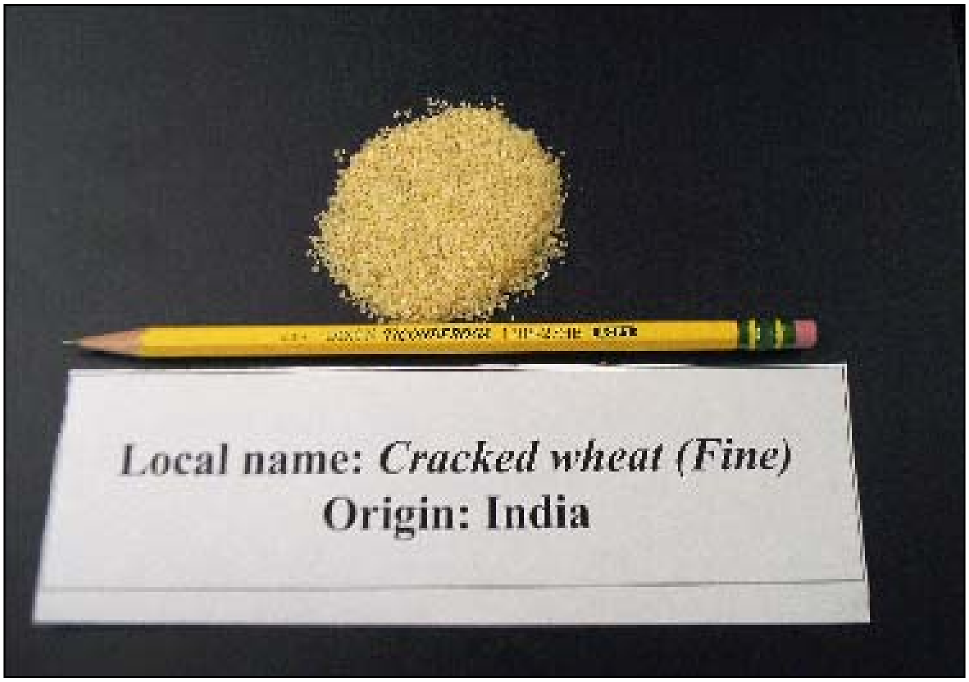
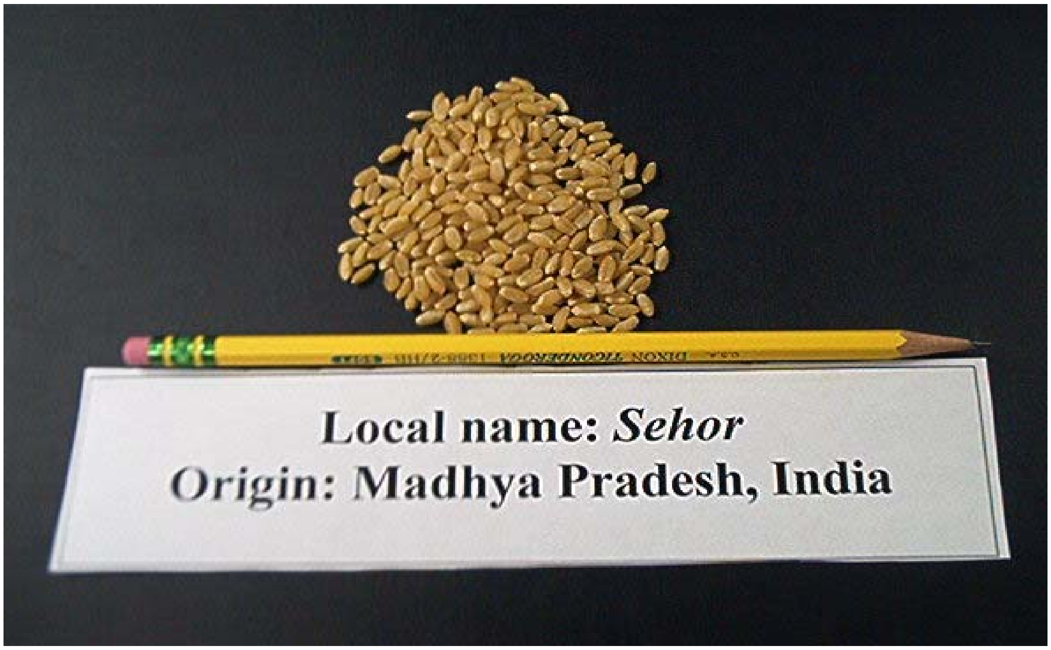


Figure 1.4: Halim (White wheat; Canada)

Figure 1.5: Lokvan (India):

Figure 1.6: Punjab (India):

Figure 1.7: Sehor (India):



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Chapter 2

Review of the literature

Introduction

Carbohydrates and its starch and the non-starch components of foods represent a major portion of the human diet. These components of carbohydrates are synthesized from plant sources. Cereals with high starch content are corn, wheat, sorghum, rice, potato, and cassava. The major cereal grains like wheat, rice, corn are important sources of many nutrients including dietary fiber, resistant starch, oligosaccharides, trace minerals and other disease preventing compounds including phytoestrogens and antioxidants (Slavin and others 1999). Cereal fibers especially, are insoluble, which also explains why they have an important role to play in disease prevention (Smith and others 1981; Trowell and Burkhit 1986). In recent years several studies have focused on the importance of resistant starch and non-starch polysaccharides like beta glucans and fructo-oligosaccharides as the substrates for colonic fermentation. Wheat bran is known to shift the fermentation of the resistant starch more distally producing short chain fatty acids, in turn exhibiting a number of physiological functions (Govers and others 2002). Consumption of insoluble fiber and resistant starch via the intake of wheat bran can strengthen the effects of the colonic physiology by increasing the transit time with a subsequent production of butyrate (Govers and others 1999). The development of new functional ingredients has the advantage that the food product manufacturers can add extra value to products the consumer is already familiar with.

Starch:

Starch is the most common storage carbohydrate in plants. Its unique chemical and physical characteristics and nutritional qualities set it apart from other carbohydrates. It is metabolized by the plants themselves, by microbes and by higher organisms so there is a great diversity of enzymes able to catalyze its hydrolysis. Starch is the predominant food reserve substance in plants and provides 70-80% of the calories consumed by humans worldwide (Whistler and Bemiller 1996). Starch from all plants occurs in the form of granules, which differ markedly in size and physical characteristics among different plant species (Whistler and Bemiller 1996). Starch molecules are composed of a mixture of two polymers: a linear polysaccharide called amylose and a highly branched polysaccharide called amylopectin (Whistler and Bemiller 1996).

The linkage between glucose molecules is important in view of the specificity of the hydrolyzing enzymes. The enzyme that catalyzes the hydrolysis of the α-1, 4 glycosidic bond is the α-amylase present in saliva or pancreatic juice. The α-amylase is an endo enzyme that cleaves both amylose & amylopectin internally producing oligosaccharides. It splits the starch into maltose, maltotriose & branched tri, tetra, & pentapolysaccharides with only small amounts of glucose (Macdonald 1994). The α-1, 6 linkages of amylopectin is not hydrolyzed by α-amylase; hence the branched oligomers remain after hydrolysis with α- amylase. These oligomers are further split by isomaltase present in the brush border of the intestines (Macdonald 1994).

Glucoamylase (amyloglucosidase) is used commercially, in combination with an α-amylase for producing D-glucose (dextrose) syrups and crystalline D-glucose. The enzyme acts upon fully gelatinized starch as an exo-enzyme, sequentially releasing single D-glucose units from the non-reducing ends of amylose and amylopectin molecules, even those joined by the α-1,6 bonds. Consequently, the enzyme can completely hydrolyze starch to D-glucose, but is used on starch that has been previously depolymerized with α- amylase to generate small fragments and more non-reducing ends (Whistler and Bemiller 1996). The β- amylase releases the disaccharide maltose sequentially from the non-reducing end of the amylose. It also attacks the non-reducing ends of amylopectin, sequentially releasing maltose, but it cannot cleave the 1-6 linkages at branch points, so it leaves a pruned amylopectin residue termed a limit dextrin, specifically a beta limit dextrin (Whistler and Bemiller 1996).

There are several debranching enzymes such as isoamylase and pullulanase that specifically catalyze hydrolysis of α- 1, 6- linkages in amylopectin, producing linear low molecular-weight molecules (Whistler and Bemiller 1996).

Carbohydrates have always been the principal source of metabolic energy for humans and the means for maintaining health of the human gastrointestinal tract. Carbohydrates are the principal providers of the bulk and the body of food products. The higher saccharides may be digestible (most starch based products) or partially digestible (retrograded amylose). Moreover, the starch polysaccharides are the only polysaccharides that can be hydrolyzed by human digestive enzymes. They of course, provide D-glucose, which is absorbed by microvilli of the small intestine to supply the principal metabolic energy of humans (Whistler and Bemiller 1996). Starch granules are susceptible to the action of digestive enzymes (alpha amylases) but the rate of enzymatic degradation is greatly increased when the starch is gelatinized.

Resistant Starch:

Resistant starch has been defined as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (Muir and others 1993). In other words, starch not hydrolyzed in the small intestine is considered resistant starch (RS); in the sense that it has resisted hydrolysis by the amylolytic enzymes secreted by the healthy human (Champ and others 1999). The rate and extent of hydrolysis in the small intestine are both important nutritionally. An altered rate of hydrolysis in the colon may lead to the desired resistant starch fermentation occurring in the different portion of the colon, with any physiological effects of the fermentation products accruing to the specific colonic region.

Four types of resistant starch have been identified (Englyst and others 1993, Skrabanja and Kreft 1998, Topping and Clifton 2001, Niba 2002). These are:

Resistant starch type 1 (RS_1): is composed of physically trapped starch.

These starch granules are physically trapped within the food matrix so that the digestive enzymes are prevented or delayed from having access to them. This can occur in whole or partly ground grains, seeds, cereals, and legumes (Lineback 1999).

Resistant starch type 2 (RS_2): refers to native resistant starch granules such as those typically found in bananas, raw potatoes and amylose maize starch.

These native starch granules are known to resist attack by α-amylase. Berry first showed that the amylose level in maize starches generally correlates with resistant starch levels (Berry 1986). Additionally, it was also reported that the granular size of high amylose starch influences the digestibility. Smaller granules have lower digestibility (Knutson and others 1982).

Resistant starch type 3 (RS_3): is comprised of retrograded starch.

The starch components (amylose and amylopectin) undergo the process of retrogradation in a time dependant process after the starch has been cooked or gelatinized. Although, RS_3 is often attributed to amylose retrogradation (Sievert 1989; Eelingen 1993 and others), retrograded amylopectin has also been shown to contribute to type 3 resistant starch (Eelingen 1994; Russell 1989 and others). Most amylose-containing starches can be processed by heat and moisture to produce some type 3 resistant starch. For example, wheat starch will produce low levels of resistant starch as a result of gelatinization and cooling in bread baking (Rabe 1992; Bjorck 1986 and others). In one recent review focusing on resistant starch type 3 (Eerlingen and others 1995) the authors stated that RS_3 was shown to be "thermally very stable", and was isolated by the TDF method (Total dietary fiber). These authors subsequently noted that "highly resistant" fractions are those that resist hydrolysis at 100°C. Sievert and others (1989) described the use of autoclave/cooling cycles to produce resistant starch. They studied the autoclaved starches and resistant starch isolated by the TDF method, and showed that the high temperature Differential Scanning Calorimetry (DSC) endotherm of the intact starches correlated with a similar endotherm from the RS. Based on polymer crystallization theory Eerlingen and others (1993) hypothesized that the formation of type 3 resistant starch can be considered as a crystallization process of amylose in a partially crystalline system. Multi-cycle autoclaving of starch-based products results in retrogradation, and consequently increased levels of $RS_{3,}$ particularly in high amylose foods (Skrabanja and Kreft 1998).

Resistant starch type 4 (RS_4): is defined as chemically modified starch.

This is a relatively newer classification of resistant starch that refers to- a chemically modified or repolymerised starch. (Croghan 1994). Chemical modification facilitates the generation of distarch phosphodiester cross-linkages which could be modulated to produce products of 40 % - 80 % resistant starch by the total dietary fiber method (Seib and Woo 1999).

All four resistant starch types can be manipulated by processing treatments, and combinations of resistant starch types are possible as well. Baking for prolonged period was shown to increase resistant starch content of wheat bread and rye bread (Rabe and Seivert 1992). Similar effects with a rise in resistant starch content are observed by dehulling legumes by steam treatment and cooking (Tovar and Melito 1996). On the other hand, methods like canning, extrusion and microwave heating have been reported to lower the levels of resistant starch (Periago and others 1996; Meance and others 1999; Marconi and others 2000). These processes utilize moist heat thereby facilitating gelatinization of starch, and with no subsequent retrogradation phase, starch susceptibility to digestion is greatly enhanced (Niba 2002). Storage techniques and conditions of starch based foods and products may influence resistant starch levels. It is also suggested that during storage, starch interacts with other food components, which inhibit starch-degrading enzymes and reduce its digestibility (Niba 2002).

In types 2 and 3, the resistance to digestion is due to the physical state of the starch molecules. Evidence is accumulating that the precise nature of a RS material may lead to differing nutritional effects (Heijnen and others 1998; Schulz and others 1993).

Beta -glucans and fructo-oligosaccharides (FOS):

Polysaccharides serve the growing plant as a structural component maintaining the tissue integrity. In foods, they control texture, water binding, and sensory properties and are important sources of nutrients and dietary fiber. One of the most important members of the dietary fiber family is β- glucan. β-glucan is a cell wall polysaccharide present predominantly in oats and barely (Marlett 1990). β-glucans consists of linear, unbranched polysaccharides composed of 1,4-beta glucan units 1,3-beta glucan units found in the endosperm cell walls. The beta-glucan molecule consists of 1, 4- linkages occurring in groups of 2 to 4 units linked by single 1,3-linkages (Dreher 1999). Among all the cereal grains, barley and oats are known to have the highest beta-glucan content (Charalampopoulos and others 2002). Beta glucan is predominantly concentrated in the inner aleurone and the subaleurone endosperm cell walls of barley (Koksel 1999), oats (Wood 1993) and wheat (Wood 1997). Significant amount of beta-glucan is known to be found in the crease area of wheat and other cereal grains (Wood 1984). The available literature shows that wheat is not recognized as a major source of beta-glucan because of its lower content, which is evaluated to be less than 1% on dry weight basis (Charalampopoulos and others 2002). However, pearling technology can be implicated to separate the aleurone layer of the wheat grain to obtain substantial amount of beta-glucan (Charalampopoulos and others 2002).

Another most important class of dietary non-digestible saccharides is that composed of short (up to 10 monomers) and medium (up to 50-60 units) chain length homopolymers (Roberfroid 1999). Some non-digestible oligosaccharides, like inulin and its hydrolysis product oligofructose, which belong to the group of fructans, are common natural food ingredients

(Roberfroid 1999). Fructo-oligosaccharides (FOS) are not digestible by enzymes such as alpha-amylase, saccharase and maltase, especially in humans (Losada and Olleros 2002). Fructo-oligosaccharides belong to the group of oligosaccharides consisting of glucose units linked together by β 1,2 glycosidic linkages; they are indigestible and highly fermentable (Roberfroid and Delzenne 1998). They are natural plant compounds found in various fruits, vegetables and cereal grains (Losada and Olleros 2002).

Physiological aspects of resistant starch, beta-glucans and fructo-oligosaccharides:

Fermentation of the resistant starch in the colon results in the formation of short chain fatty acids (SCFA), which are associated with number of health benefits (Escarpa and others 1996; Langkilde and others 1998; Topping and Clifton 2001). During the last decade it was recognized that resistant starch induces a high butyrate and propionate production (Bird and others 2000). Research has shown that impairments in butyrate supply to colon cells induce gut atrophy and functional impairments, including reduced immune responses. In contrast, enhanced butyrate supply to the colon cells induces the growth of the gut epithelium, gut cell differentiation and improvement of immune-surveillance (Cummings and Englyst 1991; Roediger 1990; Scheppach 1994).

Butyrate is known to have beneficial effects on the reduction of the risk factors involved in the etiology of colon cancer and adenoma development (Smith and others 1998). Butyrate oxidation has been shown to make up for more than 70% of the oxygen consumption by the human colonic tissue (Roediger 1980), indicating that butyrate is the prime energy substrate of the colonocytes. Butyrate is an anti-neoplastic agent in vitro, and has been implicated in the protective effect of fiber in rodents (McIntyre and others 1993, Scheppach and others 1995)

A recent human study indicated that diets containing resistant starch increased fecal bulk and concentrations of short chain fatty acids (acetate and butyrate, with butyrate increasing by the larger amount), and lowered the fecal pH (Philips and others 1995). Of special interest is the fermentation of the resistant starch in the large intestine to short chain fatty acids. In both in *vitro* and in *vivo* studies, it has been shown that starch is a good source of butyrate via fermentation (Muir and others 1993).

The results of the studies carried out to examine the effect of resistant starch on the healthy volunteers and their possible implications for cancer prevention, suggest that resistant starch has potentially important effects on bacterial metabolism in the human colon that may be relevant for cancer prevention (Hylla 1998).

Another study indicates that a high-resistant starch diet and its resultant increase in the fermentation products may be partly responsible for protecting the black population in South Africa against colorectal cancers and other large bowel diseases (Ahmed and others 2000). The results of a study carried out by Ahmed and his co-workers demonstrated that “subjects eating a high-resistant starch diet showed a significant decrease in the stool pH, a significant increase in SCFA, particularly butyrate, compared with a low resistant starch diet” (Ahmed and others 2000). This provided evidence that a high resistant starch diet induces greater fermentation than a low resistant starch diet.

Results of another study indicate that rats fed high amylose corn starch (HAS) diet along with the wheat bran had a greater excretion of the SCFA than those fed just the individual components, suggesting that incorporation of wheat bran delayed the site of fermentation of HAS to the distal part of the hind gut (Henningsson and others 2002). A study by Henker and others (1986) showed that hot traditionally cooked maize has a concentration of 18% resistant starch. Moreover cooked cooled maize contains a higher resistant starch than hot cooked maize meal (Venter 1990). The rate and extent of carbohydrate digestion is reported to be influenced by a variety of factors both intrinsic and extrinsic. There is evidence that slowly digested and absorbed carbohydrates are favorable in the dietary management of metabolic disorders, such as diabetes and hyperlipidemia (Asp 1994; Wursch 1994).

The effect of feeding of butyric acid on alleviation of diabetic status in rats was also studied (Kumar and others 2002). Butyric acid in particular is known to modulate activities of many key regulatory enzymes (Smith and others 1998), including enzymes involved in glycoconjugate metabolism (Jacobsson and others 1985; Shah and others 1992). One of the derivatives of butyric acid, 4-trans-4-methyl cyclohexyl-4-O-oxobutyric acid (JTT-608), is shown to selectively reduce glucose levels in diabetic rats (Ohta and others 1999). The benefits observed of feeding 500 mg / kg body weight (per day)of butyric acid to diabetic rats were attributed to the combined effect of, slow absorption of glucose and hence better glucose levels in the blood as a consequence of insoluble matrix formed by dietary fiber in the intestine (Nuttall 1993; Mujumdar 1995; Plaami 1997), slow release of butyric acid over a period of time by the fermentation of dietary fiber which *in situ* acts as a reservoir of butyric acid (Cummings and others 1986; Bourquin and others 1992; Cummings and Macfarlane 1991; Smith and others 1998), and supplementation of butyric acid.

In a Danish study, ten healthy, normal-weight males consumed test meals containing either 50 g starch free of resistant starch (0 % resistant starch), or 50 g starch containing a high level of resistant starch (54 % resistant starch). Postprandial concentrations of glucose, insulin, glucagons-like peptide 1 (GLP-1) and epinephrine were significantly lower following the high resistant starch meal (Raban and others 2002). These findings have important implications for diabetics as well as healthy individuals. Foods containing starch composed of high levels of resistant starch, such as energy bars, have been shown to dramatically decrease postprandial blood glucose control in subjects with type 2 diabetes (Reader and others 2002). In healthy individuals, studies have shown that resistant starch provides only about 30-70 per cent of the energy of rapidly metabolised starch (Behall and Howe 1995, 1996).

A study conducted by Japanese researchers suggests that regular additions of fructo-oligosaccharides (FOS) to the diet appear to result in a significant reduction in total cholesterol and triglycerides in the blood (Hate 1986). FOS contributes relatively few calories, which are obtained from the metabolites formed in the large intestine generating only 2 kcal/g (Lopez 1997). Following the ingestion of fructo-oligosaccharides, no changes occur in the plasmatic levels of glucose, fructose and insulin. Their consumption does not modify glycemia or the insulinemia (Lopez 1997).

Beta-glucans, fructo-oligosaccharides and resistant starch also have important physiological roles to play. In a study on rats, the total and soluble β-glucan (derived from dietary barley) appeared to be a strong predictor of the cholesterol-lowering in serum and liver of rats. The viscous property of β-glucan may result in reduced absorption, or re-absorption of lipids (Kalra and Jood 2000). Results of another study on the high 1-3, 1-4 β-glucan barley fractions in bread making and their effects on human glycemic response confirmed the effectiveness of viscous β-glucan in reducing the blood glucose levels, even in the foods with high glycemic index (Cavellero and others 2002). The reduced postprandial rise in glucose and insulin levels can be attributed to the presence of (1-3, 1-4)-β-D glucan, which is a polysaccharide component of the dietary fiber providing viscosity to a solution (Cavellero and others 2002).

Application of resistant starch, beta-glucans and fructo-oligosaccharides as functional ingredients:

Resistant starch is generally found as a component of a resistant starch - containing ingredient or food. The goal of including ingredient high in resistant starch should be to combine physical functionality, processing stability, and nutritional functionality. The physical functionality of the resistant starch-containing ingredient is required for appropriate physical characteristics of the food such as texture and water holding capacity. The processing stability is vital to conserve the nutritional functionality of the resistant starch-containing ingredient. The nutritional functionality of the resistant starch-containing ingredient can involve both- resistance to digestion in the small intestine and resistance to fermentation in the colon (Thompson 2000) in turn producing a large amount of short chain fatty acids. To fully take advantage of the resistant starch in foods, it will be important to understand the bases for enzyme resistance in the small intestine and in the colon. With such knowledge it might be possible to develop a wide range of resistant starch-containing materials for use in manufactured foods especially to be used as a functional food.

Functional foods are defined as “foods similar in appearance to conventional foods that are consumed as a part of the normal diet and have demonstrated physiological benefits and /or reduce the risk of chronic disease beyond basic nutritional functions” (German and others 1999). Resistant starch can be used for the development of functional foods in many ways. Recently, functional food research has moved progressively towards the development of dietary supplementation, introducing the concept of probiotics and prebiotics, which may affect gut microbial composition and activities (Zeimer and Gibson 1998).

Resistant starch can be exploited for the development of resistant starch based products as prebiotics and probiotics. Prebiotics have been defined by Gibson and Roberfroid (1999) as “ a food ingredient that is not hydrolyzed by the human digestive enzymes in the upper gastrointestinal tract and that beneficially affects the host selectively, stimulating the growth and/or activity of one or a limited number of bacteria in the colon that can improve host health”. In other words they are the substrates for bacteria like the bifidobacteria in the large intestine whose metabolic products are beneficial in promoting intestinal cell differentiation. A food ingredient could be categorized as a prebiotic, when it is neither hydrolyzed, nor absorbed in the upper part of the gastro-intestinal tract. They are selectively fermented by one or limited number of potentially beneficial bacteria like the lactobacilli and bifidobacteria and there by improve the colonic microflora towards a healthier composition (Kolida and others 2002). Resistant starch provides this readily fermentable substrate, subsequently leading to the formation of short chain fatty acids. Enhanced growth of the beneficial bacteria lead to enhanced immune function, improved colon health, and subsequent protection against disease (Niba 2002). Additionally, the functionality of colonic strains could be improved by the presence of specific non-digestible components of the cereal that could act as prebiotic (Charalampopoulos and others 2002). Probiotic foods on the other hand, can be defined as "those that contain single or mixed culture of microorganisms that affect beneficially the consumer's health by improving their intestinal microbial balance" (Fuller 1989). Thus the probable applications of cereals or cereal components in functional foods could be as fermentable substrates for growth of probiotic microorganisms like the lactobacilli and bifidobacteria (Charalampopoulos and others 2002). Probiotics in the form of bacterial preparations are commonly available in the market place. The utility of these probiotics however, is linked to availability of fermentable substrate (prebiotics) (Niba 2002). Thus, development of new functional ingredients has the advantage that food manufacturers can add extra value to products the consumer is already familiar with.

Observations as above have prompted the food industry to give more focus on dietary fibers and resistant starch rich food ingredients that lead to high butyrate production in a large segment of the colon. Fructans and fructo-oligosaccharides (FOS) are not digested by human alimentary enzymes, but they are fermented in the large intestine. They are of interest as potential ingredients in foods because of their effects on intestinal flora, their functionality and their reduced caloric value (Lineback 1999). Various studies have demonstrated the utility of the FOS as an efficient prebiotic (Losada and Olleros 2002). Fructo-oligosaccharides transit through the upper gastrointestinal tract intact without being absorbed. Fructo-oligosaccharides are used selectively by certain acid producing bacteria, such as bifidobacteria and the lactobacilli that are in the intestine and are considered beneficial (Hidaka and others 1986).

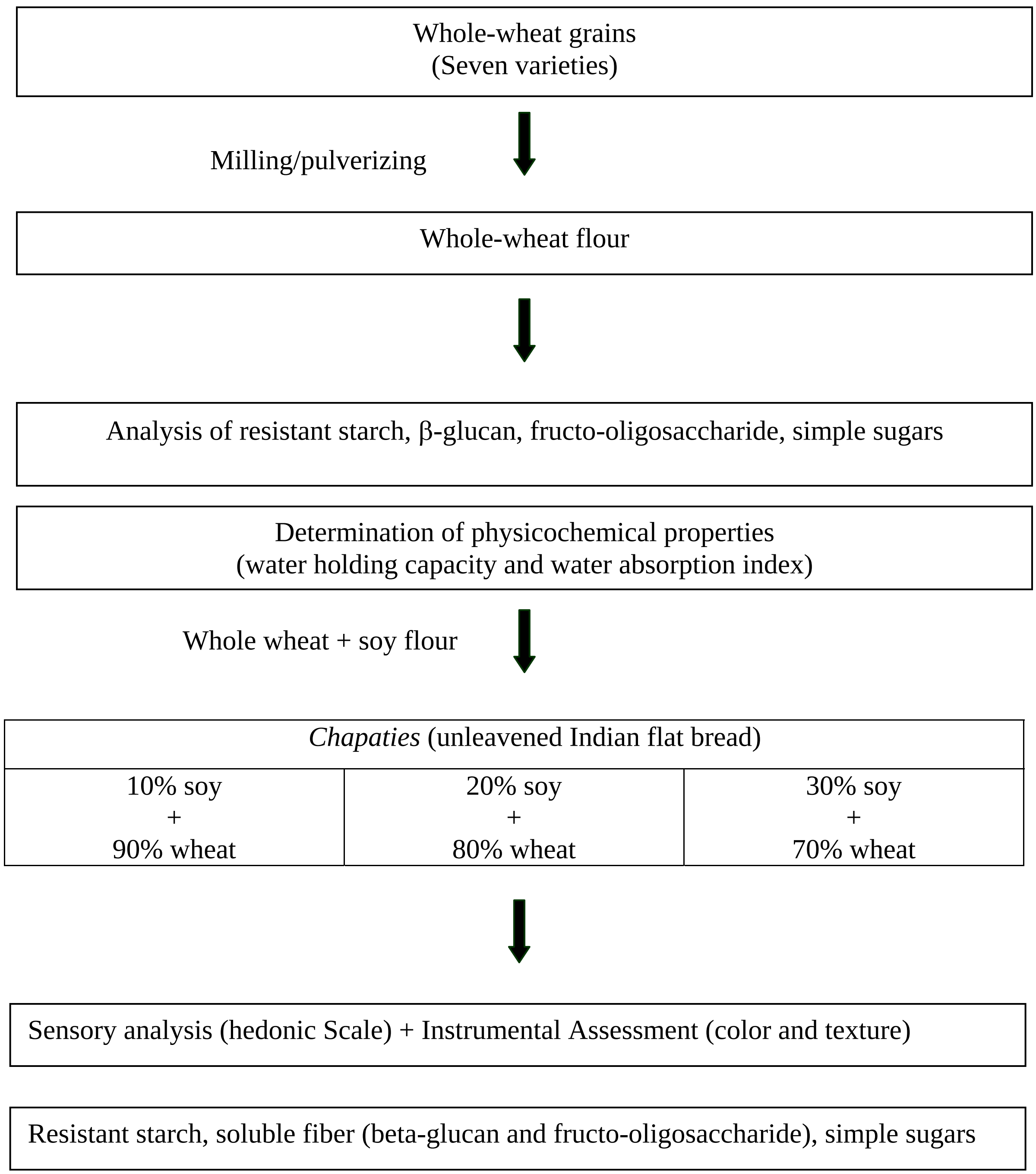
Various forms of β-glucan like the oat β-glucan have been reported to selectively support the growth of lactobacilli and bifidobacteria (probiotics) in rat experiments (Ryhanen and others 1996).

Wheat and wheat utilization in India:

Wheat is the second major cereal crop of India after rice. A number of traditional food products, other than bread and biscuits are prepared from wheat. *Chapati, phulka,* and *poori* are some of the traditional wheat-based foods consumed regularly both as breakfast and during lunch-dinner. A study which isolated and characterized resistant starch in these wheat-based products indicated that, the purified resistant starch from these foods was a linear 1, 4-linked α-D-glucan, which is derived from the highly retrograded amylose fraction of starch. The quantitative make up of wheat flour; particularly its gluten and damaged starch contents have profound influence on the final product making qualities of wheat-based foods (Tharanathan and Tharanathan 2000). Apart from cereals, pulses and legumes form a staple of Indian traditional diets. Moth beans and horse gram are two underexploited Indian legumes growing in the adverse conditions but their composition is little known. In a study carried out by Bravo and colleagues (1999), it was reported that most of the legumes like moth beans, horse gram, black gram, green gram and chick peas have a high content of non-digestible carbohydrates (37-48% of carbohydrates) (Bravo and others 1999). In the study performed by Bravo and colleagues it was found that only 76- 90% of the total starch in legumes was digestible - due to the presence of the resistant starch. The resistant starch content of boiled legumes varied between 3.4% in black gram to up to 8.3% in the haricot beans. Moth beans showed a low resistant starch value (3.9%

dry matter), lower than that of green gram and chick peas (Bravo and others 1998). A reduced starch digestibility in legumes is related to lower glucose release into the blood stream. This would result in reduced postprandial glycemic and insulinaemic responses with potential beneficial effects in the dietary management of diabetes (Jenkins and others 1988). Hence, the presence of a starch fraction resisting digestion and absorption in the small intestine could have beneficial health effects. However, it was observed that legumes are known to contain α-amylase inhibitors, which may decrease in vitro starch digestibility resulting in high resistant starch values. Another possible explanation for the different starch digestibility found in the studied seed legumes could be a decreased accessibility of the hydrolytic enzymes to their substrate (Bravo and others 1998).

Figure 2.1: Project Outline



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Chapter 3

Chemical analysis: Resistant starch, beta-glucans, fructo-oligosaccharides and simple sugars

Abstract

The objective of this study was to quantify beta-glucans, fructo-oligosaccharides and resistant starch in various Indian and Canadian wheat varieties and in the wheat-soy product-chapaties made from them. Seven wheat varieties were milled and/ or pulverized and made into chapaties. Defatted soy flour was added to the wheat flour in 10%, 20% and 30% proportion and made into chapaties. Flours and chapaties (freeze-dried, pulverized) were assayed for resistant and soluble starch, beta-glucan, fructo-oligosaccharides, and glucose/sucrose. Resistant starch content of flours was between 7.1 g/100g and 12.6 g/100g, this decreased to less than 1 g/100g when made into chapaties. The total resistant starch content in the chapaties decreased furthermore with the addition of 10%, 20% and 30% of soy flour to as high as 0.55g/100g to as low as 0.32g/100g with 30% soy flour addition. On the other hand, the soluble starch levels in control chapaties were much higher than in the flours. However, addition of defatted soy flour decreased the soluble content in the chapaties. A direct relationship between addition of soy and decrease in the total soluble starch content was observed. Desirable amounts of beta glucan and fructo-oligosaccharide content were found in the flours and chapati. Processing, roasting and addition of soy flour affected the total beta-glucan and fructo-oligosaccharides marginally. It is apparent therefore that various Indian and Canadian wheat varieties contain considerable levels of resistant starch, beta-glucans and fructo-oligosaccharides. Resistant starch is however notably reduced in chapati, possibly due to dextrinization that occurs during the roasting in chapati

preparation. This provides insight into the selection and application of wheat varieties with desirable levels of beneficial components.

Introduction

Whole cereal grains are vital sources of dietary fibers and resistant starch. The major classes of cereal grains are wheat, rice and corn (Potter 1997). Wheat accounts for one-third of the total grain production in the world (Slavin and others 1999). Whole wheat is considered to be the major staple food among the Indian population. By and large, wheat consumed by the Indian population is subjected to different milling techniques to make a desirable product for consumers. One of the most common wheat based product served as the staple food in a typical Indian diet is *chapati.* Chapaties are unleavened Indian flat breads made by milling whole-wheat grains into whole-wheat flour (*atta*) (Gujral and Pathak 2002). Flours of other cereal grains can be used in combination with wheat flour to improve the nutritional quality of the chapati. Chapaties have been made mixing soy flour and barley flour with the whole-wheat flour (Lindell and Walker 1984; Sood and others 1992; Anjum and others 1991; Leelavathi and Rao 1988) to improve the protein and soluble flour content respectively. A typical Indian diet consists of chapaties, rice, dhal (lentils), vegetable and curd. This is illustrated in Figure 3.1, page 47). Thus the characteristic prototype of an Indian diet is that of a high carbohydrate-fiber diet, with 60-70% of calories accounted for those coming from carbohydrates (Cummings and others 1986; Technical committee 1995; The Nutrition Sub-Committee 1983; Thorburn and others 1987; Sharavathy and others 2001).

The objectives of this study therefore were: to quantify the resistant starch, soluble fiber components (beta-glucan and fructo-oligosaccharides) and in various Indian wheat flour varieties; and in wheat chapaties and wheat-soy chapaties.

Figure 3.1: A typical Indian meal featuring chapaties as a staple:

Materials and Methods:

Certified wheat varieties were procured from local suppliers in India and the United States of America to obtain the Indian and Canadian wheat varieties respectively. Five Indian varieties included- Cracked wheat (coarse), cracked wheat (fine), Lokvan, Punjab and Sehor, while the two Canadian varieties were Black wheat and Halim (white wheat). The grains were cleaned and milled in a pulverizer and/or grinder to respective flours. Pulverization was carried out in the Dept of Food Science and Technology food-processing laboratory, Virginia Tech, using Mikro- Pulverizer (Serial No. 8513) using sieve of hole size 027 mm. Defatted soy bean flour (JEM®) was obtained from Sonic Extractions Limited, Indore (Madhya Pradesh) India and was stored at a temperature of - 20 ^{o}C. Wheat flours were mixed with soy-flour in the desirable proportion of 10 %, 20 % and 30 % respectively. Mixing of the two flours were done uniformly in a Black and Decker's, Smart Grind™ coffee bean grinder. Chapaties of desirable size and shape were rolled from whole-wheat flour and flour mixtures respectively. Whole-wheat grains, flour, flour mixtures, and chapaties were stored in the freezer at -20 ^{o}C until used for the analysis. They were freeze dried for 72 hours prior to analysis to estimate the percentage moisture content.

Chapati preparation:

Whole-wheat flour and/or the wheat-soy flour mixtures (20 g -30 g) were mixed in optimum quantity of water as required and kneaded thoroughly to obtain dough of desirable softness and elasticity. A pinch of salt and ½ tsp of oil were added to the flour during the dough preparation. The prepared dough was allowed to rest for half an hour. The dough was made into a ball and rolled uniformly into a flat chapati with rolling board and pin. The *chapaties* were dry roasted on a flat iron pan over a stove at high temperature for a short period of time (2-3mins) until brown from both the sides. Chapaties made were allowed to cool, and were packed in a polyethylene zip-lock bags and stored in the freezer at - 20 ^{o}C prior to analysis. Some samples were chipped and pulverized in a Black and Decker's, Smart Grind™ coffee bean grinder to a fine powder, and stored in the freezer until used for the chemical analysis.

Chemical analysis:

Determination of resistant and non-resistant (solubilised) starch:

Resistant starch content of wheat flour, wheat-soy mixture and the chapaties made was determined using the AOAC 2002.02 procedures explicated by Mc Cleary, McNally, and Rossiter (2002); Niba and Hoffman (2003). Sample material was weighed out into screw cap tubes. Sodium maleate buffer (pH= 6.0), pancreatic amylase (Megazyme International Ireland Ltd) and amyloglusidase (3 U/ml, Megazyme International Ireland Limited), was added. Samples were then incubated for 16 h at 37°C with continuous shaking in the Precision® Reciprocal shaking water bath. The tubes were treated with 50% ethanol and centrifuged in a Fisher Centrific™ centrifuge for 10 min at approximately 3000 g speed. The supernatants were then decanted and the pellets re-suspended in 50% ethanol. This procedure was repeated twice. The pellets were then treated with 2 M potassium hydroxide in an ice bath (reaction is exothermic) and stirred with a magnetic stirrer for 20 min. 1.2 M Sodium acetate buffer (pH=3.8) was added, followed by an incubation for 30 min at 50°C in a Fisher Scientific water bath. Aliquots (0.1 ml) were removed and treated with GOPOD (glucose oxidase peroxidase) reagent (Megazyme International Ireland Ltd.). These were then incubated at 50 °C in a Fisher Scientific water bath for 20 min and the absorbance read at 510 nm, against the reagent blank, with Glucose solution (1mg/0.1 ml) used as a standard using Beckman Coulter™ DU® 530 LifeScience UV/Vis Spectrophotmeter. High resistant starch samples were diluted prior to color development with GOPOD.

The supernatant solutions obtained on centrifugation of the initial incubation with the supernatants obtained from the two 50 % ethanol washings were adjusted with 100 ml water in a volumetric flask. Aliquots (0.1 ml) were removed and treated with GOPOD reagent (Megazyme International Ireland Ltd.). These were then incubated at 50°C for 20 min and the absorbance read at 510 nm, against the reagent blank, with glucose solution (1mg/0.1 ml) used as a standard. An outline of this procedure is shown in Figure 3.2.

Figure 3.2 Determination of resistant starch content

(AOAC Method 2002 02; McCleary and others, 2002; Niba and Hoffman, 2003)

Sample material

(Wheat flours and chapaties)

↓

Pancreatic amylase and amyloglucosidase in maleate buffer incubated with

agitation for 16 hours in the shaker-bath

↓

Soluble starch precipitated with ethanol

↓

Resistant starch solubilized with concentrated KOH over an ice bath

(Exothermic reaction)

↓

Resistant starch further solubilized with amyloglucosidase

↓

Aliquots treated with GOPOD (color reagent)

↓

Absorbance read at 510 nm

(Standard 1mg/ml glucose solution)

Determination of beta-glucan content:

Beta-glucan content were determined by the AOAC/AACC procedures of (McCleary and Codd 1991; McCleary and Mugford 1992; Niba and Hoffman 2003). Sample materials were treated with 50% ethanol, then sodium phosphate buffer (pH 6.5) added and the samples were incubated in a boiling water bath. The tubes were equilibrated at 50°C. Lichenase enzyme (50 U/ml; Megazyme International Ireland Ltd) was added and then further incubated for 60 min at 50°C in a Fisher Scientific water bath. Acetate buffer (pH 4.0) was then added and the tubes were centrifuged in Fisher Centrifuge at 1000 g for 10 min after which aliquots were removed and treated with β-glucosidase (2 U/ml, Megazyme International Ireland Ltd) for further 10 min. The reaction mixture was incubated with GOPOD (Megazyme International Ireland Ltd) for 20 min and the absorbance were read at 510 nm against a reagent blank. An outline of this procedure is shown in Figure 3.3.

Figure 3.3: Determination of beta-glucan content

(McCleary and Codd 1991; McCleary and Mugford 1992; Niba and Hoffman 2003

Sample material

(Wheat flours and chapaties)

↓

Treated with 50 % ethanol

↓

Sodium phosphate buffer added

Incubated in a boiling water

↓

Equilibrated at 50°C

Enzyme Lichenase (Megazyme International Ireland Ltd) added and incubated - 60 min.

↓

Addition of acetate buffer + centrifuge (10 min)

↓

Aliquots removed and treated with β-glucosidase enzyme

↓

Treated with GOPOD reagent (color reagent)

↓

Absorbance read at 510 nm against the reagent blank

Determination of Fructo-oligosaccharide:

Fructo-oligosaccharide levels in the test samples were measured by the enzymatic procedure of McCleary and Murphy 2000. Sample material was treated with distilled water, and stirred on a hot plate for until boiling. The mixture was cooled, made up to 100 ml volume in a volumetric flask and filtered through Whatman paper. Aliqouts were then treated with diluted sucrase / amylase enzyme solution (Megazyme International Ireland Ltd.) and incubated at 40°C for 20 minutes. The test mixture were then reduced with alkaline borohydride solution for 30 minutes, and further treated with acetic acid. Aliquots were treated with fructanase solution (Megazyme International Ireland Ltd.) at 40°C for 20 minutes in Fisher Scientific water bath. The test mixture were finally treated with color reagent, P-hydroxybenzoic acid hydrazide in trisodium citrate solution (PAHBAH working reagent), incubated in a boiling water for 6 minutes, and the absorbance read at 410 nm against a reagent blank. Fructo-oligosaccharide content was computed using the reference formula (McCleary and Murphy 2000). An outline of this procedure is shown in Figure 3.4.

Figure 3.4: Determination of fructo-oligosaccharide content

(McCleary and Murphy 2000)

Sample material

(Whole-wheat flours and chapaties)

↓

Treated with distilled water and stirred over hot plate (until boiling)

↓

Aliquots treated with amylase/sucrase solution and incubated at 40°C

↓

Test mixture reduced with alkaline borohydride + acetic acid.

↓

Aliquots treated with fructanase solution

↓

Test mixture treated PAHBAH (color reagent)

↓

Absorbance read at 410 nm against reagent blank

Determination of Glucose and Sucrose:

Glucose and sucrose content were determined by using the modified procedures of the AACC (1983) using a test kit from Megazyme International Ireland Ltd. Reagents used in the buffer preparation were obtained from Sigma Chemicals. Sample material to which 95% ethanol was added, were incubated for 5 min at around 85- 90 °C and then made up to 50 ml volume with 50 mM sodium acetate buffer (pH=4.5). The mixture were filtered and 1 ml aliquots were incubated either with acetate buffer for glucose determination or invertase (100 U/ml; Megazyme International Ireland Ltd.) for sucrose determination. Post incubation samples were treated with glucose oxidase peroxidase reagent (GOPOD) (Megazyme International Ireland Ltd.) for 20 min and then the absorbance of the samples were read at 510 nm against a reagent blank. Glucose solution (100 μg/0.1 ml) was used as a standard.

Data Analysis:

Duplicate and/or triplicate samples were assessed. Absorbance data were converted by reference formula (Appendix A) into g/100g dry weight for resistant starch, soluble starch, beta-glucans, fructo-oligosaccharides and simple sugars. Results are expressed as means ± standard deviation.

Results and Discussion

Various Indian and Canadian wheat varieties and chapaties made from them contain reasonable fractions of resistant starch and soluble fibers like beta-glucans and fructo-oligosaccharides. However, roasting of chapaties did bring about a critical decrease in the amount of resistant starch content as compared to the whole flour. Minimal changes were observed between the flour and chapaties with regard to the beta-glucan and fructo-oligosaccharide content. Addition of defatted soy-flour to the whole-wheat flour while preparing the chapaties bought about distinct changes in the resistant starch content but just marginal changes in beta-glucan and fructo-oligosaccharide content.

The percentage moisture content and the percentage dry matter for the seven different varieties of whole wheat grains, flour and *chapaties* is provided in Table 3.1, Table 3.2 and Table 3.3 respectively. The *chapaties* made out of fine cracked wheat flour showed a high dry matter percentage (83.45%) and a low moisture content of 16.45 %, while the chapati made out of coarse cracked wheat flour showed the least moisture content of 16.55%. The total moisture content of the *chapatie*s does seem to have an effect on the resistant starch content of the *chapaties* in addition to its resting time (Tharanathan 2001). The low content of moisture prevents the further crystallization of amylose chains thereby attributing to its low resistant starch content (Gori and others 1997). Blending the wheat flour with 10% and 20% defatted soy flour to make chapaties increased the percentage moisture content of the chapaties with a subsequent decrease in the percentage dry matter in comparison to the chapaties without made without the addition of soy flour. This can be observed from Table 3.4. A study carried out to assess the farinographic characteristics of fried snacks based on blends of wheat flour and soy flour showed that as the proportion of soy flour increased there was a slight increase in the water absorption and dough stability (Senthil and others 2002). Addition of 30 % soy flour also increased the moisture content of the chapaties as compared to that of the control. This could be attributed to increased addition of water during dough making, which in turn could be related to increased water absorption caused due to the addition of soy flour.

Table 3.1: Mean moisture and dry matter content in whole wheat grains, prior to milling*

Wheat variety	Moisture (%)	Dry matter (%)
Blackwheat	3.87	96.13
Cracked wheat (Coarse)	9.51	90.49
Cracked wheat (fine)	10.12	89.88
Halim	6.64	93.36
Lokvan	3.45	96.55
Punjab	3.74	96.26
Sehor	3.82	96.18

*Mean of two determinations.

Table 3.2: Mean moisture and dry matter content in wheat flours, after milling [*]

Wheat variety	Moisture (%)	Dry matter (%)
Blackwheat	10.10	89.90
Cracked wheat (Coarse)	16.99	83.01
Cracked wheat (fine)	11.57	88.43
Halim	10.49	89.51
Lokvan	9.75	90.25
Punjab	9.87	90.13
Sehor	9.87	90.13

[*] Mean of two determinations.

Table 3.3: Mean moisture and dry matter content in *Chapaties* [a]

Wheat variety	Moisture (%)	Dry matter (%)
Blackwheat	19.00	81
Cracked wheat (Coarse)	20.76	79.24
Cracked wheat (fine)	16.55	83.45
Halim	17.65	82.35
Lokvan	18.85	81.15
Punjab	19.81	80.19
Sehor	17.03	82.97

[a] Mean of two determinations.

Table 3.4: Mean moisture and dry matter content in wheat-soybean flour composite *chapaties* [a]

	10% soybean flour		20% soybean flour		30% soybean flour	
Wheat variety	**Moisture content (%)**	**Dry matter (%)**	**Moisture content (%)**	**Dry matter (%)**	**Moisture content (%)**	**Dry matter (%)**
Blackwheat	17.56	82.44	18.67	81.33	22.10	77.90
Cracked wheat (coarse)	21.74	78.26	24.56	75.44	26.92	73.08
Cracked wheat (fine)	21.44	78.56	23.78	76.22	24.21	75.79
Halim	21.40	78.60	23.08	76.92	25.88	74.12
Lokvan	24.79	75.21	26.36	73.64	23.51	76.49
Punjab	28.39	71.61	28.85	71.15	29.53	70.47
Sehor	26.33	73.67	29.89	70.11	26.81	73.19

[a] Mean of at least two determinations.

Resistant starch levels in wheat flours, chapaties and wheat-soy chapaties:

Resistant starch content was high in the unprocessed wheat flours as compared to the resistant starch content analyzed in the *Chapaties* (control) and the wheat-soy composite chapaties. The flour resistant starch ranged from 7.4 g/100 g in Sehor variety to as high as 12.2 g/100 g in the Halim variety as observed from Table 3.5. Resistant starch content of *chapatie*s was found to be much lower ranging from 0.6g/100g in the Lokvan variety to 1.0g/100g in coarse cracked wheat variety as seen in Table 3.5. These results are consistent to those carried out by Tharanathan and others in 2001 in their study which reported that the content of resistant starch in chapatti was the lowest as compared to the other wheat based products that they evaluated. The total resistant starch content in the chapaties decreased furthermore with the addition of 10%, 20% and 30% of soy flour to as high as 0.5 g/100 g in Halim to as low as 0.3 g/100g in Punjab variety with 30% soy flour addition as observed in Table 3.5. Halim variety recorded the highest resistant starch content in flour, which decreased to 0.8g/100g when made into chapati (control), which decreased furthermore to 0.5g/100g, 0.5g/100g and 0.5g/100g on addition of 10%, 20% and 30% of soy flour respectively. This can be observed from the graphical representation in Figure 3.5. Similar trend was observed among the other wheat varieties, with an exception of the cracked wheat varieties which showed a high variability. Limited literature is available that has examined nutritional composition of cracked wheat varieties and the effect of processing on them. Moreover, cracked wheat flour is not commonly used for preparation of chapaties. The possible reason for the low resistant starch content in *chapaties* could be attributed to the high temperature, short time roasting treatment (use to make *chapatti*s), that brings about dextrinization of starch granules which breaks the starch to smaller units of glucose and thereby improves its digestibility. Similar observations were observed by Parchure and others in 1997 where they evaluated the resistant starch content of native and processed starches of rice and amaranth (Parchure and others 1997). This dextrinization and the subsequent decrease in the resistant starch content in *chapatie*s resulted in a corresponding increase in the soluble starch content in *chapatie*s as observed from Table 3.6, unlike the soluble starch content in the wheat flours. The dry heat treatment leads to depletion of resistant starch, resulting in the breaking down of the indigestible granules and making them accessible to enzymatic digestion. The soluble starch levels in control chapaties were much higher than in the flours. However, addition of defatted soy flour decreased the soluble starch content in the chapaties. A direct relationship between addition of soy and decrease in the total soluble starch content was observed. Halim flour which had the highest resistant starch had soluble starch as low as 43.9g/100g, while in control chapaties the soluble starch content was at a higher level of 58.7g/100g. The soluble starch content decreased subsequently with addition of soy flour as depicted in Figure 3.6.

Table 3.5: Resistant starch content in wheat flour, *chapaties* and wheat soy composite *chapaties* [a].

			Percentage soy-bean flour		
Wheat variety	**Flour (g/100g)**	**Control chapati (g/100g)**	**10% (g/100g)**	**20% (g/100g)**	**30% (g/100g)**
Blackwheat	10.1 ± 0.1	0.76 ± 0.2	0.6 ± 0	0.7 ± 0.2*	0.4 ± 0.0
Crackedwheat (Coarse)	12.7 ± 0.2*	1.0 ± 0.1*	0.7 ± 0.0*	0.6 ± 0.0	0.5 ± 0.0*
Cracked wheat (Fine)	11.5 ± 0.3*	0.9 ± 0.1*	0.7 ± 0.0*	0.6 ± 0.0	0.5 ± 0.0*
Halim	12.2 ± 0.4	0.8 ± 0.2	0.5 ± 0.0	0.5 ± 0.0*	0.5 ± 0.0
Lokvan	9.4 ± 0.2	0.6 ± 0.0*	0.4 ± 0.0	0.5 ± 0.0*	0.4 ± 0.0
Punjab	7.9 ± 0.8*	0.7 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.3 ± 0.0*
Sehor	7.4 ± 0.5*	0.7 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.4 ± 0.0
Population mean	10.0 ± 1.9	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

Means with an asterisk within a column fall outside of the confidence interval for the population; $\alpha = 0.05$

Figure 3.5: Resistant starch content in flour, *chapaties* and wheat-soy *chapaties*

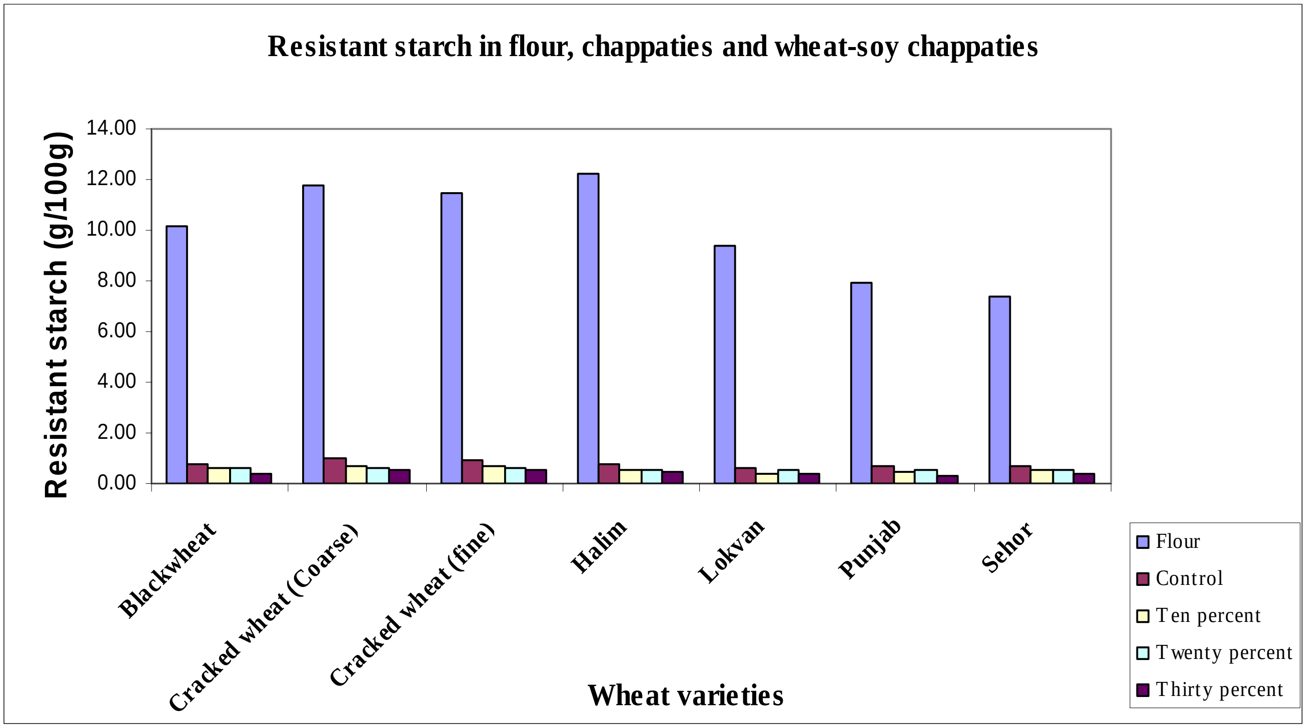


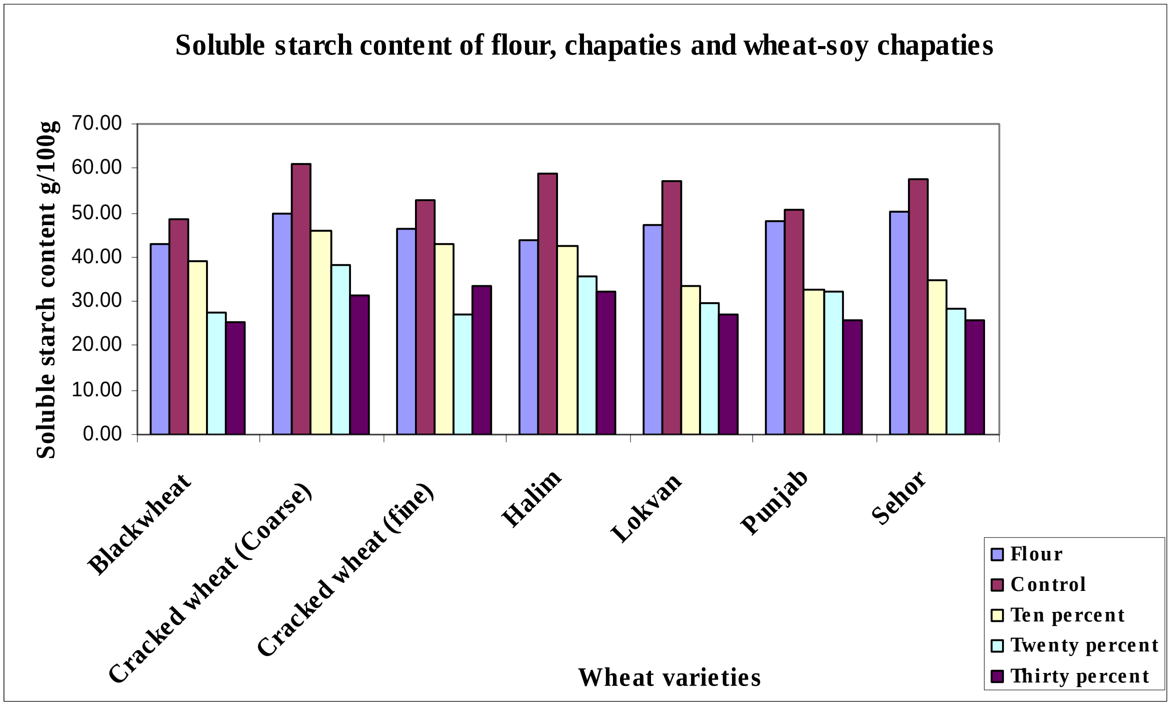
Table 3.6: Soluble starch content in flour, *chapaties* and wheat-soy *chapaties* [a]

			Percentage soy-bean flour		
Wheat variety	**Flour**	**Control *chapati***	**10% soy addition**	**20% soy addition**	**30% soy addition**
Blackwheat	43.0 ± 1.3*	48.5 ± 6.6*	38.9 ± 1.4*	27.4 ± 4.9*	25.3 ± 0.7*
Crackedwheat (Coarse)	50.0 ± 5.1	61.1 ± 1.8*	45.9 ± 0.2*	38.2 ± 0.3*	31.4 ± 3.4*
Cracked wheat (Fine)	46.2 ± 1.8	52.7 ±2.5	42.9 ± 2.2*	27.2 ± 0.8*	33.4 ± 1.0*
Halim	43.9 ± 0.7*	58.7 ± 0.3	42.5 ± 4.2	35.7 ± 2.7*	32.1 ± 0.7*
Lokvan	47.3 ± 0.8	57.3 ± 0.9	33.3 ± 3.0*	29.5 ± 1.9	26.9 ± 3.1
Punjab	48.0 ± 0.1	50.8 ± 1.6*	32.4 ± 1.2*	32.1 ± 1.9	25.7 ± 0.1*
Sehor	50.0 ± 0.3*	57.5 ± 1.5	34.7 ± 4.9	28.2 ± 3.8	25.9 ± 0.2*
Population mean	46.9 ± 2.7	55.2 ± 4.6	38.8 ± 5.3	31.1 ± 4.3	28.7 ± 3.5

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

Means with an asterisk within a column fall outside of the confidence interval for the population; $\alpha = 0.05$

Figure 3.6: Soluble starch content of flour, *chapaties* and wheat-soy *chapaties*



Beta Glucan levels in wheat flours, *chapaties* and wheat-soy *chapaties*:

The beta-glucan content of the wheat flour and the *chapatie*s were relatively lower as compared to other cereals like the barley or oats which are more extensively studied for their high beta-glucan content (Cavellero and others 2002). The mean flour beta-glucans content ranged from 1.3 g/100g to 1.5 g/100g while the mean *chapati* beta-glucan content ranged from 0.9 g/100g to 1.7 g/100g as observed from the Table 3.7. These results of minimal difference in the beta-glucans content between the flour and *chapatie*s is comparable to the study carried out by Izydorczyk and others in 2000 on barley which states that the dry heat treatments which includes roasting, do not have any effect on the solubility of the beta-glucans. However, results of another study performed by Zhang and others (1998) on oat grains show that dry heat processing like roasting increases the extractability of beta-glucans in oat grains (Zhang and others 1998). Similar effect of dry roasting could be possible with regard to wheat grains, which attributed to a marginal increase in chapati beta-glucan content as compared to that of the flour in most of the wheat varieties. Addition of soy flour to wheat flour in the proportion of 10%, 20% and 30% brought about a slight decrease in the beta glucan content of the chapaties as represented in Figure 3.7. For instance Sehor variety which had a total flour beta-glucan content of 1.4g/100g had an increase in its beta-glucan content to 1.5 g/100g when made into chapaties (control), which decreased proportionately to 1.2g/100g, 1.1g/100g, and 1.0 g/100g with addition of 10%, 20% and 30% of soy flour respectively (Table 3.7). The main purpose of adding soy flour to wheat flour was to improve the protein quality. Barley flour could be incorporated into wheat flour along with the defatted soybean flour to compensate the beta-glucan loss that occurs with addition of soy bean flour.

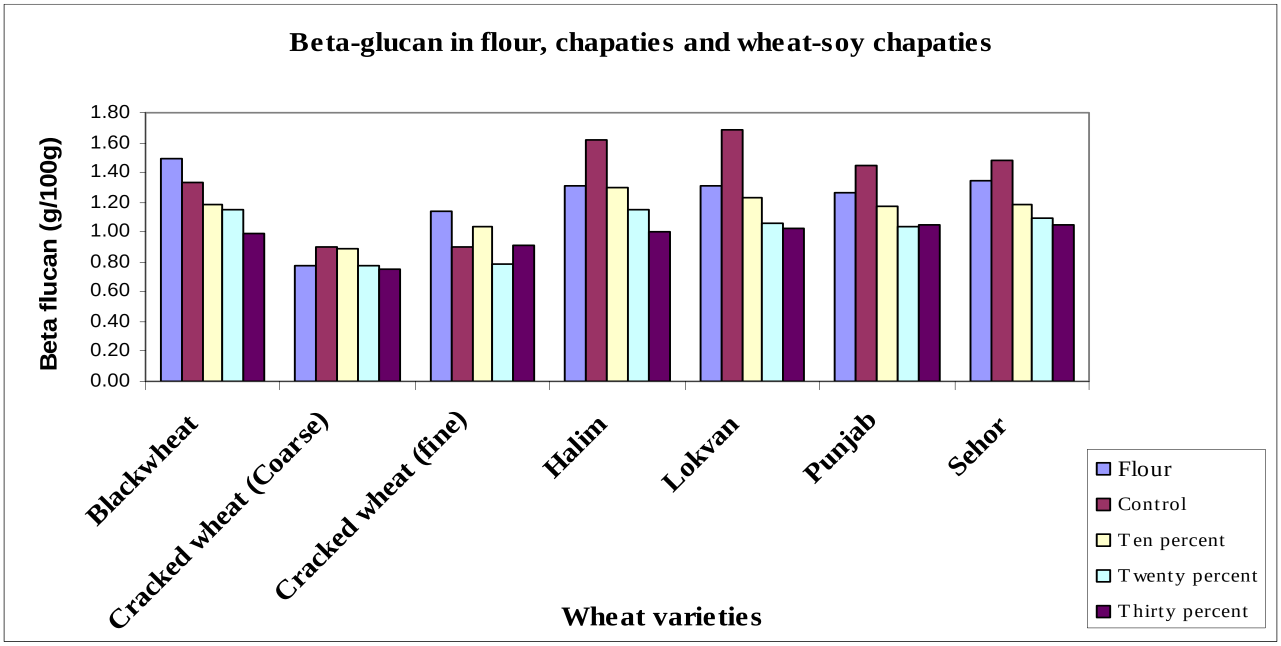
Table 3.7: Beta-glucan content in flours, chapaties and wheat-soy chapaties [a]

			Percentage soy-bean flour		
Wheat variety	**Flour**	**Control chapati**	**10% soy addition**	**20% soy addition**	**30% soy addition**
Blackwheat	1.5 ± 0.29*	1.3 ± 0.07	1.2 ± 0.01	1.2 ± 0.05*	1.0 ± 0.02
Crackedwheat (Coarse)	0.8 ± 0.12*	0.9 ± 0.07*	0.9 ± 0.08*	0.8 ± 0.02*	0.8 ± 0.00*
Cracked wheat (Fine)	1.1 ± 0.11	0.9 ±0.07*	1.0 ± 0.10	0.8 ± 0.02*	0.9 ± 0.19
Halim	1.3 ± 0.11	1.6 ± 0.19*	1.3 ± 0.08*	1.2 ± 0.00	1.0 ± 0.00
Lokvan	1.3 ± 0.02	1.7 ± 0.19*	1.2 ± 0.13	1.1 ± 0.04	1.0 ± 0.03
Punjab	1.3 ± 0.00	1.4 ± 0.07	1.2 ± 0.13	1.0 ± 0.05	1.0 ± 0.1
Sehor	1.4 ± 0.03	1.5 ± 0.00	1.2 ± 0.11	1.1 ± 0.05	1.0 ± 0.05
Population mean	1.2 ± 0.23	1.3 ± 0.32	1.1 ± 0.14	1.0 ± 0.16	1.0 ± 0.11

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

Means with an asterisk within a column fall outside of the confidence interval for the population; $\alpha = 0.05$

Figure 3.7: Beta-glucan content in flour, *chapaties* and wheat-soy *chapaties*



Fructo-oligosaccharide levels in flours, chapaties and wheat-soy chapaties

Results of the present study show that desirable amounts of fructo-oligosaccharide content were found in the flours and chapati. Fructo-oligosaccharides do form an important constituent of cereal grains (Henry and Saini 1989). However, exact distribution of these polymers in cereal grains is not fully studied.. Processing, roasting and addition of soy flour affected the total fructo-oligosaccharide content marginally. Results were highly variable among various different wheat varieties. In Blackwheat, Cracked wheat (fine) and Punjab, there was an increase in the fructo-oligosaccharide content when made into chapaties while for the other varieties there was a subsequent decrease. Blackwheat flour had a high of 1.8 g/100g fructo-oligosaccharide content which increased to 2.4 g/100g when made into chapaties. This can be observed from Table 3.8. On the other hand there were few varieties that showed a marginal decrease in the total fructo-oligosaccharide content when made into chapaties like, Cracked wheat (coarse), Halim, Lokvan and Sehor. Halim variety which showed the fructo-oligosaccharide content as high as 2.3g/100g in flour decreased minimally to 2.1g/100g when made into chapaties. Addition of defatted soy flour to wheat flour bought about nominal decrease in the fructo-oligosaccharide content as observed from Figure 3.8. Similar trend was observed in all the wheat varieties with an exception of Blackwheat, where in there was an increase with addition of 20% soy flour (Table 3.8). More research needs to be carried out to identify the occurrence of fructo-oligosaccharides in cereal grains and recognize different processing techniques that help to prevent the losses.

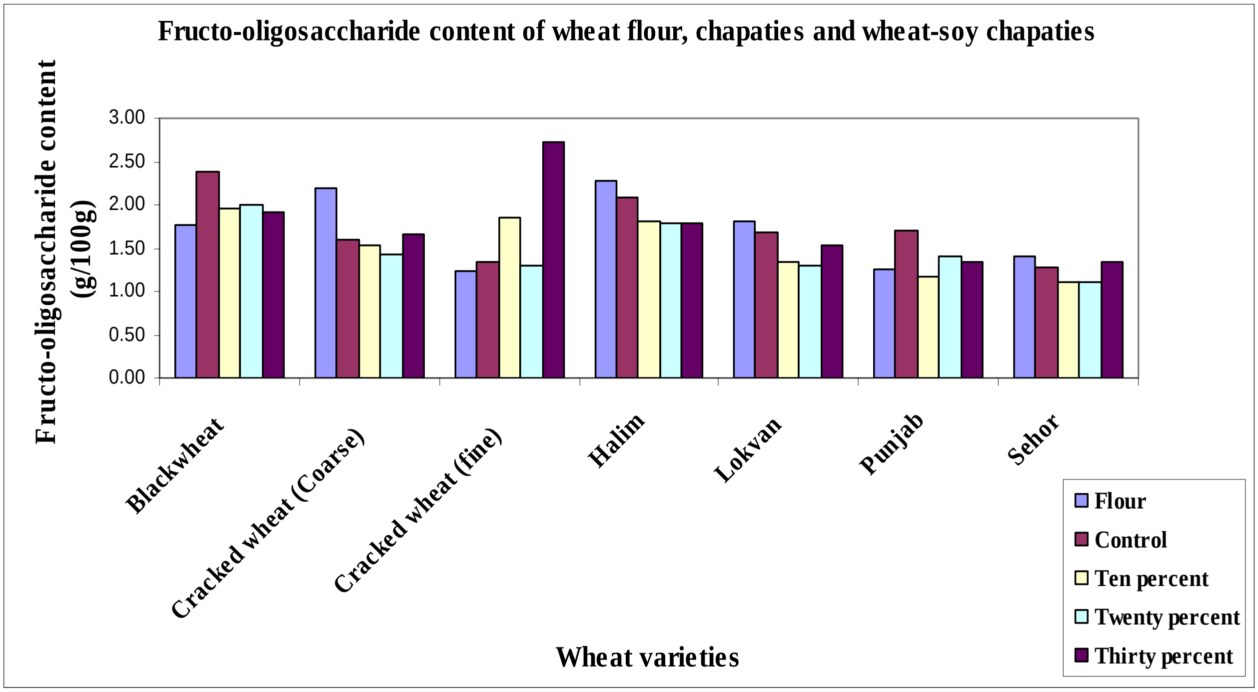
Table 3.8: Fructo-oligosaccharide content in flour, chapaties and wheat-soy chapaties (g/100g) [a]

			Percentage soy-bean flour		
Wheat variety	**Flour**	**Control chapati**	**10% soy addition**	**20% soy addition**	**30% soy addition**
Blackwheat	1.8 ± 0.07	2.4 ± 0.22*	2.0 ± 0.11*	2.0 ± 0.11*	1.9 ± 0.32
Crackedwheat (Coarse)	2.2 ± 1.45*	1.6 ± 0.15	1.5 ± 0.07	1.4 ±0.20	1.7 ± 0.25
Cracked wheat (Fine)	1.2 ± 0.08*	1.3 ± 0.04*	1.9 ± 0.76	1.3 ± 0.16	2.7 ± 0.56*
Halim	2.3 ± 0.30*	2.1 ± 0.22*	1.8 ± 0.03	1.8 ± 0.04*	1.8 ± 0.35
Lokvan	1.8 ± 0.19	1.7 ± 0.0	1.3 ± 0.10	1.3 ± 0.07	1.5 ± 0.38
Punjab	1.3 ± 0.29	1.7 ± 0.27	1.2 ± 0.17	1.4 ± 0.02	1.3 ± 0.22*
Sehor	1.4 ± 0.04	1.3 ± 0.15*	1.1 ± 0.06*	1.1 ± 0.12*	1.4 ± 0.14*
Population mean	1.7 ± 0.4	1.7 ± 0.4	1.5 ± 0.3	1.5 ± 0.3	1.8 ± 0.5

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

Means with an asterisk within a column fall outside of the confidence interval for the population; $\alpha = 0.05$

Figure 3.8: Fructo-oligosaccharide content in flour, *chapaties* and wheat-soy *chapaties*



Glucose and sucrose content in wheat flours, *chapatie*s and wheat-soy composite chapaties:

Most of the wheat flour varieties had negligible free glucose content as observed from Table 3.9, while the highest glucose content (0.1 g/100g) was observed in the variety Halim. In *chapatie*s the glucose content was minimal, almost near negligible amounts.

The levels of simple sugars assessed in the flour and *chapatie*s were minimal in the flours as well as the *chapaties*. No material was located that states changes in the simple sugar content after roasting in any cereal flour. Studies carried out on various cereal based Indian food preparations (Sharavathy and others 2001), also showed similar observations that most of the Indian foods prepared by different methods of cooking have low amounts of free sugar. Moreover, studies have been reported with regard to the effect of other processing techniques like soaking, autoclaving of cereal grains like sorghum, which bring about minimal changes in the glucose and sucrose content (FAO 1995; Niba and Hoffman 2003).

The mean sucrose content in flour was also minimal, ranging from 0.2g/100g in Sehor to 0.3g/100g in the coarse variety of the cracked wheat. In *chapatie*s it was found that the sucrose content was slightly increased as compared to the plain flour. The mean sucrose content in Sehor was as high as 0.3g/100g as seen from Table 3.10. Addition of defatted soy flour did not bring about any discrete changes in the total glucose and sucrose content. These can also be observed from Table 3.11 and Table 3.12. The results looked highly inconsistent. Probably a more sensitive method like the gas chromatography could be employed to get more accurate results.

Table 3.9: Glucose content in flours and *chapaties* [a]

Wheat variety	**Flour (g/100g)**	**Chapaties (g/100g)**
Blackwheat	N/D	N/D
Cracked wheat (Coarse)	N/D	0.1 ± 0.13
Cracked wheat (fine)	N/D	N/D
Halim	0.1 ± 0.09	N/D
Lokvan	N/D	N/D
Punjab	N/D	N/D
Sehor	N/D	N/D

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

N/D = Non–detectable levels.

Table 3.10: Sucrose content in flour and *chapaties* [a]

Wheat variety	**Flour (g/100g)**	**Chapaties (g/100g)**
Blackwheat	N/D	0.4 ± 0.14
Cracked wheat (Coarse)	N/D	0.2 ± 0.17
Cracked wheat (fine)	N/D	0.3 ± 0.03
Halim	0.1 ± 0.02	0.3 ± 0.06
Lokvan	N/D	0.4 ± 0.04
Punjab	N/D	0.4 ± 0.14
Sehor	N/D	0.4 ± 0.15

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

N/D = non-detectable

Table 3.11: Glucose content in wheat-soy *chapaties* [a]

Wheat variety	Addition of soybean flour 10%	Addition of soybean flour 20%	Addition of soybean flour 30%
Blackwheat	N/D	N/D	N/D
Cracked wheat (Coarse)	N/D	0.1 ± 0.06	N/D
Cracked wheat (fine)	N/D	0.3 ± 0.37	0.1 ± 0.05
Halim	N/D	0.1 ± 0.06	N/D
Lokvan	N/D	N/D	N/D
Punjab	N/D	0.1 ± 0.04	N/D
Sehor	N/D	0.1 ± 0.10	N/D

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

N/D = Non-detectable

Table 3.12: Sucrose content in wheat-soy *chapaties* (g/100g) [a]

Wheat variety	Addition of soybean flour 10%	Addition of soybean flour 20%	Addition of soybean flour 30%
Blackwheat	0.3 ± 0.02	0.6 ± 0.38	0.4 ± 0.02
Cracked wheat (Coarse)	0.3 ± 0.05	0.3 ± 0.10	0.3 ± 0.04
Cracked wheat (fine)	0.2 ± 0.01	N/D	0.2 ± 0.06
Halim	0.3 ± 0.03	0.4 ± 0.25	0.3 ± 0.05
Lokvan	0.2 ± 0.05	0.4 ± 0.18	0.1 ± 0.00
Punjab	0.2 ± 0.02	0.4 ± 0.22	0.3 ± 0.01
Sehor	0.2 ± 0.01	0.3 ± 0.03	0.3 ± 0.10

[a] Mean of at least two determinations; data expressed as mean ± standard deviation. Data expressed on dry weight basis.

N/D = Non-detectable

Conclusions

Results of the study reveal that various Indian and Canadian wheat varieties contain considerable levels of resistant starch, beta-glucans and fructo-oligosaccharides. Making a wise choice in selecting the right processing and cooking technique would help in preserving and/or formation of the essential components like the resistant starch and soluble fibers such as beta-glucans and fructo-oligosaccharides present in wheat.

Chapaties could be made by using composite flours like the defatted soy flour to improve the protein quality, provided it does not cost an excessive loss of other vital nutritional components.

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Chapter 4

Physicochemical properties: Water holding capacity and water absorption indices of wheat flours

Abstract

Seven varieties of whole wheat flours and wheat-soy composite flour mixtures used for preparation of chapati were analyzed for their physicochemical properties. Water holding capacity and the water absorption index were determined gravimetrically. Soy bean flour was shown to have effect on the properties and functionality of the wheat flour. Addition of soy-flour to the wheat flour increased the water absorption from 1.9g/g in control wheat flour samples to 2.3g/g in wheat-soy composite mixtures with an addition of 30% soy bean flour. Similar trends were observed in the other wheat varieties. Soy bean flour could be added to wheat flour to prepare chapaties to improve their nutritional quality. It may be concluded that incorporating desirable amount of soy-bean flour to wheat flour up to 20% with required amount of water would yield softer and more pliable chapaties.

Introduction:

Apart from the physiological benefits that carbohydrates exhibit, they are also added to foods because they have many technological functions. For instance they can act as bulking agents, humectants, gelling agents, stabilizers, thickening agents and thereby impart a desirable texture and quality to a product. Functional properties of flour and starch containing foods are very crucial in deciding their use in food industry.

The whole wheat flour used for making chapaties was obtained by grinding the whole wheat grains in mills into flour. The starch damage caused by milling, increased its water absorption, yielding softer and more pliable chapaties (Gujral and Pathak 2002). Water binding capacity is vital in maintaining texture in foods (Ju and Mittal 1995). The water sorption behavior of flour-based products is of prime importance since they are known to affect rheological and functional properties; especially of the baked products like viscosity and consistency (Muller 1973, Lorenz and Kulp 1991, Demertzis and others 1991).

Soy proteins have been identified in many food applications because they offer desirable functionalities in foods at lower costs (Lusas and Rhee 1995). Addition of soy-bean flour in varied proportions to wheat flour has been shown to improve the nutritional quality (Lindell and Walker 1984). The objective of the present study was to assess the effects of soy flour (added to whole wheat flour in 10%, 20% and 30% proportion respectively) on properties of wheat flour; objectively study the properties of chapati; and how their texture was affected by the use of wheat-soy composite flour.

Materials and Methods

Certified wheat varieties were procured from local suppliers in India and the United States of America. Five Indian varieties included- Cracked wheat (coarse), cracked wheat (fine), Lokvan, Punjab and Sehor, while the two Canadian varieties were Black wheat and Halim (white wheat). The grains were cleaned and milled in a pulverizer and/or grinder to respective flours. Pulverization was carried out in the Dept of Food Science and Technology food-processing laboratory, Virginia Tech, using Mikro- Pulverizer (Serial No. 8513) using sieve opening of 027 mm. Defatted soy-bean flour (JEM®) was obtained from Sonic Extractions Limited, Indore (Madhya Pradesh) India and was stored at a temperature of -20^{o}C. Wheat flours were mixed with soy-flour in the desirable proportion of 10 %, 20 % and 30 % and stored at - 20 ^{o}C. Mixing of the two flours were done in Black and Decker's Smart Grind™ coffee bean grinder.

Determination of water absorption index and water holding capacity:

Water holding capacity (WHC) and water absorption index (WAI) were determined by a modification of the methods of Valdez- Neibla and others (1992); Ju and Mittal (1995) and Subrahmanyam and Hoseney (1995). Flour samples (1g) were suspended in 5 ml. water in a centrifuge tube. The slurry was then shaken on a platform tube rocker for a minute at room temperature and centrifuged in Fisher Centrific™ centrifuge at speed of 3000 g for 10 minutes. The supernatant was poured carefully into an evaporating dish, and water released was weighed on a weighing scale.

Water holding capacity was calculated as follows (g / g):

$$\frac{\text{Mass of water added to sample} - \text{mass of water removed from the sample}}{\text{Mass of flour sample}}$$

Water absorption index was calculated as follows (g / g):

$$\frac{\text{Weight of wet sediment}}{\text{Initial weight of the flour sample}}$$

Results and Discussion

Water holding capacity and water absorption index:

The water holding capacity and the water absorption index in different flour samples ranged from 1.0 g/g in Sehor variety to 2.2 g/g in Punjab. Water holding capacity is an important processing parameter and has numerous implications. Results from Table 4.1 show that the water holding capacity of the wheat flour from different wheat varieties was similar for all the samples with the exception of Punjab, which showed marginally higher values than the others. This probably could be attributed to the variation in the starch granule size among the different flours, which in turn could influence the water absorption (Tian and others 1991).

Table 4.2 shows that addition of soy flour to wheat flour increased the water holding capacity of the flour mixture as compared to the control flour. This result is consistent with the studies of Senthil and colleagues (2002) which showed that, addition of soy-flour to the wheat flour increased the water absorption. Senthil and others also found that increasing the proportion of soy-flour added to the wheat flour increased the water absorption and thereby decreased the stability of the dough. Similar observations of increase in water absorption and decrease in the dough stability with addition of legume flour (also rich in proteins) were made by Appolonia (1977), and Silaula and others (1989). In the present study, the increase in the water absorption and the water holding capacity with addition of soy flour could be attributed to the higher soluble protein content in the soy flour and the water binding ability of the soy flour (Senthil and others 2002).

Table 4.1: Water absorption index of wheat flour and wheat-soy flour mixture[a]

		Percentage of soy-bean four		
Wheat variety	**Flour (g/g)**	**10% (g/g)**	**20% (g/g)**	**30% (g/g)**
Blackwheat	1.9 ± 0.0	2.1 ± 0.0	2.3 ± 0.0	2.3 ± 0.1
Crackedwheat (Coarse)	2.0 ± 0.0	2.1 ± 0.0	2.3 ± 0.1	2.3 ± 0.0
Cracked wheat (Fine)	2.0 ± 0.0	2.1 ± 0.0	2.3 ± 0.1	2.3 ± 0.1
Halim	2.0 ± 0.0	2.1 ± 0.0	2.2 ± 0.1	2.4 ± 0.0
Lokvan	2.0 ± 0.0	2.2 ± 0.0	2.3 ± 0.1	2.4 ± 0.0
Punjab	2.2 ± 0.0	2.2 ± 0.0	2.3 ± 0.0	2.5 ± 0.1
Sehor	1.0 ± 0.1	1.1 ± 0.0	1.1 ± 0.0	1.3 ± 0.1

[a] Mean of two determinations; data expressed as mean ± standard deviation.

Table 4.2: Water holding capacity of wheat flour and wheat-soy flour mixture[a]

		Soy bean flours added		
Wheat variety	**Flour (g/g)**	**10% (g/g)**	**20% (g/g)**	**30% (g/g)**
Blackwheat	1.2 ± 0.0	1.0 ± 0.0	1.2 ± 0.0	1.2 ± 0.1
Crackedwheat (Coarse)	1.2 ± 0.1	1.0 ± 0.0	1.2 ± 0.0	1.2 ± 0.1
Cracked wheat (Fine)	1.2 ± 0.1	1.0 ± 0.0	1.2 ± 0.0	1.2 ± 0.0
Halim	1.1 ± 0.0	1.0 ± 0.0	1.2 ± 0.1	1.2 ± 0.0
Lokvan	1.2 ± 0.1	2.0 ± 0.0	1.2 ± 0.1	1.2 ± 0.0
Punjab	1.3 ± 0.0	2.0 ± 0.0	1.2 ± 0.0	1.2 ± 0.1
Sehor	1.0 ± 0.1	1.0 ± 0.0	1.0 ± 0.1	1.0 ± 0.1

[a] Mean of two determinations; data expressed as mean ± standard deviation

Conclusions:

Results of the study show noticeable modification in the wheat flour functionality in the presence of soy flour. Results also indicate that the amount of water required for making dough increased with an increasing level of soy flour added.

Rheological characteristics like the water absorption index and the water holding capacity were found to correlate with the desirable texture of the chapaties. Chapaties could be made by using wheat-soy composite flour to improve the overall nutritional quality by incorporating desirable amount of soy flour up to 20% and the required amount of water to yield softer and more pliable chapaties.

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Chapter 5

Sensory Properties of *Chapati*

Abstract

Addition of defatted soy bean flour to whole wheat flour (obtained from Punjab and Sehor wheat varieties) at 10%, 20% and 30% was carried out to evaluate the sensory characteristics of the wheat-soy composite product chapati. Instrumental assessment was carried out using Stevens™ Texture Analyzer to assess texture and Hunter Color lab Colorimeter™ to assess color, to supplement the sensory evaluation. Results of the sensory evaluation (performed by a random group of 20 Indian untrained panelists) revealed that incorporation of 30% soy did produce an acceptable wheat-soy composite product chapati. Chapaties made from 30% soy flour appeared to be preferred over the chapaties made with addition of 10% and 20% of soy bean flour and that made without any soy addition. It may be concluded that chapaties made from composite flour mixtures of wheat and defatted soy-bean flour, up to 30% addition of soy bean flour are nutritionally and organoleptically acceptable.

Introduction:

Wheat is the world's most important cereal crop with regard to its production and consumption (Shewry and Tatham 1994). Traditionally in India 85-90% of the wheat is consumed chiefly in the form of homemade products such as chapaties (Dhingra and Jood 2001). Chapaties made from whole-wheat flour are an integral part of an Indian diet. Chapaties are consumed by themselves or with accompaniments like sauces, vegetables or *chutneys* (mixture of coconut and/or Indian spices) during lunch, dinner, breakfast or as an evening snack.

Although whole wheat is known to contain essential nutrients like soluble fibers (Wood 1997; Wood 1984) resistant starch (Tharanathan and Tharanathan 2000), and minerals; wheat is considered nutritionally poor in quality since all the cereal proteins lack in the essential amino acids - lysine and threonine (Dhingra and others 2001). Soybean flour is known to have high protein (38-40%); 5-6% being lysine. Addition of soy-bean flour to the whole wheat flour would thus help to improve the nutritional quality of the chapaties. In a study carried out by Dhingra and Jood (2001), it was found that addition of 10% defatted or full fat soy bean flour to wheat flour, produced acceptable breads, while the breads made from addition of 20% soy flour did not produce organoleptically acceptable bread. Singh and colleagues (1996) showed that a maximum of 20% soy flour can be incorporated to prepare acceptable quality biscuits. The present study aimed to investigate the effect of incorporation of soy flour (10%, 20% and 30% proportion) in wheat flour and evaluate the sensory characteristics of wheat-soy composite product chapati made from them.

Materials and methods:

Sample material was obtained and prepared as previously described (Chapter 3).

Wheat flours were mixed with soy-flour in the desirable proportion of 10 %, 20 % and 30 % respectively and stored at - 20 ^{o}C. Mixing of the two flours were done uniformly in a coffee grinder. Composite wheat-soy product chapati prepared using different proportions of soy flour were assayed for evaluating the sensory characteristics, textural qualities and color to determine its overall acceptability.

Texture assessment was carried out using Stevens™ Texture Analyzer and color was assessed using a Hunter™ Colorlab Colorimeter. Color parameters assessed were L*, a*, b*; where L* represents lightness, and a*, b* the color axes. Saturated index (chroma) and hue angle were determined as follows:

SATURATION INDEX (CHROMA) = $(a^{*2} + b^{*2})^{1/2}$

HUE ANGLE = $[\tan^{-1}(b^*/a^*)]$

Sensory evaluation:

Sensory evaluation of chapati samples was carried out by 20 random untrained Indian panelists. Indians, residing in and around the campus of Virginia Tech University were recruited to serve as panelists since chapati is primarily consumed by them as a part of their regular meal. An affective acceptance test using a 9 point Hedonic scale was chosen to evaluate the sensory characteristics of a chapati (Meilgaard and others 1999). The test area was centrally located in the sensory laboratory Wallace Hall, Virginia Tech and the participants were well directed. The area was also free of crowding and confusion, comfortable, quite and temperature controlled.

The sample preparation area was away from the sensory booths so as to avoid any sort of biases. Out of the total panelists chosen, 55 % of the panelists were males (11) and 45 % were females (9). From the total number of panelists that evaluated the samples 45% reported consuming chapaties several times a week, 45% consumed chapaties once every week, while 10% had a consumption pattern of eating chapaties once per month. A 3-digit random code was assigned to each sample randomly and presented to the panelists for evaluation in a balanced order.

Chapaties were made just prior to the presentation and stored in a closed box until the test. Chapaties were broken into small pieces of approximately 2-2 inches size for testing. A paper plate was marked with the designated numbers as mentioned above and presented to the panelists for testing.

Data obtained from the sensory evaluation of the chapaties was recorded in a summary sheet. The relative acceptance scores were used to infer the preference; where the samples with the highest score were considered as the preferred choice. The hedonic scale used by the panelists to scale their choices is shown in Table 5.1.

Table 5.1: Acceptance test: Hedonic scale (Rating scale 1= dislike extremely; 9= like extremely)

***Chapatti* sample**
_______ like extremely
_______ like very much
_______ like moderately
_______ like slightly
_______ neither like nor dislike
_______ dislike slightly
_______ dislike moderately
_______ dislike very much
_______ dislike extremely

Instrumental Assessment of *Chapati*:

Assessment of texture:

The texture analysis of the chapati was done using Stevens™ Texture Analyzer, to supplement the sensory evaluation. The texture analyzer helped to measure hardness, fracturability, springiness, adhesiveness, spreadibility, tensile strength and more of such properties of the *chapati* (Giese 2003). Giese (2003) also opined that “While sensory tests and descriptors are highly useful in understanding product rheology, there are problems and costs associated with training and maintaining of sensory panels. As an alternative, instruments for rheological food measurements are straightforward to use and provide consistent and significant data”.

Thus the texture analysis of the *chapati* was done by using a sample piece of *chapati* approximately 10 mm length and 10 mm thickness was compressed under following conditions: cross head speed 2 mm/sec and a distance of 10/mm.

Assessment of color:

Visual color judgment of *chapati* (by the way of sensory evaluation) could be affected by a wide variety of factors. Instrumentation to measure color would provide a subjective and consistent method of color quality control (Giese 2000). Giese (2000) recommended that “color measurement is a critical objective quality parameter that can be used for following applications: as quality index measurements of raw and processed foods for use in quality control documentation and communication; for determination of conformity of food quality to specifications; and for analyses of quality changes as a result of food processing storage and other factors (Giese 2000)". Thus, color assessment of *chapati* was performed using a Hunter™ Colorlab Colorimeter. The values were recorded as L*=lightness (where 0 = black, 100 = white), a* (-a* = greeness, +a = redness), and b* (-b* = blueness, +b* = yellowness). These values were compared to a standard white plate.

Results and Discussion:

Sensory evaluation

The scores obtained on a hedonic scale revealed that among the samples evaluated for sensory characteristics, samples with 30% soy addition were more accepted, followed by the acceptance of 20%, 10% and control. Both the wheat varieties had similar trend with respect to their acceptance. The results of the sensory test showed unusual exceptions as compared to the studies performed by researchers in past with regard to the acceptance of incorporation of soy flour in bread making (Dhingra and others 2001, Rastogi and Singh 1989). This can be observed from Table 5.2. For Punjab variety, 7.1 acceptance rate was in favor of the sample with 30% soy addition. Out of these, 30% of panelists showed a moderate likeness for this sample (30% soy flour), while 25% categorized their acceptance to the "like very much" scale, and 20% showed an extreme liking for the same. There were 5% responses that indicated a dislike for the sample. The overall acceptance level for 30% soy added *chapati* showed a marginal difference in comparison to the acceptance for the 20%, 10% and the control samples.

Some what similar trend was observed with regard to Sehor wheat variety. Out of the average acceptance rate of 7.3 for the sample with 30% soy flour, 40% panelists showed a moderate liking for that sample, while 15% showed an extreme liking for the same. Only 5% showed a strong dislike with regard to its overall acceptability.

Table 5.2: Sensory quality of wheat and wheat soy chapaties [a]

Wheat variety	Percentage soy-bean flour	Acceptability rating scale: (1= dislike extremely; 9= like extremely)
Punjab	Control (# 653)	6.8 ± 2.1
	10% soy bean flour (# 749)	6.8 ± 2.1
	20% soy bean flour (# 522)	7.0 ± 1.4
	30% soy bean flour (# 475)	7.1 ± 2.8
Sehor	Control (# 116)	5.8 ± 1.4
	10% soy bean flour (# 381)	6.7 ± 1.4
	20% soy bean flour (# 968)	7.3 ± 1.4
	30% soy bean flour (# 742)	7.3 ± 0.7

[a] Mean of 20 random panelists; data expressed as mean ± the standard deviation.

Instrumental assessment:

The results tabulated in Table 5.3 that evaluated the texture of the *chapaties* show random and inconsistent results with regard to the changes observed with addition of soy flour to the wheat flour. The results show that the control *chapaties* were relatively harder as compared to the *chapaties* made with addition of 10%, 20% and 30% soybean flour. For instance the chapaties made from Blackwheat variety without the addition of soy bean flour showed a harder texture of 1168 as compared to the chapati made with addition of 30% soy which showed much softer texture of 93.5. The variation among the various varieties could be attributed to the softening and the tenderizing effect of the soy-bean flour.

Variation in the color intensity is shown in Table 5.4. The results of the colorimeter indicate L* values in the range of 60^{s} which represent a dark color. Similarly, a high a* and b* color indicates the intensity of the color. Results also show that there were no obvious or consistent modifications observed in the color with an addition of soy bean flour in different proportions.

Table 5.3: Mean Stevens™ Texture Analyzer measures for *chapaties* *

Sample Number	Wheat variety	Mean texture (g/loads)
1	Blackwheat (control)	1168
1a		490
1b		630.5
1c		93.5
2	Cracked wheat coarse (control)	1031
2a		120
2b		74
2c		125.5
3	Cracked wheat fine (control)	183
3a		50
3b		209.5
3c		230.5
4	Halim (control)	1247
4a		234.5
4b		140.5
4c		468.5
5	Lokvan (control)	1234
5a		661.5
5b		52
5c		68
6	Punjab (control)	1037
6a		174
6b		36
6c		127
7	Sehor (control)	549
7a		36
7b		80
7c		62.5

*Mean values of two replicates.

a = addition of 10% soy bean flour

b = addition of 20% soy bean flour

c = addition of 30% soy bean flour

Table 5.4: Mean Hunter™ Colorimeter measures for *chapaties*[a]

Sample Number	Wheat variety	L*	a*	b*	Chroma (sat index) $(a*^2+b*^2)^{1/2}$	Hue angle (tan-1(b*/a*)
1	Blackwheat (control)	67.47	5.11	28.73	29.18	88.79
1a		68.71	5.66	25.83	26.44	86.29
1b		65.35	6.18	26.85	27.55	85.61
1c		65.06	6.08	26.46	27.15	85.65
2	Cracked wheat coarse (control)	69.03	3.53	29.76	29.97	92.48
2a		66.77	6.60	29.62	30.35	86.05
2b		65.80	5.79	28.50	29.08	87.26
2c		66.70	6.09	28.94	29.57	86.82
3	Cracked wheat fine (control)	71.77	2.06	28.71	28.78	95.44
3a		69.09	5.28	32.63	33.05	89.80
3b		66.73	6.06	30.06	30.66	87.33
3c		65.18	6.47	28.63	29.35	85.87
4	Halim (control)	69.44	5.01	27.74	28.19	88.63
4a		67.73	6.31	26.87	27.60	85.32
4b		68.66	5.99	27.23	27.88	86.23
4c		65.29	7.07	27.38	28.27	83.90
5	Lokvan (control)	62.22	6.27	28.19	28.88	86.10
5a		65.82	7.03	27.72	28.59	84.21
5b		62.99	7.22	27.43	28.36	83.62
5c		63.48	6.97	28.16	29.00	84.55
6	Punjab (control)	64.71	5.52	27.58	28.12	87.43
6a		67.00	6.03	28.66	29.29	86.82
6b		62.90	6.95	27.53	28.39	84.25
6c		63.61	6.61	27.00	27.80	84.73
7	Sehor (control)	67.02	4.18	25.73	26.07	89.75
7a		66.95	5.69	26.30	26.90	86.43
7b		66.42	6.30	28.22	28.91	86.02
7c		64.14	6.54	26.71	27.49	84.73

[a]Mean values of two replicates.

a = addition of 10% soy bean flour

b = addition of 20% soy bean flour

c = addition of 30% soy bean flour

Conclusion:

It may be concluded from the present study that defatted soy bean flour could be added to wheat flour up to levels of 30%. Overall the chapaties had a high acceptability (all rated above five on the hedonic scale). Addition of 30% defatted soybean flour increased the acceptability and was rated higher that the chapaties made without the addition of soy-bean flour. Addition of soy bean flour improved the quality of Sehor to a greater extent than Punjab since it showed a greater increase in acceptability rating. Hence, up to 30% soy bean flour can be added without negatively impacting the quality of the chapaties. However, this could be recommended with no overt corresponding modification in chemical quality.

References:

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General conclusions

The data presented in this thesis lead to a conclusion that there are variations in the wheat varieties assessed. A considerably high level of resistant starch was found in the raw flour, while applicable levels of soluble fibers (beta-glucans and fructo-oligosaccharides) are present in the wheat flour. Preparing *chapati* from the wheat flour causes depletion of resistant starch, and brings about a marginal depletion of the soluble fibers. Addition of up to 30% soy bean flour greatly improved the chapati acceptability. This provides insight into the selection and application of wheat varieties and the processing techniques to be used that could be better for retaining beneficial components.

APPENDIX A

Reference formulae (Megazyme methods)

1) Resistant starch content was calculated as:

A x F x 10.3/0.1 x 1/1000 x 100/W x 0.9

Where;

A	=	Absorbance at 510 nm
F	=	conversion from absorbance to mg [100 μg glucose/ absorbance of glucose standard]
10.3/0.1	=	dilution factor [0.1 ml taken from 10.3 ml]
1/1000	=	conversion form micrograms to milligrams;
W	=	sample weight in mg
0.9	=	conversion factor from glucose to starch

2) Solubilized starch content was calculated as:

A x F x 100/0.1 x 1/1000 x 100/W x 0.9

Where;

A	=	Absorbance at 510 nm
F	=	conversion from absorbance to mg [100 μg glucose/ absorbance of glucose standard]
100/0.1	=	dilution factor [0.1 ml taken from 100 ml]
1/1000	=	conversion form micrograms to milligrams;
W	=	sample weight in mg
0.9	=	conversion factor from glucose to starch

3) Beta glucan content was calculated as:

% w/w = A*F*94 (1/1000)*(100/W)*(162/180)

Where;

A	=	absorbance of test sample minus absorbance of blank
F	=	factor to convert absorbance to concentration [100/absorbance of standard]
94	=	volume correction factor [0.1 ml out of 9.4 total ml analyzed]
1/1000	=	conversion from micrograms to milligrams
100/W	=	conversion back to 100 mg of sample
W	=	weight of sample analyzed
162/180	=	factor to convert from free glucose to anhydro – glucose (as occurs in beta glucan)

4) Fructo-oligosaccharide content was calculated as:

Fructan (% w/w) = A*F*5*V*(1.1/0.2)*(100/W)*(1/1000)*(162/180)

Where:

A	=	PAHBAH absorbance of reaction solution (0.2 ml)
F	=	factor to convert fructose absorbance to concentration
5	=	factor to convert 0.2 ml as assayed to 1.0 ml
V	=	volume (ml) of extractant used (100 ml or 50 ml)
1.1/0.2	=	0.2 ml taken from 1.1 ml of enzyme digest for analysis
W	=	weight of sample extracted (mg)
100/W	=	factor to express fructan as % age flour wt
1/1000	=	conversion from micrograms to milligrams
162/180	=	factor to convert from free fructose to anhydrofructose (as occurs in fructan)

5) Simple sugars (Glucose and Sucrose) content was calculated as:

Glucose (%): (A/0.2)*F*(1/1000)*(50/500)*4*100
or
A*F*0.2

Sucrose (%): ((B-A)/0.2)*F*(1/1000)*(50/500)*4*(342/180)*100
or
(B-A)*F*0.38

Where:

A	=	absorbance for samples treated with acetate buffer
B	=	absorbance of samples treated with invertase
C	=	absorbance of samples treated with
F	=	factor to convert glucose absorbance to concentration
1/100	=	conversion from micrograms to milligrams
50/500	=	5 00 mg sample extracted with 50 ml buffer
4	=	dilution factor for filtered extract
342/180	=	conversion from glucose as measured to sucrose
100	=	factor to express glucose or sucrose as percentage

Appendix B

Human Consent form

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation.

Title of the project: Effect of soy flour addition to the wheat flour on the sensory properties of a *chapatt*i (unleavened Indian flat bread).

Principal Investigator: Anuya Vadnerkar.

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about the *chapatti*. The purpose of this research is to determine if a difference exists between the regular chapattis and the test samples with soy flour added in the ratio of 10%, 20% & 30% w/w.

II. PROCEDURES

There will be 2 sessions over a period of 1 week involving about 10 minutes at each session. You will be presented with 8 samples at each session. As a panelist, it is critical to the project that you attend each session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples.

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide the following information that may be helpful, such as improving the nutritional quality of the chapatti. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have any unknown food allergy. If you are aware of any food allergies related to consumption of soy, and/or wheat gluten kindly notify immediately.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VI. APPROVAL OF RESEARCH

This research has been approved by the Institutional Review Board (IRB) for the projects involving human subjects at Virginia Tech and by the human subject's review of the Department of Food Science and Technology.

VII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require 2 sessions tasting 8 samples of chapattis.

____

Signature/Date.

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes **(optional).**

Address:____

Phone No: ____

---(tear off)---

VIII. SUBJECT'S PERMISSION (provide tear off for human subject to keep).

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study which will require 2 sessions tasting 8 samples of chapattis.

____

Signature/Date.

Should I have any questions about this research or its conduct, I should contact:

Anuya Vadnerkar/ 540-231-7708/ anuyav@vt.edu.
Investigator/Phone (O)/ E-mail ID.

Dr. Lorraine Niba /540-231-8763.
Faculty Research adviser/ Phone (O)/ E-mail ID.

____

Chair, IRB/Phone for research Division : 540-231-6077.

Appendix C

Curriculum Vitae

ANUYA VADNERKAR

Email: anuyav@vt.edu

Current address: 306, Apartment heights drive, Apt #J3
Blacksburg, VA 24060

Phone: (540)2398377

OBJECTIVE:

To apply my academic knowledge, research experience, and laboratory skills towards a challenging position in a team oriented developmental environment in the food industry.
Focus areas: Food product development, starch/cereal/carbohydrate functionality, sensory evaluation, nutrition, research and development.

EDUCATION:

Master's (Human Nutrition Foods and Exercise): Area of Specialization – Food Science and Nutrition, Fall 2004. Overall GPA- 3.56 / 4.0.Virginia Tech, Blacksburg, VA.
Thesis*:* Identifying soluble fiber and resistant starch components in Indian wheat varieties and in wheat-soy product chapatti (unleavened Indian flat bread)".

Post Graduate Diploma (PGD): Dietetics and Hospital Food Service, May 2000. Passed with **first division**
Institute of Hotel Management Catering Technology and Applied Nutrition (IHMCTAN). Mumbai, India.
Dissertation: "Therapeutic Power of Spices".

Bachelors of Home Science: Dietetics, June 1999. Passed with **first class- grade A**
Sir Vithaldas Thackersay College of Home Science (Autonomous) Mumbai, India.

PROFESSIONAL AND CLINICAL EXPERIENCE:

- **Nutrition Educator: Kelloggs India Limited**. Mumbai, India (July 2001- September 2001)
- **Junior Dietitian (Intern):** King Edward Memorial Hospital. Mumbai, India (September 2000-December 2000)
- **Junior Dietitian (Intern):** Holy Spirit Hospital. Mumbai, India (May 2000- August 2000)
- **Undergraduate Dietitian (Intern):** Lilavati Hospital and Medical Research Centre.Mumbai, India. (April 1999- June 1999)

RELEVANT COURSEWORK:

- Food Product Development
- Carbohydrates and Pigments in Foods
- Food Microbiology and Food Preservation
- Meal Management
- Biometry
- Sensory Evaluation of Foods
- Lipids and Colloids in Foods
- Carbohydrate and Energy Metabolism
- Human Physiology
- Biochemistry in Life Sciences

PROJECT LEADERSHIPS AND ORGANIZATIONAL SKILLS:

Sensory Evaluation:

- "Effect of light oxidation and refrigerated storage on physiochemical and sensory properties of organic mango nectar juice"- developed, conducted & communicated results in an oral & a written document
- Group project: Consumer Sensory Testing (Affective - Preference and Hedonic tests) on Chocolate Chips Ahoy at Virginia Tech campus
- Participated as a sensory panelist and attended various training sessions for Quality Descriptive Analysis

Symposium- Role of Carbohydrates as Functional Foods:

- Definition of functional foods – prebiotics and probiotics
- Physiological and functional role of resistant starch, beta glucans and fructo-oligosaccharides as prebiotics and probiotics

RESEARCH AND LABORATORY SKILLS:

- Enzymatic analysis of soluble fiber and starch components in foods
- pH calibration techniques, titrations, preparation of buffers
- Sensory evaluation techniques/tests
- Physio-chemical analysis of cereal flours and gums
- Instrumental (textural and color) assessment
- Food cooking techniques, quantity food production

ORAL COMMUNICATION AND TEAM INTERACTIONS (ACADEMIC & OTHER WORK EXPERIENCE):

- **Graduate Teaching Assistant:** Methods of Human Nutritional Assessment, Virginia Tech, VA. (Spring 2004)
- **Graduate Teaching Assistant:** Metabolic Nutrition; Virginia Tech, VA (Fall 2003)
- **Graduate Teaching Assistant:** Meal Management and Food Communication; Virginia Tech VA. (Spring 2003)
- **Graduate Teaching Assistant:** Basic Foods and Nutrition; Virginia Tech, VA (Fall 2002)
- **Diet Counselor:** "Pace Fitness Centre" Mumbai, India. (March 2002- June 2002)
- **Diet Counselor: "**Figure in Health Care Centre and Gymnasium" Mumbai, India (July 2001-January 2002)

PROFESSIONAL AFFILIATIONS / LEADERSHIP:

Student member: Institute of Food Technologists (IFT).
Student member: American Association of Cereal Chemists (AACC).
King Edward Memorial Hospital. Mumbai, India:

- Conducted community awareness exhibition "Medical Nutritional Therapy for Diabetics".
- Volunteered at a geriatric exhibition "Healthy Aging".
- Volunteered at a cardiac camp (A community nutrition program).

Institute of Hotel management Catering Technology and Applied Nutrition. Mumbai, India:

- Participated and conducted an exhibition and seminar on "Stress and its Implication on Health".

- Presented a dissertation on the topic “Therapeutic Power of Spices” **(Awarded Certificate of Merit).**

PAPERS / PUBLICATIONS:

Poster Presentation: “Resistant starch and beta-glucan content of wheat flat bread (chapati) from various Indian wheat varieties” Niba L, Vadnerkar A; at **AACC- TIA (Tortilla Industry Association), Joint Annual Meeting to be held in September 2004, San Diego.**

HONORS AND ACTIVITIES:

- **P. Howard Massey Food and Nutrition Scholar Award 2004**, Department of Human Nutrition Foods and Exercise. Virginia Tech.
- Selected as a **Session monitor** for 2004 IFT Annual Meeting and Food Expo, Las Vegas, Nevada.
- Certificate of Completion: Training in Human Subject’s Protection in Research.
- Awarded **certificate of merit** for my Post Graduate Diploma dissertation-“Therapeutic power of Spices”
- **Ranked first** in SNDT Women’s University for securing highest marks in **English.**
- Invited as a judge for the “Low Calorie Cooking Contest”- organized by “Figure in Health Care Centre”.

COMPUTER SKILLS:
Microsoft word, excel, power point and its applications, statistical data analysis.