# ENHANCING BREAD USING HIGH TEMPERATURE EXTRUDED LENTIL FLOURS

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Abhay Gupta

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Enhancing Bread using High Temperature Extruded Lentil flours

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# ABSTRACT

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Wheat proteins play a major role in determination of dough rheological properties and quality of baked bread. The main objective of this study was to evaluate the effects of extrusion speeds and lentil flour concentration in wheat flour on dough rheological properties, staleness and sensory attributes. Wheat flour was fortified with 5%, 10%, 15% and 20% lentil flour extruded at 350rpm, 400rpm and 450rpm extrusion speeds. The wheat flour sample (control) was also fortified with non-extruded lentil flour to evaluate the effects of extruded and non-extruded lentil flours on bread characteristics. Bread staling was determined by measuring hardness of the crumb and samples were compared to control. Overall consumer acceptability was evaluated using a nine-point hedonic scale sensory analysis to determine the best wheat flour to lentil flour ratio. Extrusion speeds did not have any effects on overall acceptability of bread. Breads baked from wheat flour fortified with 10% lentil flour were most accepted by sensory panelists in terms of overall acceptability. Therefore, 10% lentil flour breads were tested for staleness and compared to the control sample. Lentil breads had no significant difference in staleness on day one as compared to the control sample. However, on day four and day six, it was observed that lentil breads staled faster than the control bread. It can be concluded that bread with lentil flour had poorer shelf life as compared to breads with only wheat flour.

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## LIST OF ABBREVIATIONS

AACCI ..................................... American Association of Cereal Chemists International

BU ..................................................................................................... Barbender Units

°C ........................................................................................................................ °C

cc ........................................................................................................ Cubic Centimeter

DDT ........................................................................... Dough Development Time

EX ........................................................................................................... Extensibility

F ............................................................................................................... Fahrenheit

G ....................................................................................................................... Grams

GG ............................................................................................................... Guar Gum

GI ............................................................................................................ Gluten Index

GS ........................................................................................................ Gluten Subunit

HPLC ........................................................ High Pressure Liquid Chromatography

Hr ......................................................................................................................... Hour

HMW ............................................................................. High Molecular Weight

IDF ............................................................................... Insoluble Dietary Fiber

IRB ...................................................................... Institutional Review Board

LBG ................................................................................... Locust Bean Gum

LMW ............................................................................. Low Molecular Weight

Mm ............................................................................................................ Millimeter

NDF ......................................................................................... Neutral Dietary Fiber

P ....................................................................................... High Methoxyl Pectin

PER ................................................................................. Protein Efficiency Ratio pH........................................................... Potential for Hydrogen Ion Concentration

Rpm.................................................................................... Rotation per minute

SDS .................................................................................Sodium Dodecyl Sulfate

TDF.......................................................................................... Total Dietary Fiber

XG............................................................................................Xanthan Gum

# INTRODUCTION

Bread is a staple food prepared in many countries by baking wheat flour dough. In some cuisines, bread also is steamed or fried. Common wheat (*Triticum aestivum)* is mainly used for making bread, cakes and pastries. The quality of bread depends on the quality of wheat used as a raw product, which largely depends on the genotype, environment and fertilizer used (McNeal et al. 1971).

Legumes are an important, but inexpensive source of proteins grown in many parts of the world. Lentils (*Lens culinaris*) contribute 2.4% of the world total production for grain legumes. The composition of lentils is approximately 65% to 70% carbohydrates and 25% to 30% proteins. Lentil proteins have digestibility of 93% (Dowsley et al. 1999, Bhattacharya et al. 2005). It is considered to be one of the best and cheapest sources of proteins available (Adsule 1989). Lentil flour has the potential to enhance food products as it contains high quantities of proteins and is gluten free (Swanson 1990). Legumin and vicilin are two main globulins of which legume proteins are comprised. Legume proteins are considered storage proteins (Swanson 1990).

Proteins play an important role in the final texture characteristics of bread. An increase in protein content leads to increased dough strength and enhanced baking quality (Vereijken et al. 2000). Gliadin and glutenin are the two main proteins in wheat flour. Gliadin, a mixture of single polypeptide proteins, is soluble in 60% to 70% aqueous ethanol. Gliadin proteins consist of polypeptide amino acid chains. The molecular weight of the gliadin proteins ranges from 25,000 to 100,000 (Jackson et al. 1983). While glutenin is composed of polypeptide chains linked together by disulfide bonds and are soluble in dilute acid or alkali. Molecular weight ranges from 100,000 to several million (Weegels et al. 1996). Gliadin is low in lysine content compared to glutenin (Caldwell 1983). Lentil proteins are also considered a good source of amino acids, particularly lysine. They contain approximately 4.66 to 6.34 g of lysine per 100 g of proteins (Verma et al. 1977). In conjunction with cereal protein that is relatively high in methionine and cysteine and low in lysine, lentils provide good protein quality in diets (Longnecker et al. 2002). Lentils are also a very good source of dietary fiber, which adds to the nutritive value of lentils. Total dietary fiber content in lentils is approximately 15.8%, approximately 85% to 89% of which is insoluble dietary fiber (Ramulu and Rao 1997). In contrast, common wheat (*Triticum aestivum*) contains 1.8 to 2.3 grams of fiber per 100 grams, which supports the use of lentils as a fiber source (Duke 1983).

Optimum conditions for the extraction of lentil flours, which passed through a 0.79 mm screen, were pH 9.0 at 30°C for the Matilda variety (Chun 2007). While optimal conditions for Digger and Cobber were pH 8.5 at 35°C (Chun 2007). These conditions enable a high yield of lentil starches and proteins, with minimal changes or damage to their quality (Chun 2007). Cooking quality of lentils is largely dependent on the physical properties of the seed. Seeds with a thinner seed coat have shorter cooking time compared to seeds with a thicker seed coat (Hughes and Swanson 1986). Positive effects on physicochemical and textural characteristics of bread by fortifying wheat flour with other types of flour were found by Harinder et al. (2004).

## Hypothesis

Better dough rheological properties and enhanced protein content were hypothesized upon fortifying wheat flour with lentil flour. However, evaluations of sensory attributes and staleness of the product also are of significant consideration in this study.

## Specific Objectives

In this study, wheat flour was fortified with extruded lentil flour (ELF) (350, 400 and 450 rpm) and nonextruded lentil flours (NELF). The main objectives of this study are as follows:

1. To observe effects of extrusion speeds and lentil flour concentration in wheat flour on dough rheological properties;
2. To observe effects of extrusion speeds and lentil flour concentration in wheat flour on staleness of bread;
3. To observe effects of extrusion speeds and lentil flour concentration in wheat flour on sensory attributes of bread; and
4. To evaluate the best wheat flour to lentil flour ratio based on overall acceptability.

# LITERATURE REVIEW

## Wheat Proteins

Flour extracted from common wheat *(Triticum aestivum),* which is also called bread wheat, is used in bread making. Proteins are a major factor influencing the quality of the finished product. An increase in protein content leads to increased dough strength and enhanced baking quality (Vereijken et al. 2000).

### Classification of Wheat Proteins

Wheat proteins are divided primarily into two types: soluble proteins (albumin, globulin and peptides) and insoluble proteins (gliadin and glutenin). Gliadin and glutenin together are also known as gluten proteins (Sahari et al. 2005).

Osborne (1907) classified the wheat proteins based on solubility properties. Albumin and globulin were separated from the other proteins by extracting the flour with low concentration salt solution. Then, gliadin was extracted with an aqueous alcohol solution (70% v/v). Glutenin can be solublized in a base solution and the leftover residue is insoluble glutenin complex.

### Protein Quality

Protein quality of wheat flour can be determined by the amino acid profile. Protein quality of wheat flour is one of the major factors determining the quality of the final product. Anjum et al. (2004) studied amino acid composition of spring wheat and losses of lysine during chapatti baking. They found the limiting amino acid lysine was further reduced upon baking the flour.

### Gliadin

Gliadin, a mixture of single polypeptide proteins, is soluble in 60% to 70% aqueous ethanol. Gliadin proteins are made up of polypeptide chains consisting of amino acids. The molecular weight of the gliadin proteins ranges from 25,000 to 100,000 (Jackson et al. 1983). Gliadin is low in lysine content but contains adequate concentrations of cysteine and tryptophan (Caldwell 1983).

**Glutenin**

Glutenin is composed of polypeptide chains linked by disulfide bonds. It is soluble in dilute acid or alkali (Weegels et al. 1996). The molecular weight of glutenin is higher compared to gliadin proteins. It ranges from 100,000 to several million. Glutenin proteins provide good resistance and elasticity to the dough (Weegels et al. 1996).

**Lysine**

Lysine is the limiting amino acid in wheat proteins. It is one of the eight essential amino acids that serve as the building blocks of proteins. It helps in body growth along with reduction of cholesterol levels in the bloodstream. It also produces cartinine, which converts fatty acids into energy (Calhoun et al. 1959).

Hussein et al. (1979) evaluated quality of wheat grains and eight related products by dose response bioassay. They concluded baladi bread and biscuits prepared from white or dark flour enriched with gluten were low in lysine content with chemical scores ranging between 20 and 49. They also found bread prepared from dark flour and cooked wheat was higher in protein quality compared to other formulations. This shows protein quality was more dependent on cooking of wheat instead of protein content. This agrees with the observation of Pomeranz (1961) who found no correlation between protein content and lysine concentration in protein. He studied the lysine content of bread supplemented with soy flour, wheat gluten, dry yeast and wheat germ. In his study, Pomeranz tested three commercially milled flours and six experimentally milled flours. He found lysine content of high extraction flours was higher when compared to white flours. Calhoun et al. (1959) studied availability of lysine in wheat flour, bread, and gluten. They found the availability of lysine in wheat, flour, and bread was 75%, 72%, and 76%, respectively, with the gluten basal diet. With the amino acid basal diet, 78%, 80%, 83% and 80% availabilities were found, respectively, for wheat, flour, bread, and gluten.

## Dough Rheology of Wheat Flours

Rheological properties of dough influence the dough behavior during its processing and the quality of the finished product. Rheological properties depend on gluten quality and starch – protein interactions (Metakovsky et al. 1997).

Linlaud et al. (2009) studied the effect of hydrocolloids on water absorption of wheat flour by Farinograph and textural characteristics of dough. In this study, they examined the influence of guar gum (GG), xanthan gum (XG), high-methoxyl pectin (P), locust bean gum (LBG), and a mixture of locust bean gum and xanthan gum (LBG+XG) on water absorption. Various techniques were used to test water absorption such as Farinograph, water imbibing capacity, SDS sedimentation test and sucrose solvent retention capacity. It was found that addition of XG and LBG+XG increased absorption values. The addition of guar gum led to higher stability of dough, which was opposite in the case of high methoxyl pectin. Overall incorporation of hydrocolloid led to rheological changes in dough, but the degree of change depended on structure and concentration of the hydrocolloid along with the amount of water added.

Holtekjolen et al. (2008) investigated water absorption capacity and resulting baking performance by baking breads that contained 40% barley flour. Different barley varieties varied in polysaccharide content. They observed large differences in water absorption capacity for different barley – wheat mixtures. Therefore, large differences were also observed in baking performances of the samples. They concluded that different polysaccharide content affected the water absorption capacity and baking quality of the sample flours. They also found β-glucan was the most important factor for the baking performance due to its major influence on the water absorption capacity. Increasing barley content in the sample led to reduction of gluten, which affected the baking performance significantly. This is in agreement with the observations made by Kim et al. (2007), who studied the dough rheology of various wheat flours and concluded that strong wheat flours had greater resistance to stretching when compared to weak wheat flour indicating dough strength is related to gluten quality.

Vetrimani et al. (2004) studied the effect of extraction rate of wheat flour on the quality of vermicelli. They concluded the water absorption of vermicelli dough increased from 35% to 42.5% with increase in flour extraction rate. There was no change in color with increases in extraction rate from 66% to 80%. They reported that making vermicelli from 100% extraction rate flour may result in a product of better nutritional quality.

Brun et al. (2006) examined the capability of mixolab by testing water absorption, development time, stability and softening. In this study, 30 samples of different types of flour from around the world were collected and subjected to rheological tests on mixolab and Farinograph were conducted simultaneously. The obtained results demonstrated no significant differences between values of mixolab and Farinograph. Therefore, it was concluded that mixolab results are comparable to that of Farinograph. In a similar study, Koksel et al. (2009) investigated potential utilization of mixolab for quality evaluation of bread wheat genotypes. The results obtained from the mixolab and Farinograph were found to be identical. Therefore, it was concluded that mixolab was equivalent to Farinograph for dough rheological testing.

### Effects of Baking on Protein Starch Matrix

Protein and starch molecules are bound to each other in a matrix in wheat. The strength of the protein starch matrix depends on the hardness of wheat. For instance, the strength of bonds between protein and starch molecules in durum wheat is higher than common wheat (Anjum et al. 2006). On comparing the changes by scanning electron microscopy, soft wheat shatters more when compared to durum wheat. There is a clear separation of starch granules and protein granules in the soft wheat as opposed to durum wheat where no clear-cut separation is found. Moreover, the amount of starch damage is less in soft wheat when compared to durum wheat (Muhammad et al. 2003).

Park et al. (2008) studied the effects of starch granule size distribution of hard red winter and hard red spring wheat on mixing and bread making quality. They found protein content was inversely correlated with parameters of B – granules (< 10 μm in diameter). Moreover, crumb grain score also showed inverse correlations with B – granules content. They concluded there is an optimum range of B – granules for different protein content flour to produce bread with better crumbs. These observations are in agreement with the observations made by Lelievre (1987) who studied the effects of starch particle size and protein concentration on bread making performance. In this study, starch granules of three size classes were prepared by sedimentation fractionation. Starch granules were recombined with gluten at protein concentrations of 8%, 12% and 16% respectively. Protein – starch interactions were formed and contributed in forming loaf texture and volume. Starch fraction affected toughness of the breadcrumb. The softness of chew was found to be dependent on the protein level. Bites and chew properties were affected by protein content.

Prabhashankar (2002) studied the changes that take place in starch and protein molecules of South Indian parotta (an unleavened flatbread) by scanning electron microscopy and electrophoresis. A fine matrix of proteins and soluble solids with starch granules was observed at the processing stage of parotta dough into a thin sheet. Clear differences were observed between the layers of parotta. In the outer layer, the extent of starch molecule distortion was higher than the inner layer. Moreover, electrophoretic patterns also revealed less intense bands were observed in HMW regions of baked parotta (Indian flat bread). Indrani et al. (2007) also studied effects of whey protein concentrate on dough quality. They observed fine gluten matrix in Indian parotta. They concluded that parotta fortified with 5% whey protein concentrate was softer in texture compared to control parotta.

## Lentils and Their Classification

Lentils, commonly referred to as red dhal, masur, burssum, lenteja, lentille, mansoor or split peas, are botanically classified as *Lens culinaris* (Dowsley et al. 1999). The genus Lens is composed of four main species: *L. culinaris, L. odemensis, L. nigricans* and *L. ervoides.* There are two subspecies of Lens culinaris: the cultivated lentil ssp. *culinaris* and the wild ssp. *orientalis* (Dowsley et al. 1999). Lentils are free growing sub-erect to erect plants depending on the growing condition. Lentils are divided into two types based on color: 1.) Green lentils (brown, yellow, and Chilean, Continental or Macrosperma lentils) have a green to brown seed coat with yellow cotyledons and 2.) Red lentils

(Microsperma or Persian lentils) have a pale grey to dark seed coat with red cotyledons (Dowsley et al. 1999).

## Nutritional Value of Lentils

Lentils are a good source of proteins, vitamins and minerals. They have heterogeneous seeds with varied chemical composition. Lentils contain approximately two-thirds carbohydrates and approximately 24% to 31% of highly digestible protein with a protein digestibility of 93% (Bhattacharya et al. 2005). This is also in agreement with the observations made by Khan et al. (1979) who described the protein quality of lentil by the Protein Efficiency Ratio method. They found the cooked lentil had a value of 2.17. Verma et al. (1977) also used the PER value to describe the protein quality of lentils, and found the PER value obtained for seven different varieties of lentils in Ludhiana, India ranged from 0.95 to 1.27, compared to that of the standard skim milk powder, which had a PER value of 3.13. Both Khan et al. (1979) and Verma et al. (1977) concluded lentils have good protein digestibility. Porres et al. (2002) evaluated the digestibility of protein components in lentil seeds. They reported autoclaving is not a good technique to improve the protein digestibility. It was found that the autoclave method increases leucine and lysine absorption and decreases tyrosine and methionine absorption.

Lentils are a good source of amino acids, especially lysine. Verma et al. (1977) found lentils generally contained 4.66 to 6.34 g of lysine per 100 g of proteins. Adsule (1989) added the lentil proteins, like other legumes, are generally deficient in sulfur amino acids: methionine (0.48 to 0.66 g/100g of protein), cysteine (0.46 to 0.59 g/100g of protein) and tryptophan (0.85 to 1.14 g/100g of protein). Longnecker et al. (2002) found upon using lentils in conjunction with cereal proteins that are relatively high in methionine and cysteine and low in lysine provides a balanced diet with good protein quality.

Lentils are also considered an excellent source of dietary fiber (Sotomayor et al. 1999, Rosin et al. 2002). Sotomayor et al. (1999) reported raw lentil generally had approximately 51% (dry matter) starch concentration, while Ramulu and Rao (1997) found the total dietary fiber content present in lentil is approximately 15.8%, approximately 85% to 89% of which is insoluble dietary fiber (IDF). The results of this study were found to be identical with the results of studies done by Rosin et al. (2002), who found lentil generally had 59.00 ± 0.2% (dry matter) starch, approximately 15% (dry matter) total dietary fiber (with about 13% IDF) and approximately 5% (dry matter) resistant starch.

## Physical Properties of Lentils

Amin et al. (2004) studied physical properties of lentil seeds including diameter, thickness, unit mass, volume, bulk density and porosity. They reported some physical properties of lentil seed increased linearly with increase in moisture content from 10.3% to 21. 0%. For example, in their study, the diameter increased from 3.84 to 4.06 mm, thickness increased from 2.18 to 2.48 mm, porosity increased from 34.5% to 37.0%, mass of 1000 seeds increased from 20 to 25.5 g and angle of repose increased from 24.8° to 27.8°, while bulk density and kernel density decreased linearly with increased moisture content. Bhattacharya et al. (2005) also reported the bulk densities of whole lentil seed decreased with increasing moisture contents. Moreover, Carmen (1996) reported physical properties such as diameter, thickness, unit mass, volume, bulk density and porosity increased with increasing moisture content of Turkish lentil seeds.

## Extrusion of Lentil Flour

Berrios et al. (2009) determined carbohydrate composition of raw and extruded pulse flours. They found extrusion processing did not affect the concentration of total available carbohydrates. However, extruded pulse flours showed a significant increase in fiber content. This indicated extrusion processing of lentil, chickpea or dry pea is beneficial in producing value added, nutritious snack products with high fiber content. This conclusion agrees with the observations made by Antila et al. (1983), who studied effects of extrusion cooking on the quality of bread by using lower quality ingredients. They found extrusion had positive effects on flatbreads produced by lower quality ingredients.

Chun (2007) studied the physio-chemistry and rheology of Australian lentil flour and analyzed their implications on extrusion. It was found that among three varieties of Australian lentils, Maltida had the largest physical dimensions and protein content. No differences in physical or chemical properties were observed between Digger and Cobber varieties. The optimum conditions for the extraction of lentil flours, which passed through a 0.79 mm screen, were pH 9.0 at 30°C for Matilda and pH 8.5 at 35°C for both Digger and Cobber (Chun, 2007). These conditions enable a high yield of lentil starches and proteins, with minimal changes or damage to their quality. It was also concluded that lentil starch had a better expansion ratio compared to wheat starch upon extrusion. The lentil flours had poorer pasting quality and expansion, possibly because of interference by their lipid and protein components. Starch rheological behavior was greatly affected by the amylose/amylopectin ratio and the molecular weight of starches, while flour behavior was more influenced by the interfering components (Chun 2007).

Li and Lee (2000) studied the effect of extrusion temperature on the solubility and molecular weight of lentil bean flour proteins containing low cysteine residues. They extruded the lentil flour at die temperatures of 135, 160, and 175 °C. They reported the soluble protein content in the extrudates decreased by 40.1% in the extracting buffer (1% sodium dodecyl sulfate in 50 mM sodium phosphate buffer, pH 6.9) as the extrusion die temperature was increased to 175 °C. Most insoluble proteins extruded at temperature up to 175 °C could be resolublized by sonication. It was also observed that temperature of extrusion did not have much effect on the solubility and molecular weight of the lentil proteins.

## Cooking Quality of Lentils

Shorter cooking time is one of the most important aspects of processing. Lentils generally have shorter cooking time than other legumes (Hughes and Swanson 1986). Cooking time of lentils is dependent on the structure of the kernel, especially thickness of the seed coat. Lentils with thinner seed coat have shorter cooking time when compared to peas and beans, which have thick seed coats (Hughes and Swanson 1986). Tang et al. (1994) reported cooking quality of lentils was also dependent on the initial moisture content of the seed as it affects the moisture uptake in moisture absorption process. Moisture migration occurs through seed coat in seeds with higher moisture content while low moisture content results in reduced moisture migration in seeds (Tang et al. 1994). Due to low moisture content, permeability of seed coat is reduced and hilum opening is closed due to hygroscopic shrinkage (Tang et al. 1994). Soaking lentils also affects fiber content. Rehman and Shah (2004) reported that soaking lentils in $NaHCO_3$ increases neutral detergent fiber (NDF) and reduces acid detergent fiber (ADF). Cooking lentils by various methods for various time period leads to reduction of various dietary fiber components. Ordinary and microwave cooking reduced NDF content by 21.7% to 27.3% and 21.0% to 24.5%, respectively. Upon pressure-cooking, 28.5% to 35.3% reduction in NDF content was observed. It was concluded that pressure-cooking led to maximal loss of NDF content. Therefore, lentils should be cooked either by ordinary cooking or microwave cooking in order to minimize fiber losses.

Singh and Rao (1995) studied the quick cooking quality of pigeon pea dhal as influenced by soaking solutions and enzyme treatment. They found cooking quality of dhal can be influenced by pre-soaking it in different types of soaking solutions. Soaking pulses in salt solution instead of water reduces cooking time. In this study, various salt solutions were evaluated, and sodium bicarbonate ($NaHCO_3$) was found to be most effective in reducing cooking time in pigeon pea dhal. Although it was also reported that soaking dhal in a salt solution of more than 1% can lead to low consumer acceptance. Pectinase enzyme treatment was found to be more effective compared to salt solution in terms of consumer acceptance. Observations made by Singh and Rao (1995) were in agreement with the observations made by Abou – Samaha et al. (1984) who concluded soaking lentils in saline water leads to reduction of cooking time and losses. Although Prodanov et al. (2004) reported pre-soaking of lentils prior to cooking leads to loss of nutritional quality. He studied the influence of soaking and cooking on the thiamine, riboflavin and niacin contents of legumes. They found vitamins such as thiamine, riboflavin and niacin were lost upon soaking lentils prior to cooking.

## Protein Functionality of Lentils

Swanson (1990) studied pea and lentil protein extraction and functionality. His study demonstrated that peas and lentils can be used as protein sources for flours, concentrates and isolates. He also found pea and lentil protein extracts have comparable protein functionality compared to soy protein extracts. Pea protein isolates exhibited better foaming properties but needed to be more concentrated compared to soy protein isolates. Bora (2003) studied effects of acetylation on functional properties of lentils. He reported acetylation leads to a shift in the isoelectric pH (towards neutral or alkaline) of the acetylated lentils globulins compared to native globulins. This results in an improved water absorption capacity and decreased oil absorption capacity due to increased solubility of the globulins at neutral to alkaline pH. He concluded acetylation results in improved relative viscosity, emulsifying activity and the foaming capacity of lentil globulins.

## Chemistry of Wheat and Lentil Starches

Starch is a carbohydrate comprising of a large number of glucose units linked by glycosidic bonds (Brown et al. 2005). It is a white, tasteless and odorless powder that is insoluble in cold water or alcohol. It consists of amylose and amylopectin (Brown et al. 2005). Extrusion temperature has significant effects on starch properties (Bhattacharya et al. 1987). They reported that increases in extrusion temperature led to increases in starch gelatinization. This is in agreement with observations made by Ibanoglu et al. (1996) who studied effects of barrel temperature and screw speeds on starch gelatinization and reported that screw speed did not have any significant effects on starch gelatinization. Starch gelatinization is a process that breaks down intermolecular bonds of starch molecules in the presence of water and heat (Stanley et al. 2001). This leads to incorporation of more water into the structure. Therefore, upon heating, starch granules swell and ultimately burst releasing starch liquid. This leads to better digestion of starch (Bhattacharya et al. 1987). Swelling of starch also leads to thickening of the liquid.

Starch retrogradation is the reversal of starch gelatinization. It occurs if gelatinized starch is kept at low temperature for a long time. This results in rearrangement of amylopectin and amylose structures, which leads to formation of more crystalline structure (Elliason et al. 1993). Starch retrogradation directly affects staling of bread; faster retrogradation, leads to faster staling (Gonzalez et al. 2002). Retardation in starch retrogradation can lead to retardation in staling speeds of breads. Gujral et al. (2003) reported that effects of starch hydrolyzing enzymes (alpha amylase) of intermediate thermostability and cyclodextrin glycoxyl transferase [CGTase] on starch addition leads to reduction of amylopectin retrogradation.

## Effects of Fortification of Wheat Flour with Other Flours

Several studies have been conducted in the past to evaluate changes in the nutritional value upon fortifying wheat flour with other flours. Attempts have also been made to determine the best ratio in terms of consumer acceptability. Kailasapathy et al. (1985) studied the changes in nutritional value of wheat bread on fortifying wheat flour with full fat winged bean flour. They observed that upon increasing bean flour concentration from 0% to 20% there was an increase of 63% in protein, 153% in fat, 130% in calcium, 97% in phosphorus, and 105% in iron contents. A significant increment in the amounts of histidine and lysine along with other essential amino acids was also observed by increasing bean flour concentration from 0% to 15%. Similarly, bread made from 15% bean flour concentration showed higher protein efficiency ratio compared to 5% bean four concentration or pure wheat flour. Khetarpaul et al. (2009) studied the effects of composite flour fortification to wheat flour on the quality characteristics of unleavened bread. They also found the unleavened bread prepared from composite flour had better protein quality and moisture content. It was also observed that the unleavened bread had better sensory attributes when compared to the unleavened bread prepared from unprocessed flour.

Therefore, it was concluded that incorporation of soy, sorghum, maize, rice and pearl millet flour to wheat flour could enhance the nutrient content of unleavened bread. Harinder et al. (2004) observed that increases in pigeonpea flour content in wheat flour led to increases in protein and mineral content of the baked products. In their study, they examined baking properties of wheat flour blended together with pigeonpea flour to bake bread, chapatti and cookies. Pigeonpea flour was substituted for wheat flour at various levels for bread and chapatti making and up to 50% for cookie making. They also reported wheat flour with 10% pigeon pea flour was the best formula for bread from high loaf volume and loaf quality standpoint. Although Tyagi et al. (2006) reported, for biscuits, 15% is the optimal concentration of mustard flour in wheat flour from a nutritional, sensory and textural standpoint. Because of mustard flour incorporation, protein content of biscuits increased 2.5 times along with increase in fiber content and decrease in fat. Tonella et al. (1985) found no significant difference in the overall acceptability of bread baked from fortified samples or unfortified samples upon fortifying wheat flour with lysine and methionine.

Khoshgoftarmanesh et al. (2010) studied nutritional quality and sensory attributes of bread fortified with iron and zinc. They found fortifying wheat flour with iron and zinc led to enhanced nutritional quality of bread. They observed however, fortifying wheat flour with ferrous sulfate resulted in poor color and taste, which impaired the overall acceptability of bread. They concluded fortifying wheat flour with zinc led to better nutritional quality and overall acceptability, but that was not the case with ferrous sulfate.

Albaldawi et al. (2005) studied the effect of flour fortification with haem liposome on bread and bread dough. They observed significant improvement in the stability and rheological characteristics of the dough. Fortification of haem liposome also led to an increase in fat content and improvement in uniformity of loaf volume and crumb.

Therefore, it was concluded that haem liposome can serve as a good supplement in order to increase nutritional quality of bread.

# MATERIALS AND METHODS

## Materials

Lentils were obtained from Premium Pulses Incorporation at Minot, ND. Lentils were then ground to flour using a Fitzmill and extruded using a Wenger twin-screw extruder TX 52 at screw speeds of 350rpm, 400rpm and 450 rpm. Unextruded lentil flour was also used in dough rheology tests and baking studies. Three lots of wheat flour were purchased from Food Services of America.

## Methods

### Grinding of Lentils

Lentils were ground using Fitzmill operated at an auger feed rate of 17 rpm and mill speed of 7200 rpm. Flour that passed through a 20-mesh sieve was used in the extrusion experiments.

### Extrusion of Lentil Flour

Lentil flour was extruded using a Wenger twin-screw TX-52 extruder located at the Northern Crops Institute. Lentils were dried using an ambient drying method. In this method, lentils were left at room temperature for 6 – 10 hours. Extrusion conditions included barrel length of 130 cm and twin screws of 5.25 cm in diameter. Flour was extruded at screw speeds of 350rpm, 400rpm and 450 rpm at hydration level of 42%. Lentils were extruded at a barrel temperature of 50 °C and die temperature of 105 °C. Extrudates were ground again using a Fitzmill. However, flour that passed through a 40-mesh sieve was used in baking studies.

### Sampling

Common wheat flour was blended with lentil flours extruded at different rotation speeds (350rpm, 400rpm and 450 rpm) and unextruded lentil flour at concentration levels of 0% (control), 5%, 10%, 15%, and 20 %.

## Flour Quality Evaluation

### Moisture Content

Moisture content of flours plays a vital role in evaluation of rheological properties of dough. An air oven method (AACCI Method 44 – 15 A, AACC 2000) was used to determine moisture content of the flours. In this method, 2 g of flours were weighed in tarred moisture dishes. Dishes were covered and tarred weights were subtracted. Weight of the samples was recorded. Dishes were uncovered and put in an air oven at 135°C for 60 minutes. Dishes were covered immediately, cooled in a dessicator and weighed again. Previous weight was subtracted from this weight and percent moisture was calculated.

### Ash Content

The basic ash determination method (AACC Method – 01.01, AACC 2000) was used to determine ash content of the flours. In this method, 3 g of sample was weighed in ashing dishes. Dishes were put in a muffle furnace at 550°C for 15 hours. Dishes were weighed after the 15 hours and the previous weight was subtracted to calculate the ash percentage. Percent Ash weight of residue sample weight $=\times 100$

### Protein Content

Protein content of the flour was determined using AACCI standard method 46 – 30, (AACCI 2000). In this method, protein content of the sample flours and control flours was determined using a Leco protein analyzer. Protein percentage was calculated as crude protein percent, which equals the percent Nitrogen x 5.7 for wheat flour and 6.25 for lentil flour.

## Dough Rheology – Mixolab Analysis

Water absorption levels of each sample dictated the amount of water added during the baking process of the dough. Water absorption of the sample and control flours was determined using Mixolab. Mixolab was used to determine dough rheological properties instead of Farinograph because of its time efficiency and accuracy. Mixolab works as Farinograph and Rapid Visco Analyzer. In this, 50 grams of sample being tested was placed into the blades of the mixolab. Based on the moisture content of the sample, mixolab software calculated the amount of water to be added in the sample in order to analyze its rheological properties. Water was automatically fed via integrated water circuit based on the hydration level and moisture content of the sample. Blades ran in opposite direction for approximately 45 minutes to ensure enough dough development. The resistant torques exerted on the blades was measured by a sensor located on the axis of one of the blades. A hydration level of 60% was selected as default at base moisture level of 14%. If the water absorption level fell outside the tolerance level of 1 nm to 1.2 nm, the test was redone using a different amount of sample suggested by mixolab software to obtain water absorption levels within the tolerance levels. After obtaining the correct sample size, water absorption level was recorded automatically by mixolab software.

## Baking Performance of Lentil Flour Bread

After determination of water absorption capacity of the sample flours, samples were baked using the straight dough method used at Northern Crops Institute, North Dakota State University. The following formula was used in order to bake the bread:

| Name of Ingredient: | Amount in g |
|---|---|
| Sample Flour | 100 g |
| Instant Yeast | 0.87 g |
| Salt | 1.74 g |
| Sugar | 5.22 g |
| Oil | 3.48 g |
| Baker's Emplex Supreme | 0.43 g |
| Water | Based on Water absorption capacity |

In this process, ingredients were mixed on a Hobart A – 120T mixer. Dough was mixed between three to five minutes or until it was thoroughly mixed. Dough was fermented for 1 hour at 30 °C. Dough was scaled to 100 g, rounded and covered in a plastic container and allowed to rest for 15 minutes. It was sheeted and molded on a Moline sheeter/molder. Bread was panned and proofed for 60 minutes at 40 °C at 85% RH. Loaves were then baked at 198.8 °C for 24 minutes. Bread was cooled for 1 hour before being bagged.

**Sensory Evaluation of Breads**

Sensory attributes of breads were evaluated using a nine-point hedonic scale (see appendix for IRB approval). A sensory panel of eight panelists from different ethnic backgrounds was recruited in order to analyze the overall acceptability of the new bread. Bread samples were given random codes in order to prevent biased results. Sensory tests were conducted twice each day (morning session and afternoon session). Based on the results of the sensory evaluation, the most accepted bread was selected for further analysis. IRB forms and approval are shown in Appendix I.

## Texture Analysis of Breads

Bread samples that received the most satisfactory results were evaluated for hardness on day one, four and six using a Brookfield Texture Analyzer (AACC Method 74-09.01) in order to determine staleness. In this method, two slices of 25 mm thickness total (2 slices of 12.5 mm thickness) were put on the texture analyzer. A cylindrical probe was selected for the test. The compression plunger was set to 1 mm above the center of the sample. Crosshead speed was set to 100mm/min and 40% compression depth was selected. After measuring the reading, the slice was discarded and the plunger was brought back to the original position.

## Statistical Analysis

All experiments were replicated three times to ensure the accuracy of results. Results were analyzed using two way ANOVA (Analysis of Variance). F test was significant at $P \leq 0.05$. Means were separated by Fischer's Least Significant Difference (LSD) at $P = 0.05$. SAS software was used to statistically analyze all data.

# RESULTS AND DISCUSSION

## Physicochemical Characteristics of Flours

Bread wheat (*Triticum aestivum)* flour (control) was fortified with high temperature extruded lentil flour (samples). Moisture, ash and protein contents of sample flours were determined. Results of sample flours were compared to the control. Table 1 shows effects of extrusion speeds on physicochemical properties of flours and Table 2 shows effects of lentil flour concentration on physicochemical properties of flours.

### Moisture Content

Moisture content was tested by using an air oven (AACC Method 44 – 15 A, AACC 2000). From Table 1 it can be observed that extrusion speeds had no significant effect on the moisture content of flours. This could be due to equivalent moisture content between extruded flour and unextruded flour (Table 1).

Table 2 shows significant differences in moisture content of flours based on lentil flour concentration. With the increase in lentil flour concentration in flours, moisture content of sample flours decreased. The decrease in sample moisture content with an increase in lentil flour percentage was expected because moisture content of pure lentil flour was 10.9% compared to 12.7% of pure wheat flour.

### Ash Content

Lentil flour percentage in the samples also played an important role in influencing ash content. Table 1 shows that extrusion speeds had no effect on the ash content. This observation was in agreement with the observations made by Obatolu et al. (2005), who reported that screw speeds were not related to ash content of flours. Table 2 shows the higher the percentage of lentil flour the higher the ash content. The means of ash

**Table 1. Effects of Extrusion Speeds on Physicochemical Properties of Wheat Flours Blended with Lentil Flours**

| Extrusion Speed (rpm) of Lentil Flours | Moisture % | Ash % | Protein % |
|---|---|---|---|
| 350 | 12.4a | 0.7a | 14.6a |
| 400 | 12.2a | 0.7a | 14.7a |
| 450 | 12.4a | 0.7a | 14.6a |
| Unextruded | 12.4a | 0.7a | 14.9a |

[a] Values followed by the same letter in the same column are not significantly different from each other
[b] Values lentil flour % were averaged to analyze effect of extrusion speeds

**Table 2. Effects of Lentil Flour Concentration on Physicochemical Properties of Flours**

| Lentil Flour Concentration (%) | Moisture % | Ash % | Protein % |
|---|---|---|---|
| 5 | 12.5a | 0.6d | 14.2c |
| 10 | 12.4ab | 0.7c | 14.5bc |
| 15 | 12.2b | 0.8b | 14.9ab |
| 20 | 12.2b | 0.9a | 15.1a |
| Control (wheat Flour) | 12.7c | 0.5e | 13.9d |

[a] Values followed by the same letter in the same column are not significantly different from each other
[b] Values of extrusion speeds were averaged to analyze effect of lentil flour %

content based on lentil flour concentration were significantly different from each other. This was also expected because pure lentil flour has an ash content of 2.4% when compared to wheat flour (control) 0.5% (Table 1).

**Protein Content**

Table 1 shows the extrusion speeds have no significant effect on protein percentage of the flours. This was expected because extrusion affects protein quality and dough rheological properties instead of protein content (Chun et al. 2007). Table 2 shows a significant increase in protein content as the amount of lentil flour in wheat flour was increased. Therefore, it can be concluded from the observations that lentil flour percentage had a significant effect on the protein content of the flours while extrusion speeds had no effect. This was expected, since the protein content of pure lentil flour was 25.3%, which is very high compared to the protein content of wheat flour (13.9%). Increase in protein content in wheat flour by fortifying it with lentil flour is beneficial because lentil flour is high in lysine content, an essential amino acid for the human body (Li et al. 2000).

## Dough Rheology – Mixolab Properties of Flours

Rheological properties of sample flours and control were evaluated with the Mixolab. Results of Mixolab analysis of sample and control flours are given in Table 3 and Table 4 and discussed as follows.

**Water Absorption**

The amount of water added in the bread formulation was based on the water absorption of the samples. Water absorption of wheat flour (control) was 62.4%, whereas water absorption for unextruded lentil flour was found to be 61.7% (Table 3). Extrusion speeds had little effect on the water absorption of samples although significant differences were observed between water absorption of extruded flours and unextruded flours. Water absorption of unextruded flours was found to be much lower compared to that of extruded flours. This could be due to starch gelatinization of flour during the extrusion process (Rayas – Duarte et al. 1998). Ibanoglu et al. (1996) studied effects of barrel temperature and screw speeds on starch gelatinization and reported that screw speed had no significant effects on starch gelatinization. This also agrees with the observations made by Lazou et al. (2009) who reported that extrusion temperature was the main factor affecting water absorption. Table 4 shows that lentil flour concentration had significant effects on water absorption. Water absorption significantly increased from 5% lentil flour concentration to 20% lentil flour concentration (Table 4). This was expected since the lentil flour was extruded and extrusion leads to slowing of starch retrogradation because during retrogradation amylose, amylopectin molecules retrograde and rearrange themselves back to a more crystalline structure, which leads to expulsion of water and decrease in water absorption. This is opposite of starch gelatinization, which leads to an increase in water absorption due to realignment of amylose and amylopectin losing their crystalline structure. Therefore, dough formed by fortifying wheat flour with lentil flour led to increased water absorption (Gonzalez et al. 2002).

**Dough Amplitude**

Dough amplitude is a measure of dough elasticity, measured by the curve width. The higher the value of dough amplitude, the higher is the elasticity (Marco et al. 2008). Dough elasticity is mainly dependent on gluten composition (Shewry et al. 2002). Results of dough amplitude of sample and control flours are shown in Tables 3 and 4. Table 3 shows no significant differences were observed in dough amplitude due to extrusion speeds

**Table 3. Effects of Extrusion Speeds on Dough Rheology of Wheat Flours Blended with Lentil Flours**

| Extrusion Speed (rpm) of Lentil Flours | Water Absorption % | Amplitude % | Stability (Sec) |
|---|---|---|---|
| 350 | 66.3a | 0.08a | 324.5b |
| 400 | 66.2a | 0.08a | 297.4bc |
| 450 | 66.9a | 0.08a | 294.5c |
| Unextruded | 61.7b | 0.09b | 513.7a |

a. Values followed by the same letter in the same column are not significantly different from each other
b. Values of lentil flour % were averaged to analyze effect of extrusion speeds

**Table 4. Effects of Lentil Flour Concentration on Dough Rheology of Flour Blends**

| Lentil Flour Concentration (%) | Water Absorption % | Amplitude % | Stability (Sec) |
|---|---|---|---|
| 5 | 63.7c | 0.08a | 519.1a |
| 10 | 65.1b | 0.08a | 376.2b |
| 15 | 66.3a | 0.07b | 268.9c |
| 20 | 66.4a | 0.07b | 265.9c |
| Control (Bread Flour) | 62.4d | 0.08a | 616.3d |

a. Values followed by the same letter in the same column are not significantly different from each other
b. Values of extrusion speeds were averaged to analyze effect of lentil flour %

amplitudes of samples fortified with unextruded lentil flour and extruded lentil flour. The results were expected because extrusion of lentil flour leads to disruption of the gluten network, which leads to a decrease in dough elasticity (Esselink et al. 2003). Table 4 shows the control had better dough elasticity compared to sample flours fortified with lentil flour.

This could be due to a decrease in gluten percentage by addition of lentil flours (Shewry et al. 2002; Swanson et al. 1990). It can also be observed that an increase of lentil flour concentration in samples led to a further decrease of dough amplitude, which led to decrease in gluten that weakens dough amplitude (Shewry et al. 2002).

**Dough Stability**

Dough stability is dough resistance to breakdown during kneading. It gives a measure of dough strength. The higher the stability, the stronger the dough (Chun et al. 2007). Effects of extrusion on dough stability are shown in Table 3 and effects of lentil flour concentration on dough stability are shown in Table 4. Table 3 shows significant differences in dough stability due to extrusion speeds. Significant differences in dough stability were also observed between extruded and unextruded flours. Samples fortified with unextruded lentil flour had higher dough stability, (i.e., dough strength, as compared to samples fortified with extruded lentil flours). Dough stability of sample flours fortified with extruded lentil flours ranged from 178.6 sec to 508.3 seconds while for sample flours fortified with unextruded lentil flours ranged from 477.3 seconds to 575.7 seconds. These results were expected due to higher starch viscosity in unextruded flour samples compared to extruded flours (Nwabueze 2006). Table 3 also shows extrusion speeds had a significant effect on dough stability. On increasing screw speed, a decrease in dough stability was observed. This could be due to a decrease in starch viscosity due to higher shear and stress at high screw speeds (Guha et al. 1998). Table 4 shows effects of lentil flour concentration on dough stability. It can be seen from Table 4 that lentil flour concentration had a significant effect on dough stability. Samples fortified with lentil flour at various concentrations were significantly different from each other. The higher the lentil flour concentration, the lower the dough stability, and the weaker the dough strength. Samples with lentil flour concentration of 5% had the highest stability. Stability ranged from 492 seconds to 575 seconds for the lentil blends. The strength of flours with 5% lentil flour concentration was highest while samples with lentil flour concentrations of 20% had the lowest stability (i.e., weakest dough strength). This was as expected because pure wheat flour (control) had a stability of 616.3 seconds, which is higher than all of the samples. This is due to higher gluten content in wheat flour when compared to lentil flour (Abang et al. 2009). Abang et al. (2009) found a positive correlation between gluten content and dough strength. Therefore, upon increasing the lentil flour concentration in samples, the gluten became diluted and stability decreased significantly.

**Sensory Evaluation of Breads**

After determination of water absorption with the mixolab by the optimized straight dough baking method (AACC Method 10-10.03), two replicates of samples were baked to ensure accuracy of the data for statistical analyses. Baked bread samples were tested for overall acceptability by eight sensory panelists. Results of sensory evaluation based on extrusion speeds and lentil flour percentage are shown in Table 5 and Table 6. Table 5 shows effects of extrusion speeds on consumer acceptability of baked breads. Extrusion speeds did not have any significant effects on consumer acceptability, although significant differences were observed between extruded and unextruded lentil flours.

Breads baked from extruded lentil flour had better overall acceptability when compared to breads baked from unextruded lentil flours. This was expected because extrusion of flours leads to better texture characteristics of bread (McWatters et al. 2004). Table 6 shows effects of lentil flour concentration on consumer acceptability of baked breads. Lentil flour concentration had a significant effect on consumer acceptability. Based on the sensory evaluation, bread baked from wheat flour fortified with 10% lentil flour was found most suitable in terms of overall acceptance with the highest rating ranging from 4.75 to 7.31. General comments by sensory panelists for bread samples with 10% of lentil flour concentration supported acceptability. Most sensory panelists liked the taste and aroma of these breads. Although breads baked with 15% lentil flour concentration had the poorest comments. Most sensory panelists found the flavor too strong.

Breads baked with 15% lentil flour concentration were least acceptable with ratings ranging from 3.18 to 4.43. Extrusion speeds had no significant effect on overall acceptability of breads. Therefore, breads baked with 10% lentil flour concentration at 450rpm extrusion speeds were selected for texture analysis.

**Table 5. Effects of Extrusion on Consumer Acceptability of Baked Breads made from Wheat Flours Blended with Lentil Flours**

| Extrusion Speeds (rpm) of Lentil Flour | Sensory Evaluation Rating |
|---|---|
| 350 | 5.9a |
| 400 | 5.7a |
| 450 | 5.4a |
| Unextruded | 4.5b |

a. Values followed by the same letter in the same column are not significantly different from each other
b. Values of lentil flour % were averaged to analyze effect of extrusion speeds

**Table 6. Effects of Lentil Flour Concentration on Consumer Acceptability of Breads**

| Lentil Flour Concentration (%) | Sensory Evaluation Rating |
|---|---|
| 5 | 5.5b |
| 10 | 6.6a |
| 15 | 4.0c |
| Control (Bread Flour) | 7.2d |

a. Values followed by the same letter in the same column are not significantly different from each other

b. Values of extrusion speeds were averaged to analyze effect of lentil flour %

## Staleness of Breads

Based on results of the nine-point hedonic scale sensory analysis, it was observed that extrusion speeds had no significant effect on the consumer acceptability of breads (Table 5). It was also observed that breads baked from wheat flour fortified with 10% lentil flour were most accepted by the sensory panelists (Table 6). Therefore, 10% lentil flour breads along with control were tested for staleness by measuring hardness of the bread at one, four and six days using a Brookfield Texture Analyzer (AACC Method 74-09.01). Results of staleness analysis showed no significant difference was observed in hardness on day one between control and 10% lentil bread (Table 7). Table 7 also shows staleness analysis on day four and day six. On day four and six, bread baked from wheat flour fortified with 10% lentil flour concentration was found to be significantly harder when compared to the control bread. Hardness of sample bread increased faster as compared to control bread from day one to day six. This indicates breads baked from lentil flour fortified flour staled quicker than control bread. This was according to expectations since staleness is directly related to starch retrogradation and lentil starches retrograde faster when compared to wheat flour starch (Gonzalez et al. 2002). It has been reported that retrogradation of lentil starches can be reduced by chemical modifications (Hoover & Sosulski, 1986). Karim et al. (2000) studied various methods to analyze starch retrogradation characteristics. They concluded it is advisable to analyze starch retrogradation by two methods and compare the results to ensure accuracy.

Several researchers have been working to improve the staleness of different bread types. Leon et al. (2002) studied the effect of enzyme mixture to retard firming of bread. They reported that using enzyme mixtures led to inhibition of amylopectin retrogradation, and therefore had a positive effect on bread firmness. This also agrees with the observations of Gujral et al. (2003) who found similar results by using starch hydrolyzing enzymes (alpha amylase of intermediate thermostability and cyclodextrin glycoxyl transferase [CGTase]) on rice flour breads.

**Table 7. Texture Analysis of Breads on Day 1, 4 and 6**

| Lentil Flour % @450 rpm | Staleness (Day 1) | Staleness (Day 4) | Staleness (Day 6) |
|---|---|---|---|
| 10 | 249.3a | 396.7a | 590.0a |
| Control | 238.6a | 355.3b | 513.0b |

[a] Values followed by the same letter in the same column are not significantly different from each other

## Summary and Conclusion

Based on the results obtained during the study, it can be concluded that fortifying wheat flour with extruded lentil flour had significant effects on dough water absorption. It also had significant effects on overall acceptability of breads due to better textural properties. Although, extrusion speeds had little effect on water absorption and overall acceptability. Increasing the lentil flour concentration (10%) in flour blends led to better water absorption. Extruded lentil flour concentration in wheat lentil flour blends was found best from an overall acceptability standpoint. Although, faster staling in breads baked from flour blends was observed when compared to wheat flour bread. Therefore, studies on retarding the speed of staling in lentil flour fortified bread are necessary.

## RECOMMENDATIONS FOR FUTURE RESEARCH

The research demonstrated that scheduling experiments could improve time efficiency. Likewise, the number of panelists should be 20 to ensure better statistical analysis of results.

There is ample scope for future research on this topic. The following list provides recommendations for future research:

1. effects of lentil flour concentration and extrusion speeds can be observed on protein quality of breads by total amino acid profile with special emphasis on availability of lysine;
2. effects of lentil flour concentration and extrusion speeds can be investigated on total dietary fiber, soluble dietary fiber and insoluble dietary fiber as lentils are a good source of dietary fiber; and
3. effects of dough conditioners and sugars should be investigated on lentil flour breads in order to retard speed of staling.

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# APPENDIX A

## GRAPHS FOR ANALYSES OF SAMPLE BREADS AND CONTROL BREADS

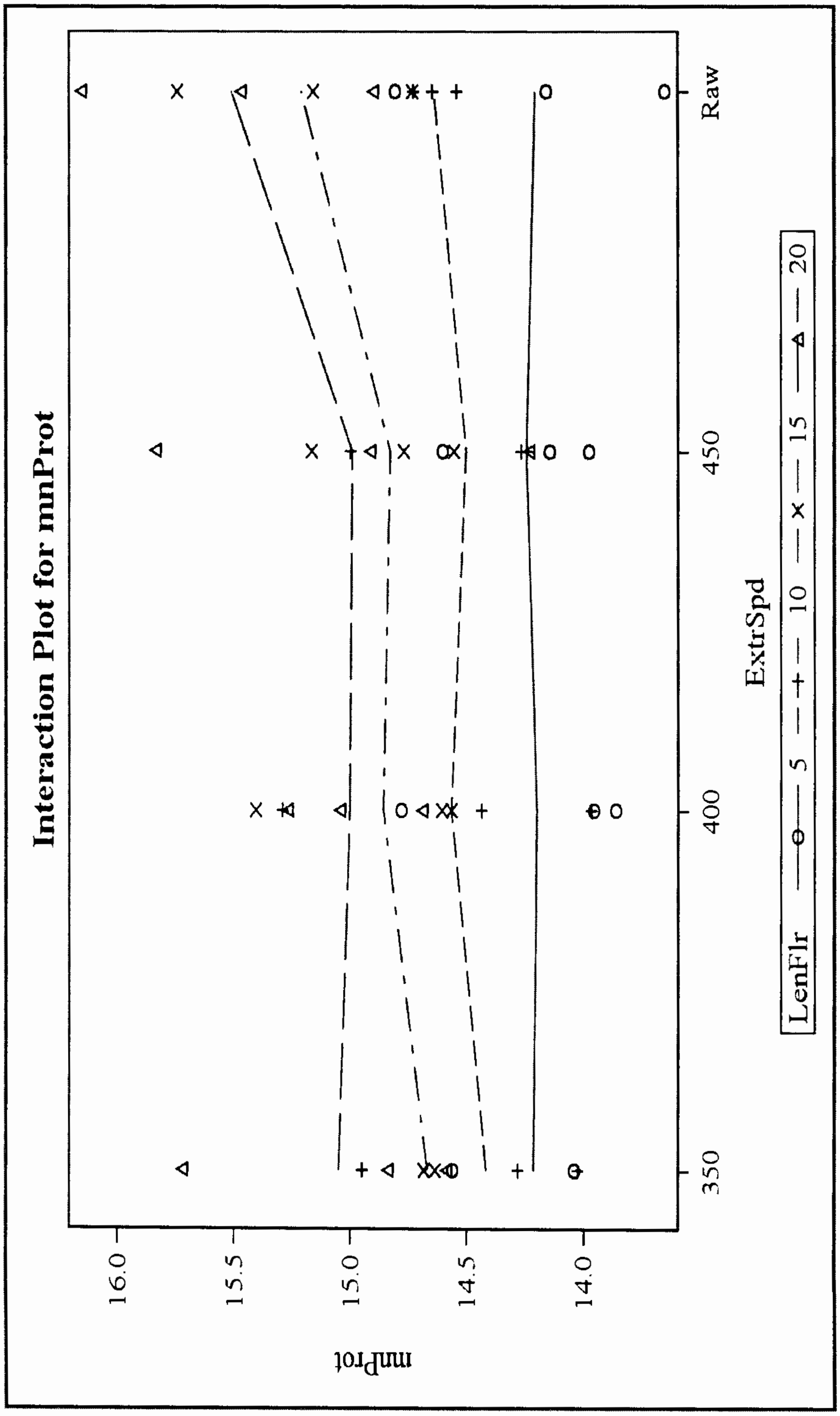


**Figure 1. Interaction Plot of Protein Content of Flours Versus Extrusion Speeds**

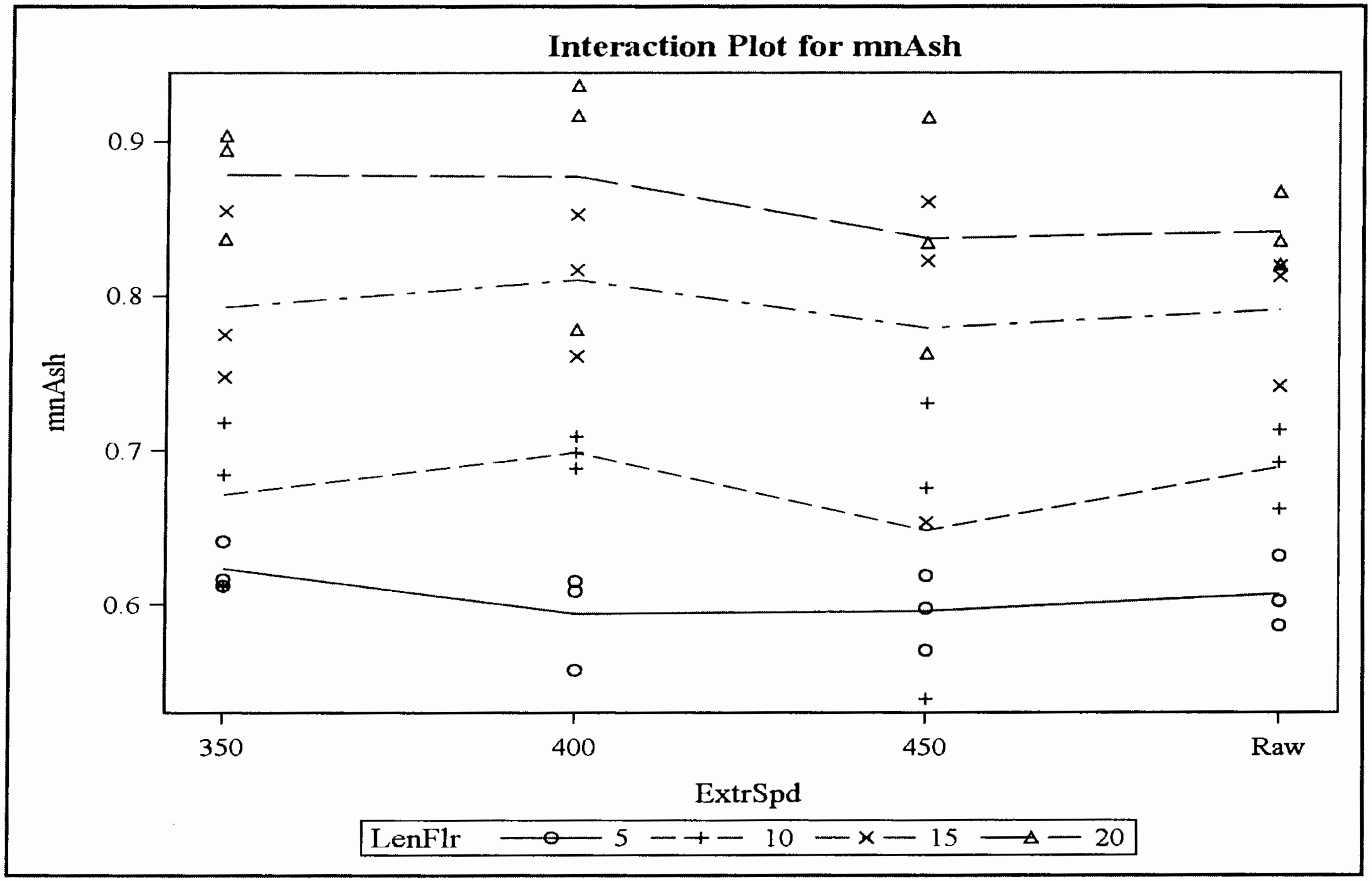


**Figure 2. Interaction Plot of Ash Content of Flours Versus Extrusion Speeds**

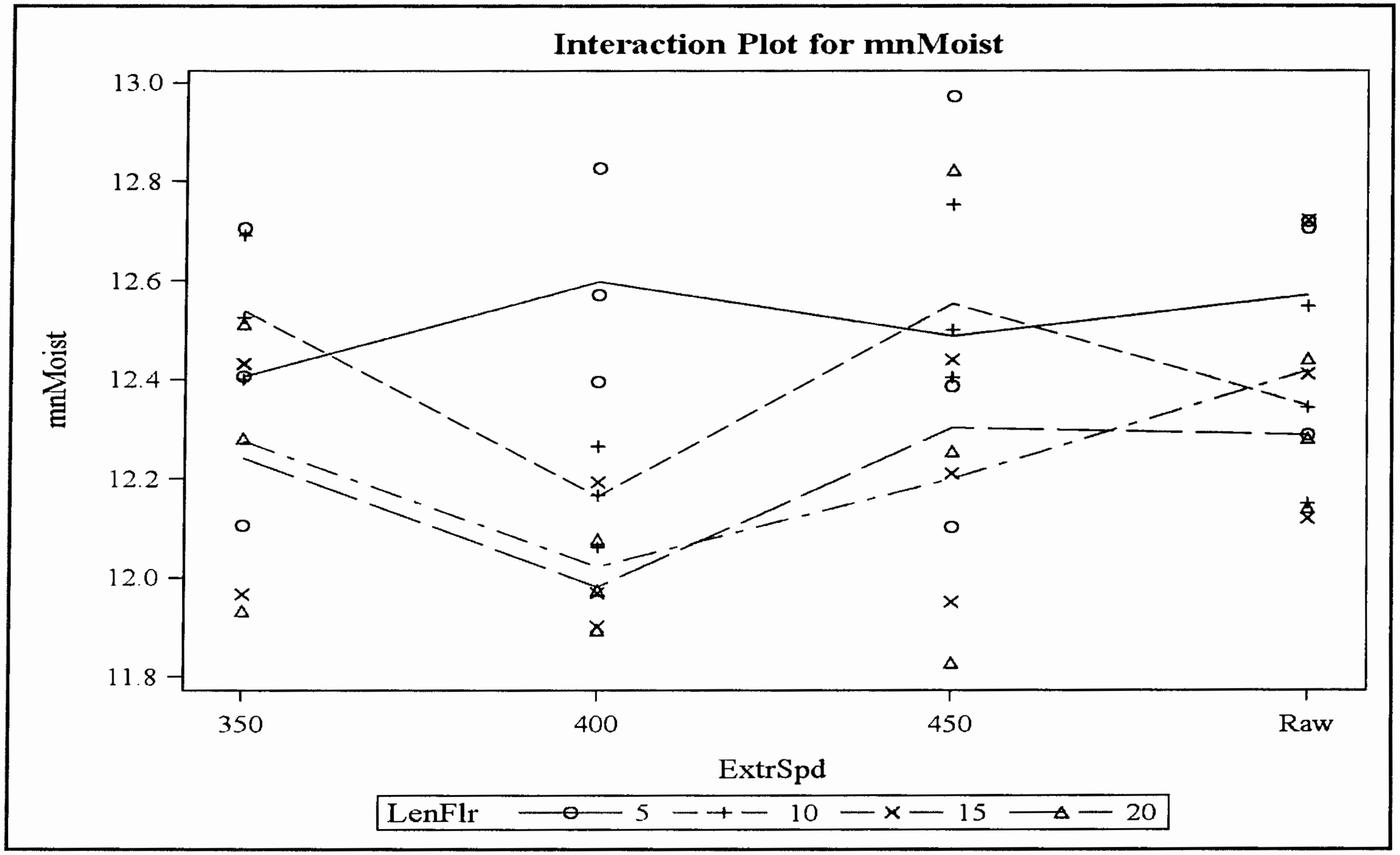


**Figure 3. Interaction Plot of Moisture Content of Flours Versus Extrusion Speeds**

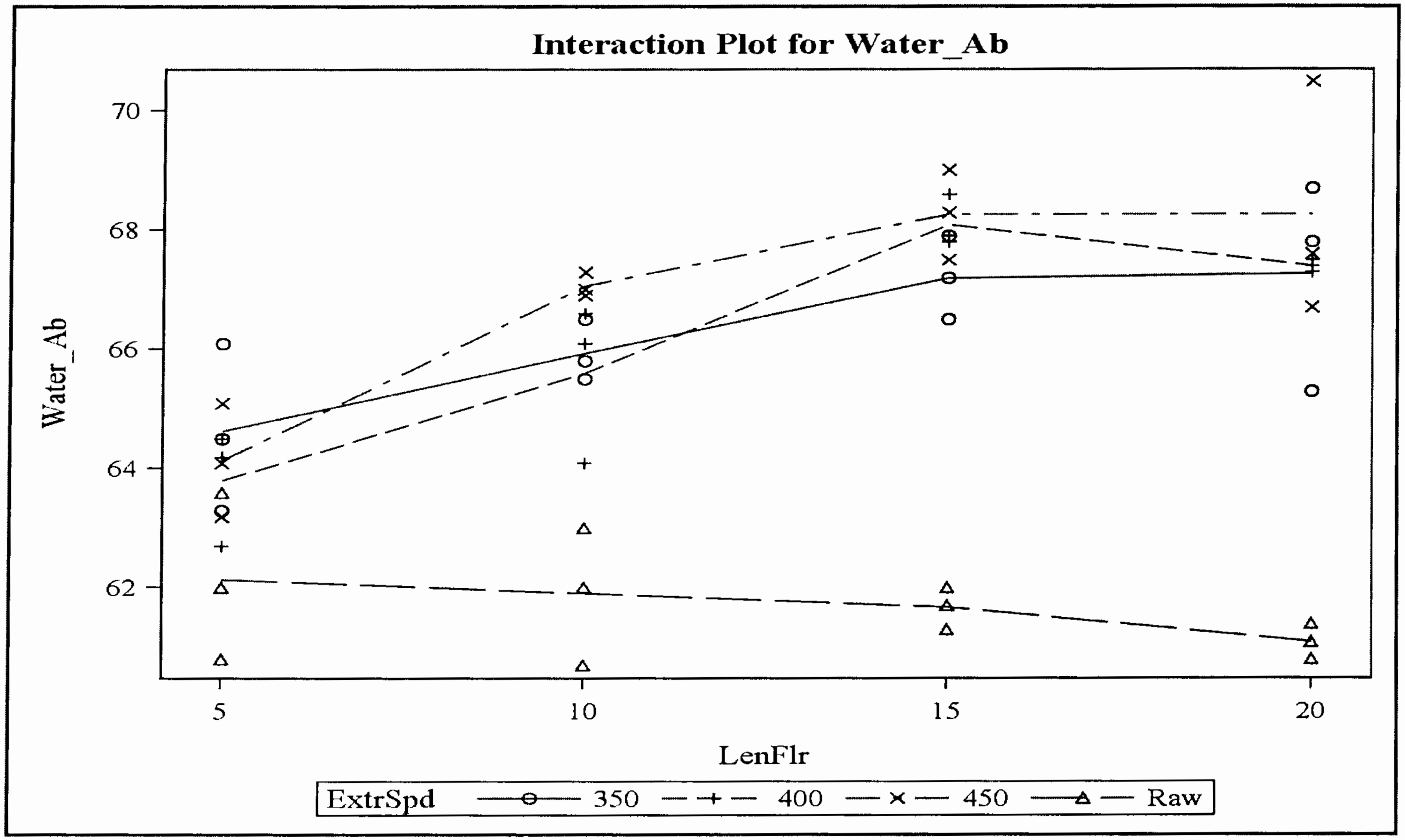


**Figure 4. Interaction Plot of Water Absorption Versus Lentil Flour %**

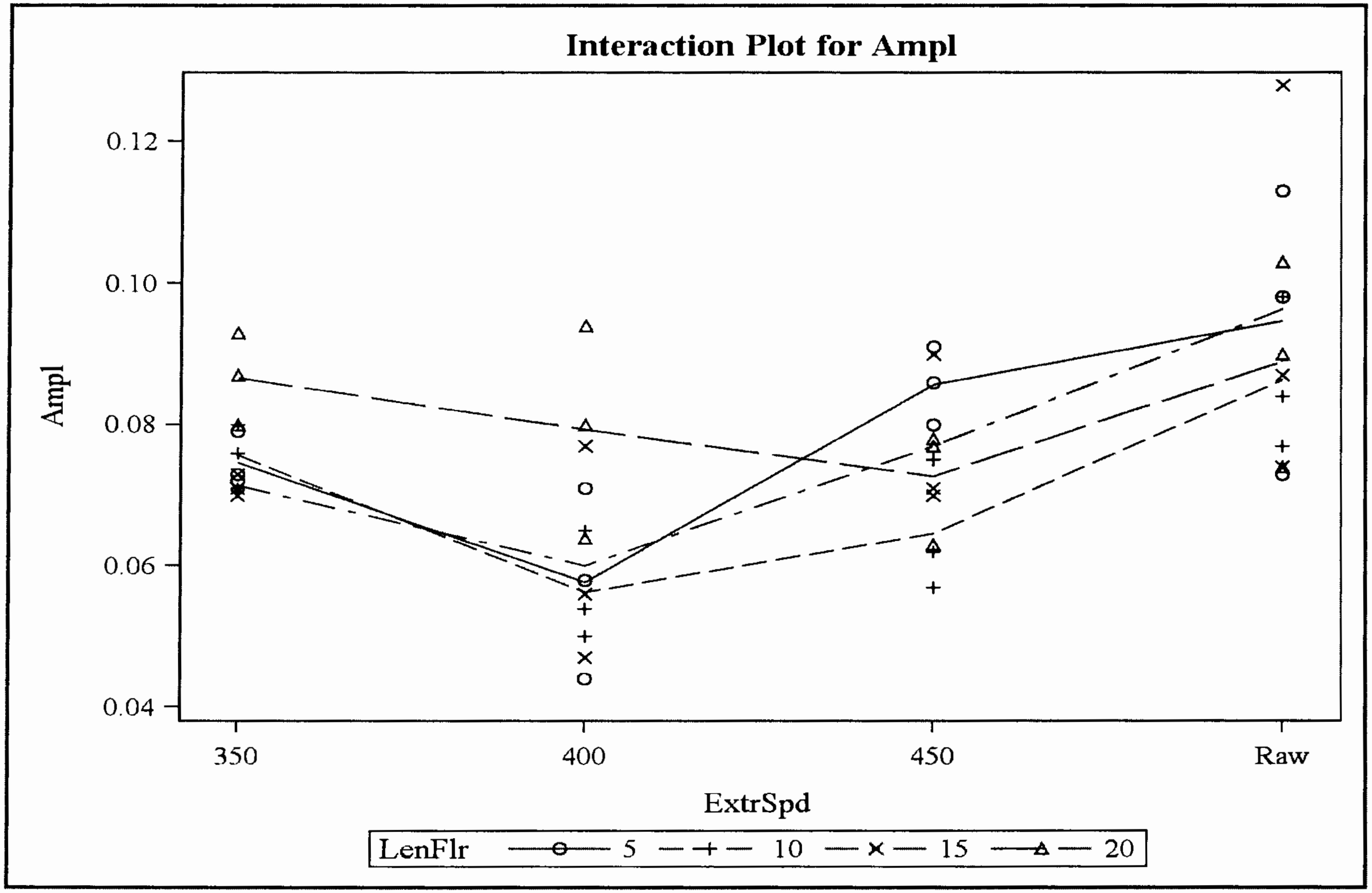


**Figure 5. Interaction Plot of Amplitude Versus Lentil Flour and Extrusion Speeds**

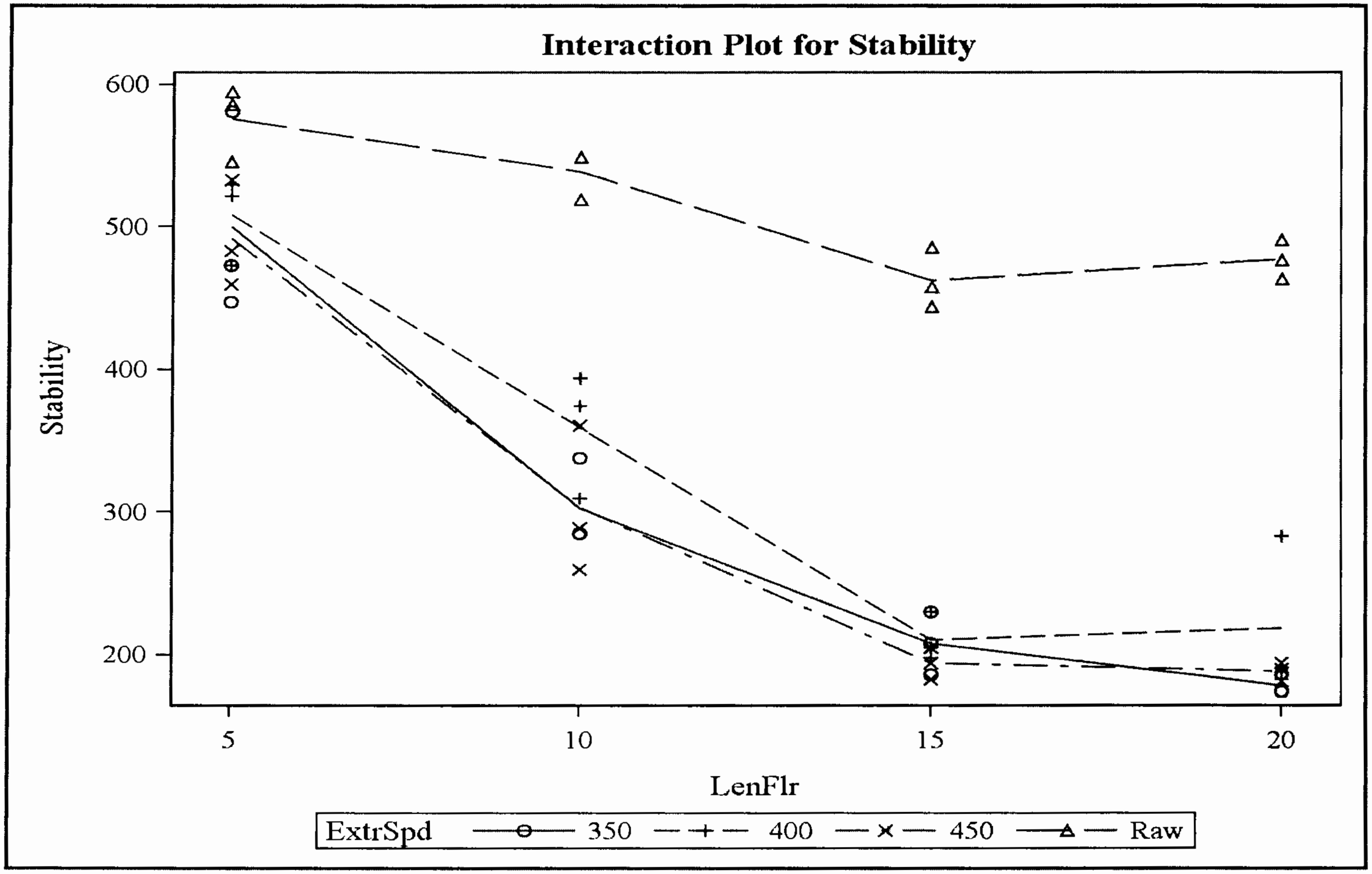


**Figure 6. Interaction Plot of Stability Versus Lentil Flour % and Extrusion Speeds**

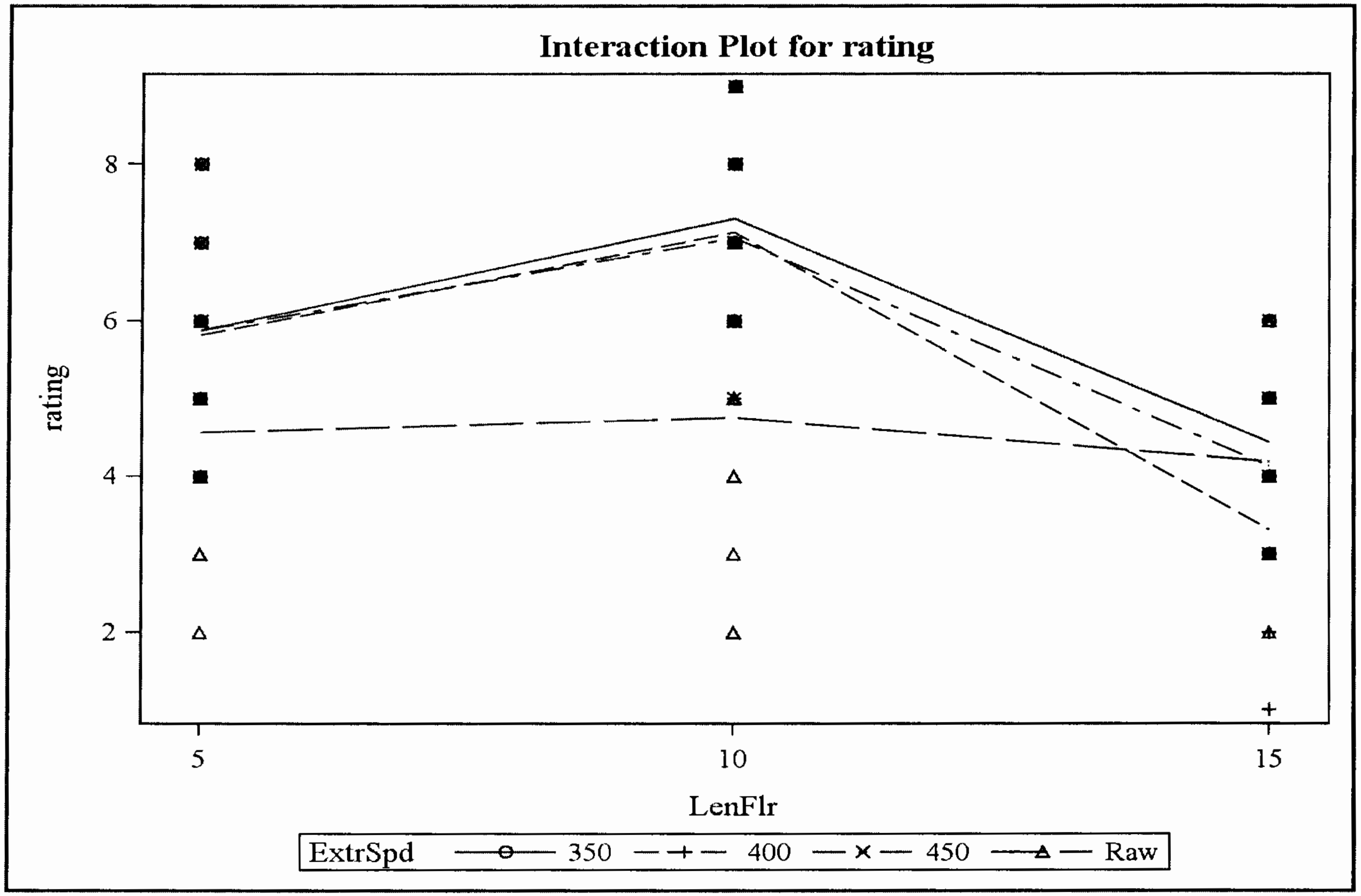


**Figure 7. Interaction Plot of Sensory Evaluation Ratings Based on Lentil Flour % and Extrusion Speeds.**

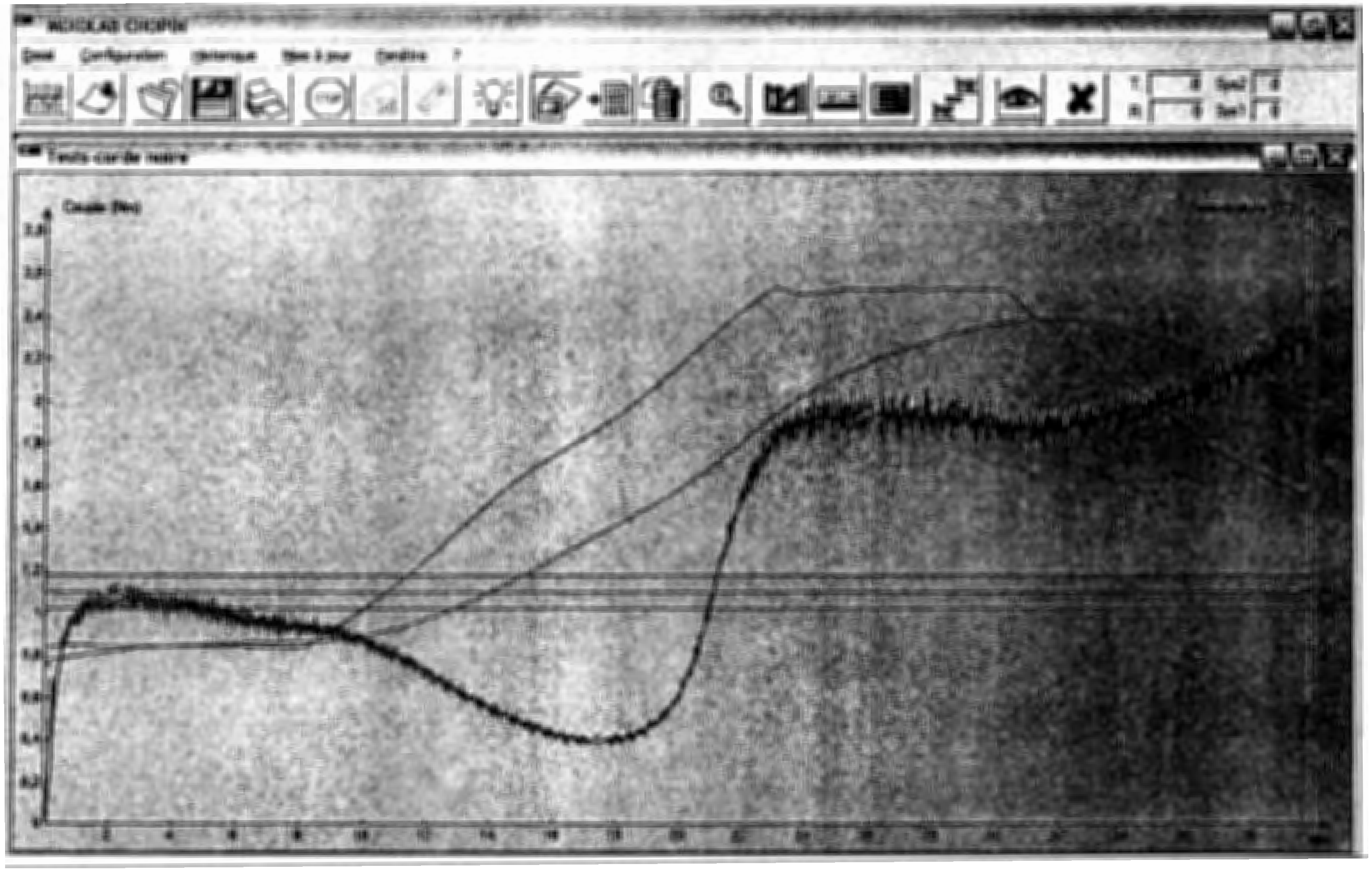

Figure 8. Example of Mixolab Graph

# APPENDIX B

# SENSORY EVALUATION FORM AND IRB APPROVAL

## 1. Score Sheet for 9 point Hedonic Scale Test

QUESTIONNAIRE FOR SCORING

NAME ______________________ DATE__________________________

Evaluate these samples for overall acceptability and rate the samples FROM "Like Extremely" to "Dislike Extremely".

| __________ | __________ | __________ |
|---|---|---|
| ______ Like Extremely | ______ Like Extremely | _____ Like Extremely |
| ______ Like Very Much | ______ Like Very Much | _____Like Very Much |
| ______ Like Moderately | ______ Like Moderately | _____Like moderately |
| ______ Like Slightly | ______ Like Slightly | _____ Like Slightly |
| ______ Neither Like nor Dislike | ______ Neither Like nor Dislike | _____ Neither Like nor Dislike |
| ______ Dislike Slightly | ______ Dislike Slightly | _____ Dislike Slightly |
| ______ Dislike Moderately | ______ Dislike Moderately | _____ Dislike Moderately |
| ______ Dislike Very Much | ______ Dislike Very Much | _____ Dislike Very Much |
| ______ Dislike Extremely | ______ Dislike Extremely | _____ Dislike Extremely |

Comments:

## 2. IRB Approval Form

NDSU NORTH DAKOTA STATE UNIVERSITY

*Institutional Review Board*
*Office of the Vice President for Research, Creative Activities and Technology Transfer*
*NDSU Dept. 4000*
*1735 NDSU Research Park Drive*
*Research 1, P.O. Box 6050*
*Fargo, ND 58108-6050*

*701.231.8995*
*Fax 701.231.8098*

*Federalwide Assurance #FWA00002439*
*Expires April 24, 2011*

February 2, 2010

Dr. Khalil Khan
Dept. of Cereal and Food Sciences
IACC 366

**Re:** IRB Certification of Human Research Project:

**"Enhancement of Bread using High Termperature Extruded Lentil Flours"**
Protocol **#AG10170**

Co-investigator(s) and research team: **Clifford Hall, Abhay Gupta**

Study site(s): **NDSU** Funding: **n/a**

It has been determined that this human subjects research project qualifies for exempt status (category # 6) in accordance with federal regulations (Code of Federal Regulations, Title 45, Part 46, *Protection of Human Subjects*). This determination is based on the protocol form received 2/1/2010 and consent/information sheet received 2/1/2010.

Please also note the following:

- This determination of exemption expires 3 years from this date. If you wish to continue the research after 2/1/2013, submit a new protocol several weeks prior to this date.
- The project must be conducted as described in the approved protocol. If you wish to make changes, pre-approval is to be obtained from the IRB, unless the changes are necessary to eliminate an apparent immediate hazard to subjects. A *Protocol Amendment Request Form* is available on the IRB website.
- Prompt, written notification must be made to the IRB of any adverse events, complaints, or unanticipated problems involving risks to subjects or others related to this project.
- Any significant new findings that may affect the risks and benefits to participation will be reported in writing to the participants and the IRB.
- Research records may be subject to a random or directed audit at any time to verify compliance with IRB policies.

Thank you for complying with NDSU IRB procedures; best wishes for success with your project.

Sincerely,

Kristy Shirley

Kristy Shirley, CIP
Research Compliance Administrator

NDSU is an equal opportunity institution.

## 3. Consent Form

# North Dakota State University

School of Food Systems,

Dept of Cereal and Food Sciences

1250 Bolley Drive, 113 Harris Hall

Fargo, ND 58108-6050

701-231-7711

### Enhancement of Bread using High Temperature Extruded Lentil Flours

**INFORMED CONSENT**

Dear Sensory Panelist,

I (Abhay Gupta) am a master's degree candidate in Department of Cereal and Food Science at North Dakota State University. You are invited to participate in a study to evaluate the sensory characteristics of lentil fortified breads. The following information is provided for you to decide whether you wish to participate in the present study. Your participation is entirely your choice, and you may change your mind or quit participating at any time, with no penalty to you.

**PURPOSE OF THE STUDY:**

The purpose of this study is to understand consumer acceptability and staleness of bread fortified with lentil flours. The goal to enhance nutritional quality of white pan bread is the basis for the project.

**EXPLANATIONS OF PROCEDURES:**

In this sensory evaluation, you will be asked to taste breads with and without lentil flours. During the evaluation you will be given samples on plates marked with different numbers. The instructions to complete the sensory evaluation are provided on the form given during the sensory panel. In short, you will be asked to mark on a scale the degree of acceptance or staleness. The 9 point scale is in increments from "Like Extremely" to "Dislike extremely" based on acceptability or a 6 point scale in increments from "Extremely firm" to "Extremely soft". The entire sensory evaluation should take less than 30 minutes.

**PARTICIPANT INFORMATION:**

All the information obtained during the test will remain confidential. Your identity will not be revealed in the experiment results. Only group comparisons will be made and reported in summary form. Furthermore, we will not collect signatures on the score sheet thus preserving your anonymity.

You may refuse to sign this form and not participate in this study. You should be aware that even if you agree to participate, you are free to withdraw at any time. If you do withdraw from this study, it will not affect your relationship with this unit, the services it may provide to you, or North Dakota State University.

**POTENTIAL BENEFITS AND RISKS:**

Results of this test will be helpful in determining the best formula for the new bread. No direct benefit will received from participation in the study. However, improvements in white bread formulations would be expected to help the population in general. It is not possible to identify all potential risks in research procedures, but the researcher(s) have taken reasonable safeguards to minimize any known risks. If you are known to be sensitive to any food or food ingredient, or have had violent allergic reactions to drugs, chemicals, or food ingredients, you should not participate in this study.

**CONTACT INFORMATION:**

You have rights as a research participant. If you have questions about your rights or complaints about this research, you may talk to the researcher or contact the NDSU Human Research

Protection Program at 701.231.8908, ndsu.irb@ndsu.edu, or by mail at: NDSU HRPP Office, NDSU Dept 4000, PO Box 6050, Fargo, ND 58108-6050.

If you have any questions about this project, please call me at Abhay.Gupta@ndsu.edu or Dr. Khalil Khan (Professor) at khalil.khan@ndsu.edu, 701-231-7729, or by mail at: School of Food Systems, Dept of Cereal and Food Sciences, Dept 7640, PO Box 6050, Fargo, ND 58108-6050.

If you wish participate in this study please sign below.

Signature: ______________________ Date:__________________

Print Name:______________________