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Title: EXTRACTION OF LENTIL PROTEINS AND THEIR USE IN SUPPLEMENTATION OF BREAD.

Abstract approved

Dr. Allen F. Anglemier

The purpose of this study was to extract proteins from lentils for use in bread making to improve nutritional quality of bread. Three solvents, distilled water, 0.7 M NaCl and 0.05 M NaOH solutions, were used for extraction. Extractions were done at an alkaline pH near 10 for 30 min. Proteins were recovered by acid precipitation using 1 N HCl. Macro-Kjeldahl determinations were run on the precipitated material to determine protein content.

The results showed that extractions were effective using either distilled water adjusted to pH 10.0 or 0.05 M NaOH. Protein recoveries of 66.01-68.79% and 64.01-66.62% were obtained by these two procedures respectively. These are simple methods that do not require sophisticated equipment. Thus they would be practical and economical for use on a large scale.

For bread supplementation studies, lentil proteins were extracted by distilled water with pH of the slurries adjusted to pH near 10. The extracted lentil proteins were used to replace white wheat flour at levels of 0, 5, 7.5 and 10% on a dry weight basis. Breads were made using the

the straight dough method. Sodium-stearoyl-2-lactylate was used as a dough conditioner. Specific volume, color, texture and protein content of the breads were evaluated objectively. The breads were also subjected to sensory evaluation for color, texture, moistness, flavor and overall desirability.

Specific volume decreased as the level of lentil protein increased. The control bread had the highest specific volume which was significantly higher ($p < 0.05$) than the lentil supplemented breads.

Bread crumb became darker in color with increasing lentil protein levels as shown by a decrease in Hunter L values. Hunter 'a' values increased in breads with higher lentil protein contents but there was little change in Hunter 'b' values.

Lentil proteins resulted in a decrease in crumb compressibility. There was a linear relation between levels of protein replacement and protein contents of the breads. The 5%, 7.5% and 10% lentil protein replacements increased the protein contents of the breads from 11.44% to 13.-2%, 13.80% and 14.59% respectively.

Although the lentil breads received lower sensory evaluation scores than the control breads, they were judged as acceptable at the 5%, 7.5% and 10% substitution levels.

These findings are significant because the supplemented breads have higher nutritional value. This would help reduce nutritional deficiency problems especially in areas where protein malnutrition exists.

Extraction of Lentil Proteins and Their Use in Supplementation of Bread

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To: Khalil

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INTRODUCTION

Nutritional deficiency is a problem especially in areas where animal proteins are scarce and expensive. Efforts have been and are being made to reduce this problem by increasing the quality of foods consumed. One of the efforts has been to produce high protein breads by supplementing wheat flour with legume flours to improve the nutritional quality of bread.

Substituting 5% of wheat flour in commercially baked bread with vegetable protein flour would increase available protein in bread by 12-15% without altering the appearance, texture, or flavor. This slight and economically practical increase in protein would make the difference between good nutrition and malnutrition to over 36% of the world's population who depend on bread for more than 50% of their calories. It is more efficient to add more protein to a basic food people already eat than to try to upgrade diets by providing more food. The cost of adding protein at this level would be less than 1/5 of a cent per one pound loaf (Baldwin, 1979).

Lentil has 2.4 times as much protein as wheat flour. It appears to be a good protein supplement for bread because of its high lysine content which is lacking in wheat. Methionine which is the limiting amino acid in lentils can be complemented by the higher level present in wheat flour to provide a more complete protein than either component alone.

The purpose of this study was to extract lentil proteins by a relatively simple but effective means to make high protein breads. The effects of supplementing wheat flour with lentil protein on the characteristics and the acceptability of the breads were also investigated.

REVIEW OF LITERATURE

Lentil (Lens culinaris Medik) is a legume, grown mainly in India, Pakistan, Iraq, Egypt, Sudan, Somalia, Canada, Mexico, and South American countries. Besides being consumed in the producing countries, lentils are also exported to other countries. They are relatively inexpensive and are therefore a desirable source of food proteins. Lentils are consumed directly as foods in many parts of the world where animal proteins are scarce and expensive (Wolf, 1977). In India and Pakistan, lentils are used to prepare a favorite dish called curry. In Sudan and Mexico, they are used to make soups. Lentils can also be cooked with rice or meats. There is a high potential for future production of lentils in Eastern Washington and Idaho. If high-yielding and disease resistant cultivars become available, production could increase significantly in areas where growing conditions are not so favorable (Bhatty et al., 1976).

It may be possible to use lentil powder in composite flours or as ingredients in manufactured foods (Anderson and Romo, 1976). Lentils can possibly function as a thickening agent in heat-treated sauces, as a protein supplement to increase nutritional quality of cereal products, or as a binding agent in processed meats.

Chemical Composition of Lentils

Lentils have received little nutritional attention, although a number of studies have reported the gross chemical composition. The following table shows the proximate analysis of lentils in comparison with soybean and wheat.

Table 1. Proximate chemical composition of unprocessed lentils, soybean, and wheat stored under commercial conditions.

Substance	Lentils[a] %	Soybean[b] %	Wheat[c] %
Carbohydrate	63.1	33.5	72.1
Protein	28.6	34.1	12.0
Lipid	0.7	17.7	2.1
Moisture	4.5	10.0	12.0
Fiber[d]	(4.4)	(4.5)	(2.5)
Ash	3.1	4.7	1.8

[a]Bhatty et al. (1976).
[b]Kawamura (1967).
[c]Inglett (1974).
[d]Fiber is a part of total carbohydrate content.

Lentils contain about 70% as much protein as soybean and more than twice that of wheat. Carbohydrate content of lentils is less than wheat but almost twice that of soybean. The low lipid content (< 1.0%) facilitates extraction of lentil proteins because it minimizes undesirable emulsion formation that occurs occasionally during the initial grinding and mixing steps in the extraction of protein from seeds.

Nutritional Properties

Amino Acid Pattern

Lysine is the limiting amino acid in wheat protein. Lentil protein has 3-4 times as much lysine as wheat protein. Because of its amino

acid pattern, lentil protein appears to be a good supplement for a cereal based product such as bread. The following table shows the amino acid composition of lentil protein in relation to wheat flour and the FAO/WHO Provisional Pattern.

Table 2. Essential amino acid profile of lentil and wheat flour proteins in comparison with FAO/WHO Provisional Pattern (g amino acid/100 g protein).

Amino Acid	FAO/WHO[a] Provisional Pattern	Lentil[b] protein	White[c] wheat flour
Lys	5.5	5.9 - 7.5	1.9
Trp	1.0	0.8 - 1.0	1.1
Ile	4.0	3.3 - 3.8	4.2
Val	5.0	3.8 - 4.2	4.3
Met & Cys	3.5	1.7 - 2.2	3.3
Thr	4.0	3.0 - 3.4	3.0
Leu	7.0	6.1 - 7.0	7.0
Phe & Tyr	6.0	5.0 - 6.5	7.8

[a]Satterlee et al. (1979).
[b]Bhatty et al. (1976); McCurdy et al. (1978).
[c]Wookey (1979).

The major essential amino acids in lentil proteins are leucine, and lysine. These levels are adequate according to the Provisional Pattern (Table 2). However, levels of other essential amino acids such as isoleucine, valine, methionine, and threonine are not adequate. On the other hand, lysine is limiting in wheat protein while the isoleucine level is adequate and methionine level is marginal. Tryptophan, phenylalanine,

and tyrosine levels are adequate in both cases. If lentil protein and wheat are mixed together, the limiting amino acid in one can be supplemented by the other. Nutritional quality of mixed or blended plant protein increases when one essential amino acid pattern supplements the other.

Growth and Nutritional Evaluation Studies

McCurdy et al. (1978) used the protozoan Tetrahymena pyriformis to assess protein quality in lentils and found that the protein in whole lentil powder supported 15-19% of the growth obtained with casein. The legumin fractions provided slightly less growth while the albumins supported almost three times more growth than whole lentil powder. It was postulated that the bioavailability of essential amino acids supplied by lentil powder was inferior to the utilization of the essential amino acids in the casein standard due to low solubility or unfavorable within the seed. Essential amino acid supplementation studies showed the sulfur-containing amino acids to be first limiting amino acids. There was significant increase in Tetrahymena growth when 0.5-1.0% methionine was added. Bhatty et al. (1976) reported that because of limiting concentrations of methionine and cystine, lentil protein gave a chemical score of 35, a protein score of 46, and an essential amino acid index of 63 compared to 100 for egg protein. Nutritional value of lentil protein is improved by supplementing with methionine.

Protein Extraction

Considerable work has been done on extraction of proteins from various plant sources although little has been completed on lentils

specifically. Proteins are classified into four different fractions based on their solubility properties namely globulins, albumins, prolamines, and glutelins.

Globulins are soluble in the presence of salts but are insoluble in water at their isoelectric points. They are rich in ionizable amino acids such as arginine, lysine, aspartic and glutamic acids. Presence of lysine and tryptophan makes globulins of high nutritional value. Globulins contribute to the hydration and emulsifying properties in food systems. Catsimpoolas and Meyer (1970) reported that the globulin fraction of soybean had excellent gelling ability.

Albumins are soluble in pure water and are of nutritional value due to presence of lysine, tryptophan, and the sulfur-containing amino acids. They have better emulsion and foaming capacity but less emulsion and foaming stabilizing effects than globulins (Satterlee et al., 1975).

Prolamines are soluble in 70-80% ethanol. They are characterized by high levels of proline which has low nutritional value. Prolamines of wheat protein consist largely of gliadin which contributes to the extensible properties of bread dough.

Glutelins are soluble in dilute acid or alkali. They are high in asparagine and glutamine but lacking in ionizable and essential amino acids. Thus their nutritive value is low. In wheat protein, the glutelin fraction consists mainly of glutenin which contributes to elastic properties in bread dough.

Extraction of Lentil Proteins

Like other legume seeds, the bulk of proteins in lentils is

globulins (47%). Saint-Clair (1972) and Bhatty et al. (1976) reported that other proteins in lentils were albumins (3.8-26%), prolamines (2-3.1%), and glutelins (8-14.9%).

Protein solubility or extractability is influenced significantly by pH (Anderson and Romo, 1976; Fan and Sosulski, 1974; Shehata et al., 1978). The basic principle employed in the extraction of protein is to complete the extraction at a pH where solubility is high and then recover the protein by acid precipitation at a pH where its solubility is minimum (the isoelectric point).

Anderson and Romo (1976) studied the influence of extraction medium pH on protein content of lentil powder and reported that solubility of lentil protein increased rapidly in the pH range of 5.5 to 7.5 and increased slowly thereafter at higher pH. Region of minimum solubility was reported at pH 4.4-5.5. Fan and Sosulski (1974) assessed the influence of pH on the extraction and precipitation characteristics of the nitrogen in nine legume flours including that of lentils and found that solubility of lentil protein was high at a pH range of 7-11. Minimum solubility occurred at a broad pH range between pH 3-5. Figure 1 shows pH solubility profiles of lentil protein obtained in the two studies. The solubility curves in the basic pH region are similar but a major difference exists in the shape of the curves in the acid region. Fan and Sosulski reported that lentil protein was only partially soluble at pH 2-3 but more soluble than in its region of minimum solubility while Anderson and Romo reported relatively high solubility at pH 2.6.

The effect of solvents, solvent concentration, solvent-flour ratios, particle size and extraction time on yield of lentil protein was studied

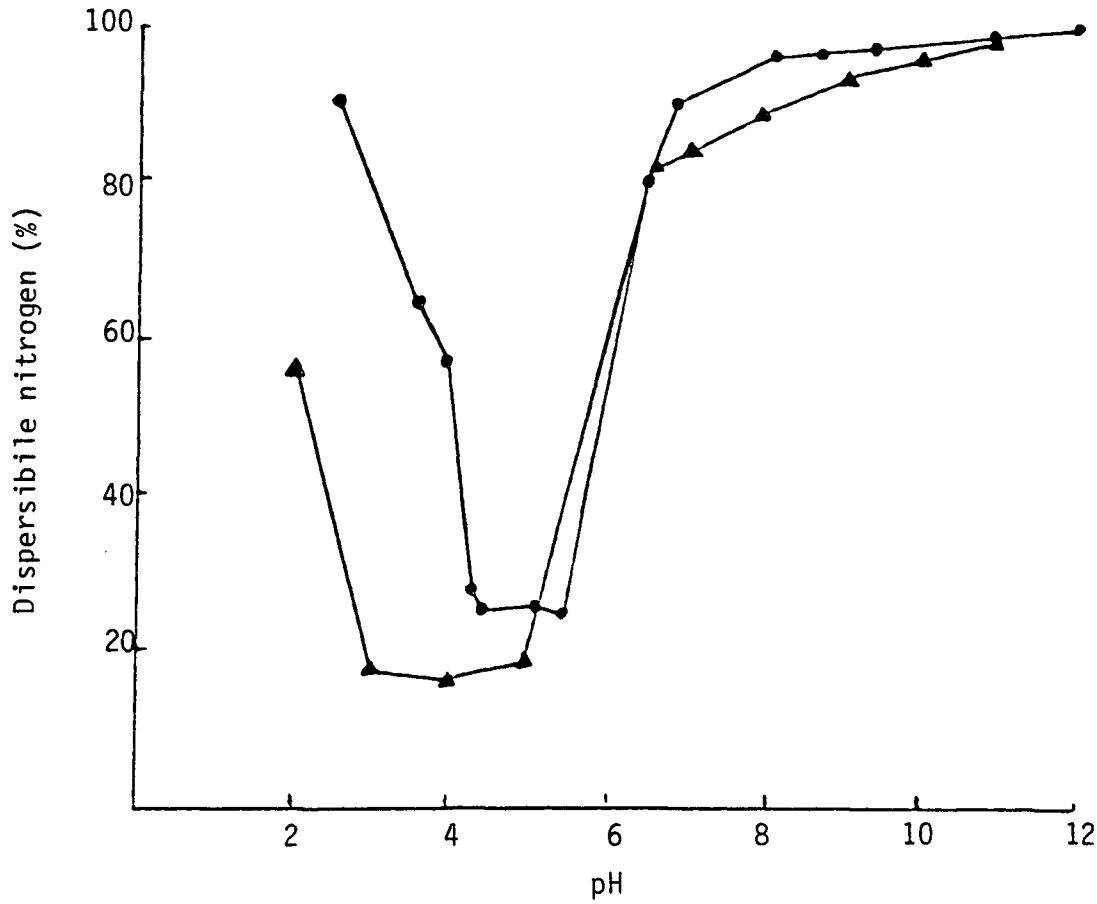

Figure 1. Protein pH - solubility profiles of lentil powder.

●—● Profile obtained by Anderson and Romo (1976).

▲—▲ Profile obtained by Fan and Sosulki (1974).

by Odeh et al. (1979). NaOH solutions were found to achieve maximum extractability. NaCl was about as efficient as water and yielded about one-third less extractable protein than NaOH. Protein extractability increased with NaOH as concentration increased from 0.01 to 0.03 M. With NaCl, 0.2 M and 0.3 M gave similar results but 0.5 M gave slightly higher protein extractability. Optimum extraction was at a flour to solvent ratio of 1:10. More proteins were extracted from finely ground than coarse ground lentil seeds due to greater surface contact with extractants. Efficient extraction time was found to be 30 min.

In a study done by Shehata et al. (1978) lentil proteins were extracted with 0.1 M phosphate buffer, pH 7.4. Four methods of precipitating the proteins were examined, namely: dehydration by acetone, direct freeze drying, acidification to the isoelectric point alone, or aided by heating. The latter method gave the highest protein recovery (66.73%). The recovery for the other methods ranged from 57.21 to 29.51%. In general, there are two major methods of recovering proteins from the extraction medium:

1. Fractionation by means of dialysis through a membrane against distilled water. The extract is then freeze dried.
2. Acid precipitation at the minimum solubility, followed by centrifugation to separate the protein precipitate from the supernatant.

Extraction of Other Legume Proteins

Several extractants for isolating proteins from legume flours have been studied. These include water, dilute calcium hydroxide solution

(Patel and Johnson, 1974) and solutions of other salts including sodium chloride, sodium hydroxide, sodium sulfate, disodium phosphate, trisodium citrate, and sodium carbonate (Pant and Tulsiani, 1969; Hang et al., 1970; Satterlee et al., 1975; Kazazis and Kalaissakis, 1979). Sodium hydroxide solution (0.01 - 0.05 M, pH 8-11) was reported to be an excellent solvent for extracting nitrogenous constituents of legume seeds. Kazazis and Kalaissakis (1979) found that nitrogen from vetch seeds (Vicia sativa) was extracted most efficiently by 0.05 M sodium hydroxide, pH 11.1. Proteins of considerable purity from Phaseolus seeds were reported to be best extracted with 0.25 - 1.00 N sodium chloride solution, pH 5.85 - 6.10 (Pant and Tulsani, 1969).

Distilled or tap water, having the pH adjusted by adding HCl, NaOH or Na_3PO_4 has been used by several workers to determine nitrogen extractability or for isolating proteins (Rhee et al., 1972, 1973; Maneepun et al., 1974; Fan and Sosulski, 1974; Thompson, 1977; Manak et al., 1980). Maneepun et al. (1974) extracted lima bean proteins with distilled water by adjusting the slurry to pH 7.2 with the addition of Na_3PO_4 solution. The proteins were precipitated between pH 4 and 5. Extraction at pH 9 and 25°C using a 1:15 flour to solvent ratio for 20 min, followed by isoelectric precipitation at pH 4 were the conditions established for the preparation of mung bean protein isolate (Thompson, 1977). Manak et al. (1980) extracted soy, peanut, and cottonseed proteins satisfactorily with tap water using a 1:10 flour to solvent ratio at pH 9, 8, and 7, respectively.

In extraction studies done by Fan and Sosulski (1974) different legume flours were dispersed in distilled water and the pH was adjusted

between 2 and 11 with 1 N HCl or 0.2% NaOH. The study showed that mung bean, field pea, soybean, faba bean, chick pea and lima bean had narrow apparent isoelectric points while lentil, pea bean and lupine had broad apparent isoelectric points. Lima bean protein appears to be unique because it was highly soluble at its apparent isoelectric point. For each legume, the nitrogen extraction profile had a steep portion between pH 5-7. Protein isolates were obtained by extraction with 0.02% NaOH for 1 hr and precipitated at pH 4.5 by the addition of concentrated HCl. Soybean, faba bean, and lupine produced high protein yields while lima bean and pea bean produced low yields. Extraction at pH 12 for 2 hr was found to be too harsh because of alkaline hydrolysis.

Factors influencing the extractability of safflower protein (Carthamus tintorius L.) were investigated by Betschart (1975). She found the major protein fractions to be soluble either in salt or dilute alkali. A fraction of the protein, higher in lysine than the original meal, could be extracted initially with water while a second extraction at an alkaline pH extracted the remaining protein. In this study, extraction was maximized within 45 min. Approximately 80% of the N was extracted at temperature from 20-42°C. Extractability increased only slightly at a temperature range of 60-70°C and at 90°C the proteins began to precipitate. Varying the solvent to meal ratio had negligible effect on N extractability.

Functional Properties

Functional properties of protein denote any physicochemical property which affects the processing and behavior of protein in food systems.

Factors that influence the functional properties are: the source of protein; processing treatments or preparation methods; and environmental conditions such as temperature, pH and ionic strength (Kinsella, 1976).

There are several general classes of protein functionality in food systems namely organoleptic, hydration, surface, structural, textural and rheological. Organoleptic properties are concerned with color, mouth-feel, odor and flavor. Hydration properties include solubility, water absorption, water-holding capacity, swelling, thickening, viscosity, and dough formation. Surface properties include whipping, foaming, binding, and emulsification. Structural properties involve elasticity. Textural characteristics includes viscosity and network cross-binding. Rheological properties include stickiness, gelation, and dough formation (Kinsella, 1976). Some of the more important functional properties involved in bread dough are discussed in the following section.

Protein Functionality in Bread Making

In bread making, proteins are extremely important for dough formation. Only cereal proteins, specifically wheat gluten, have the ability to form true doughs (i.e., an extensible, viscoelastic protein network) upon mixing with an appropriate amount of water (Kinsella, 1976). Dough development encompasses both physical and chemical changes, principally gluten formation, modification of organic constituents by yeast and enzymes, effects of mixing and expansion due to fermentation (Fowler and Priestley, 1980). During dough development, gluten is formed from gliadin and glutenin on the addition of water. Other components such as starch are embedded in the gliadin-glutenin matrix. Properties of gliadin and glutenin differ. Gliadin contains intramolecular disulfide

bonds and is extensible but inelastic. It is responsible for gluten strength which controls loaf volume. Glutenin contains both intra- and intermolecular disulfide bonds. It is elastic as a result of disulfide interchange reactions but is inextensible. Glutenin determines mixing requirements of bread dough (Hoseney and Finney, 1974).

Mixing gliadin and glutenin in the presence of water gives a viscoelastic property which is intermediate between the individual properties of gliadin and glutenin. Mixing with water causes hydration of the polypeptides which in turn causes swelling of the proteins and facilitates intra- and intermolecular association of the polypeptides (Kinsella, 1976). According to Fowler and Priestly (1980), sulfhydryl groups in glutenin react with strained disulfide bonds in other glutenin molecules to increase the elastic nature of the dough. Crosslinks are thus formed between gluten polypeptide chains. This mobility is imparted by exchange reactions of reactive disulfide bonds with free sulfhydryl groups. Amide groups, hydrogen bonding and hydrophobic associations also contribute to the viscoelastic properties (Kinsella, 1976; Pomeranz, 1973).

After mixing, dough is allowed to rise (ferment). Fermentation time depends on three factors namely quality of yeast, amount of moisture and temperature (80-100°F). The higher the temperature, the faster the dough rises. During fermentation, carbon dioxide is evolved and trapped in the gluten framework. After the dough has doubled in size, it is punched down. The dough is molded and proofed in baking pans before baking to allow the dough to rise again. During this time, more interchange between disulfide bonds and sulfhydryl groups takes place and

more carbon dioxide is trapped by the gluten framework.

Many physical and chemical changes occur during baking. In the early stages, there is rapid expansion of CO_2. Heat has softening effect on gluten enabling it to expand rapidly. This causes sudden increase in loaf volume which is called oven spring (Griswold, 1962). Starch gelatinizes at 65-68°C. At 75°C coagulation of gluten is initiated and at 90°C coagulation is completed. Bread structure is formed with a porous nature. Chemical changes occurring during baking produce the distinctive flavor and crust color. Browning of crust is due to Maillard reaction involving amines and reducing sugar.

Proteins thus have many functional properties in bread making such as dough formation, elasticity, viscosity, structural, textural, flavor, and color.

Protein Supplementation of Bread

Many workers have found that substituting or fortifying wheat flour with vegetable proteins induced adverse effects on dough properties and subsequent bread quality including: altered water absorption and mixing properties; modified gluten complex; changed fermentation rate; poor color and flavor; and decreased volume (Tsen et al., 1971; Aidoo, 1973; Pomeranz, 1973; Yousseff et al., 1976; Thompson, 1977; Appolonia, 1977, 1978; Fleming and Sosulski, 1977, 1979; Lorenz et al., 1979; Repetsky and Klein, 1981).

Tsen et al. (1971) reported that about 1% extra water was required for each 1% soy flour added to wheat flour dough. Earlier workers

observed similar findings. Lorenz et al. (1979) who worked with faba bean flour and protein concentrate also found that water absorption increased as amount of faba bean product increased. Optimum mixing time was shorter for protein supplemented dough than control dough (Tsen et al., 1971; Hoseney, 1974). Dough stabilities decreased as percentage of supplements increased. Lorenz et al. (1979) also stated that doughs with high protein substitution were quite sticky and difficult to handle.

Effect of lentil flour or rheological properties and bread characteristics was reported by Appolonia (1977). This investigator found that increasing the percentage of lentil flour in a lentil-wheat flour blend resulted in decreased water absorption. The effect was similar to that of mung bean but different from soy and faba bean mentioned earlier. Dough stability and specific volume of bread decreased with increase percentage of lentil flour in the blend. However, this effect was reduced by addition of 1% sodium stearoyl-2-lactylate (SSL). Tsen et al. (1971) reported that SSL forms a complex with gluten that stabilizes the gluten network in dough. Aidoo (1973) who studied the interactions of wheat proteins and soy proteins with surfactants reported that surfactants such as SSL could maintain or enhance the integrity of wheat protein to accommodate soy proteins in the gluten matrix.

MATERIALS AND METHODS

Lentil Protein Extraction

Lentils were obtained from a local commercial store. They were milled into a fine powder in an analytical mill for 1.5 min. Three different methods were used for extraction which are designated as Method I, Method II, and Method III.

Method I

Distilled water was used as the starting solvent for extraction. Lentil powder (20 g) was added gradually to 200 ml distilled water with continuous agitation. Extraction was carried out for 30 min at an alkaline pH near 10 by adding 1N NaOH to the slurry at 10 min intervals. Ascorbic acid was added at a level of 0.25% to reduce discoloration due to NaOH.

Method II

Lentil powder (20 g) was added gradually to 200 ml of 0.7 M NaCl and stirred continuously. Extraction was carried out for 30 min at pH near 10 by adding 1N NaOH at 10 min intervals.

Method III

Lentil powder (20 g) was dispersed in 200 ml of 0.05 M NaOH and agitated continuously for 30 min. No pH adjustment was necessary

because the medium was already alkaline, pH > 12. Ascorbic acid was added at a level of 0.25%.

In each case, flour to solvent ratio was 1:10. After 30 min of extraction, the mixtures were centrifuged at 9,000 x G at 4°C for 15 min. The supernatant from each extraction method was divided into two equal portions for acid precipitation.

Acid Precipitation

Proteins were recovered by acid precipitation using 1 N HCl. One portion was precipitated at pH 4.0 and the other portion was precipitated at pH 4.4. They were again centrifuged at 9,000 x G at 4°C for 15 min. Supernatants were poured away and protein extracts were collected and weighed. Yields were calculated by dividing weights of extracts obtained by weight of lentil powder used for extraction and multiplying the results by 100 to give yield in g/100 g powder.

Protein Determination

Macro-Kjehdahl protein determinations were run to determine protein content of the extracts (AOAC, 1960). Protein content of the lentil powder was also determined. Percentage protein recovered using the three extraction methods was calculated. Yield was multiplied by percent protein in extract and the result divided by g protein in lentil powder. The extractions were repeated 5 times as were protein determinations.

The three methods were evaluated and the best one was used to prepare protein extracts for the bread supplementation studies.

Moisture determination of lentil protein extracts was also

conducted *in vacuo* (AOAC, 1960).

Bread Supplementation Studies

Appropriate ingredients were obtained from a local store. White bread flour was obtained from a local bakery. It had a protein content of 11.6% and a moisture level of 11.62%. Preliminary studies on bread making were done following the straight dough method described by American Association of Cereal Chemists (AACC, 1969).

Bread Preparation

A standard white bread recipe was followed (AACC, 1962; Cambell, 1979). A dough conditioner, sodium stearoyl-2-lactylate (SSL) was added at a 1% level to improve loaf volume. Bread doughs were made where white wheat flour was substituted with lentil proteins at 5%, 7.5%, and 10% levels on a dry weight basis. Amount of flour and water was adjusted accordingly following this supplementation. Table 3 shows the amount of ingredients used for each bread formulation.

The four bread formulations were made on the same day. Ingredients for the four bread samples were weighed separately. For the control bread, yeast was sprinkled onto 25 ml of lukewarm water (110°F) containing one-half of the sugar. It was set aside to form a suspension. Salt was mixed with flour in a mixing bowl and shortening was rubbed in. Yeast suspension, sugar and salt solution were added to the flour mixture in the bowl with an additional 15 ml lukewarm water.

Table 3. Amount of ingredients in each bread formulation.

Ingredients		Lentil protein supplementation[a] 0%	5%	7.5%	10%
Protein extract[b]	(g)	-	33.4	50.1	66.8
Bread flour, white	(g)	100	95	92.5	90
Sugar	(g)	5	5	5	5
Yeast, active dry	(g)	2	2	2	2
Salt	(g)	2	2	2	2
Shortening	(g)	3	3	3	3
Water	(ml)	65	45	35	25
SSL	(g)	1	1	1	1

[a]Percentage protein on dry basis.
[b]Wet weight.

The mixture was mixed for a total of 9 min using a Kitchen Aid Mixer (Hobart Inc.). The speed of the mixer was increased gradually. Mixing time was 1 min at speed 2, 4 min at speed 4, 2 min at speed 6, and 2 min at speed 8.

The dough was formed into a ball and placed in a greased bowl and incubated at 95°F to ferment for 135 min. The dough was punched down after it had doubled in volume (after 85 min). It was allowed to rise again for another 50 min.

The dough was molded according to standard procedure outlined by Griswold (1962). The dough was placed in a 6" x 3 1/4" x 2 1/8" baking pan and proofed for 100 min at 95°F and then baked at 425°F for 20 min. A pan of water was placed in the oven to provide uniform heating.

The next bread sample (5% lentil protein supplementation) was prepared while the previous dough was being incubated. Protein extract was distributed in flour in which SSL and shortening had been mixed. Yeast suspension and salt and sugar solution were added. The mixture was mixed in a similar manner as for the control bread. The same procedures were repeated for 7.5% and 10% supplemented bread samples. The experimental design was that all factors were kept constant. The only variation was the level of lentil protein supplementation.

Objective Evaluation

Objective evaluations were done on specific volume, crumb and crust color, texture and protein content of the breads.

Specific Volume

Weight and volume of the breads were measured 1 hr after they had been baked. Volume was measured by rapeseed displacement (Campbell *et al.*, 1979). Dividing volume of bread by its weight gave specific volume.

Color

Crumb and crust color was measured using Hunter Color Difference Meter, model D25-2 (Hunter Associates Laboratory Inc.). The instrument was standardized against a standard white tile on reflectance mode, arrangement I. The calibrated values were: L = 94.0, a = -0.9, b = 1.2. Zero scale adjustment was done by means of a black tile. Samples were placed in a 1.5 cm cell for color evaluation. Hunter L values

indicate degree of lightness of the object. Hunter 'a' and 'b' can have positive or negative values: Hunter 'a' values indicate green-red color, Hunter 'b' values indicate blue-yellow color (Appendix Figure A.1).

Texture

The bread was cut into slices of 1 cm thickness. Crumb texture was measured using Instron Universal Testing Instrument, model 1132. Diameter of compression probe was 3.55 cm, cross head speed was 10 cm/min, chart speed was 7.874 cm/min, and gear ratio was 2:1. Texture was measured in terms of crumb compressibility. Compressibility was determined from pound force needed to compress the crumb to 25% depression.

Protein Determination

Protein content of the breads was determined using macro-Kjehdahl method (AOAC, 1960).

Sensory Evaluation

Sensory evaluation was conducted to assess acceptability of the bread products. The four different bread samples were made one day prior to evaluation. They were cut into slices of uniform thickness (1 cm). The sensory evaluation was conducted at Food Science and Technology Flavorium. Panelists comprised of students, faculty, and staff of Food Science and Technology Department, and 5 Malaysian students. In total, there were 50 different judges. The breads were

evaluated in terms of crumb color, texture, moistness, flavor, and overall desirability. The scores of 1-9 were used, 9 being the maximum score possible. Appendix B shows a sample of the evaluation sheet used.

Statistical Analysis

Statistical analysis was run using Apple II Plus Computer System. One-way analysis of variance was conducted using Software Pac No. 3, "Statistical Analysis Package" (Basic Business Software Co., Inc.). Duncan's Multiple Range test was carried out manually to determine whether the means were significantly different (Duncan, 1955).

RESULTS AND DISCUSSION

Lentil Protein Extraction

The initial pH of each solvent was taken at the beginning of each extraction. Distilled water had the lowest pH, ranging from 4.50 to 5.02. The 0.7 M sodium chloride solution had a pH range of 6.62 to 7.10 while the 0.05 M sodium hydroxide solution ranged from pH 12.21 to 12.38. The distilled water increased to pH 6.31-6.47 upon adding lentil powder. This was lower than pH of 6.5 - 6.8 given by a water-soybean meal slurry (Whitaker and Tannenbaum, 1977). Upon adding lentil powder to sodium chloride and sodium hydroxide solutions, the pH decreased to 6.00 - 6.03 and 11.93 - 12.00 respectively. In methods I and II, 1 N NaOH was added to increase the pH of the slurry to 10. The lentil proteins had a buffering effect because the pH decreased again shortly thereafter. Thus, 1 N NaOH was added at 10 minute intervals to maintain the extraction medium near pH 10. Final pH readings ranged from 9.75 - 9.88 for method I, 9.8 - 9.9 for method II, and 9.72 - 10.28 for method III.

Acid precipitation was done using 1 N HCl to recover the proteins at pH 4.0 which has been reported as the apparent isoelectric range for lentil proteins (Fan and Sosulski, 1974). Table 4 shows these results. Anderson and Romo (1976) reported that solubility of lentil protein was low at pH 4.4. Thus, acid precipitation to recover the proteins was also done at this pH and these results are shown in Table 5.

Table 4. Effect of different solvents on the extraction of lentil proteins followed by acid precipitation at pH 4.0.

Effect	Method I Distilled Water Mean[a]	S.D.	Method II 0.7M NaCl Mean[a]	S.D.	Method III 0.05M NaOH Mean[a]	S.D.
Yield extract[b] g/100 g powder	110.90[d]	± 9.13	70.90[c]	± 9.13	102.30[d]	± 13.45
Protein in extracts, %	14.97[c]	± 0.50	15.59[c]	± 0.68	15.82[c]	± 1.0
Protein recovered, %	66.01[d]	± 3.75	43.84[c]	± 3.82	64.01[d]	± 4.96

[a]Mean of 5 replicate determinations.

[b]Wet weight basis.

[c,d]Means in the same row not followed by the same letter are significantly different at $p < 0.05$.

Table 5. Effect of different solvents on the extraction of lentil proteins followed by acid precipitation at pH 4.4.

Effect	Method I Distilled Water Mean[a]	S.D.	Method II 0.7M NaCl Mean[a]	S.D.	Method III 0.05M NaOH Mean[a]	S.D.
Yield extract[b] g/100 g powder	113.90[d]	± 7.72	70.00[c]	± 18.26	108.92[d]	± 16.16
Protein in extracts, %	15.21[c]	± 0.88	15.17[c]	± 1.01	15.56[c]	± 1.58
Protein recovered, %	68.79[d]	± 2.55	42.23[c]	± 11.18	66.62[d]	± 3.56

[a]Mean of 5 replicate determinations.

[b]Wet weight basis.

[c,d]Means in the same row not followed by the same letter are significantly different at $p < 0.05$.

Yield

It was interesting to find that using distilled water as the extraction medium adjusted to pH 9-10 gave as good a yield as extraction using 0.05 M sodium hydroxide solution. Mean of five replicate extractions showed a slightly higher yield of lentil protein extracted using the first method (Tables 4 and 5). Statistical analysis showed that the difference between Method I and Method III was not significant. However, yield obtained by using sodium chloride as extractant (Method II) was low. It was significantly lower ($p < 0.05$) than the yields obtained by the other two methods. The extracts obtained were moist. Moisture determination showed that the extracts contained 77.7-83.5% moisture. Apparently, water was absorbed during the process and trapped in the protein network.

Percentage Protein in Extracts

The percentage of proteins in the extracts obtained by the three methods did not differ significantly.

Protein Recovery

In terms of percentage protein recovered, the pattern was similar to that of yield of lentil protein extracts obtained. However, in calculating percentage of protein recovered, yield percentage of protein in extracts and percentage of protein in original lentil powder were taken into account. There seemed to be a positive correlation between yield and percentage protein recovered. Mean of five replicate determinations showed a slightly higher percentage of protein recovered by Method I

(66.01%) than by Method III (64.01%) (Tables 4 and 5). Statistical analysis showed that the difference in percentage of protein recovered by Method I and Method III was not significant. Percentage of protein recovered by Methods I and II was significantly higher ($p < 0.05$) than that obtained by Method II which used the sodium chloride solution.

The two precipitation pH's of 4.0 and 4.4 had no significant effect on yield, percentage of protein in extracts, and percentage of protein recovered when Method I and Method III were compared. This was in agreement with the findings of Odeh et al. (1979) who found that the maximum protein precipitation occurred at pH values between 4.0 - 4.5. However, Method II showed greater variation in the yield and percentage of protein recovered with acid precipitation at pH 4.4 (Table 5).

pH Effect

Odeh et al. (1979) solubilized 36% of total proteins in lentil extracted using water and a higher value of 57% when using 0.03 M NaOH solution. Protein extractability seemed to be affected by pH of the medium. In their study, pH of the slurry was not adjusted with alkali when water was used as the extractant. Increasing the pH of slurry to 9-10 helped to increase extractability of lentil protein when distilled water was the extractant. This explains the higher value of 66.01% protein recovered in this study with the acid precipitation done at similar pH of 4.0 as in their study. The influence of medium extraction pH on the extractability of lentil proteins was in agreement with findings of other workers (Fan and Sosulski, 1974; Shehata et al., 1978). Shehata et al. (1978) used 0.1 M phosphate buffer, pH 7.4 for extraction and obtained

59.51% protein recovery by acid precipitaton at pH 4.5.

Fan and Sosulki (1974) used 0.2% NaOH as solvent, 1:10 flour to solvent ratio, extraction time of 1 hour and obtained 72.8% protein recovery. Their value was higher because the residue was extracted twice whereas in this study, extraction was done only once and the residue was not re-extracted. This was to make the method simpler.

With 0.07 M NaCl, total protein recovered in this study was 42.23% - 43.84%, which was slightly higher than solubilities of 40.9 and 41.9% in 0.6 M and 0.8 M NaCl respectively, as reported by Bhatty et al. (1976). At pH 4.5, the insolubility of soybean proteins can be overcome by adding sodium or calcium chloride. With 0.7 N sodium chloride, some proteins are still soluble at this pH (Wolf, 1977). Lentil proteins probably behave in a similar manner as soybean proteins in the presence of sodium chloride at pH of minimum solubility, therefore less proteins were precipitated. This may explain the relatively low yield of protein extracted and protein recovered using 0.7 M sodium chloride as extractant.

Evaluation of Extraction Methods

The extraction methods used were simple and did not required sophisticated or expensive instruments. The results showed that Methods I and III produced good yields of protein extracts with high protein recovery. Yield of protein extract as well as protein recovery obtained using Method II was rather low, therefore this method was not efficient. Method III, using 0.05 M NaOH solution, required preparation of the solution at the desired concentration. Method I, using distilled water was cheaper and simpler, in fact it gave slightly higher yield and protein recovery. For

our purposes, it was the preferred method because it was the most effective and was chosen to be used in preparation of protein extracts for the next part of this study.

Findings of this study were of significance because both Methods I and III could be applied on a large scale since they are practical and economical.

Limitations of the Study

There were limitations to this study. Adding 1 N NaOH to the slurry in Method I to adjust pH near 10 caused some discoloration. Method III, using 0.05 M NaOH for extraction gave extracts a slightly darker color. Heating may increase yield and protein recovery but this was not done.

Suggestions for Future Research

A suggestion for future research is to adjust pH to less than 10 to reduce discoloration but yet retain high protein recovery. Future research should determine which components cause the color problem and find ways to minimize it during extract preparation. Another suggestion is to apply heat during precipitation to increase protein recovery and to determine if protein functionality is affected by this heat.

Effects of Lentil Protein Supplementation on Bread Characteristics

Preliminary studies indicated that bread volume was low when wheat flour was supplemented with lentil proteins. Addition of the dough conditioner, sodium stearoyl-2-lactylate (SSL) improved the volume. The

experimental procedures were designed so that all factors were kept constant except for the degree of supplementation; thus SSL was also added to the control bread.

The dough mixture with 5% lentil protein was sticky. The one with 7.5% lentil protein was stickier and the one with 10%, the stickiest. These observations were similar to the findings reported by Lorenz et al. (1979) and some other workers. In this study, one mixing time was used. Optimum mixing time is influenced by the quantity and quality of gluten. As level of protein supplementation was increased, amount of wheat flour in the blends decreased, therefore quantity of gluten in the mixtures became less and less. With less amount of gluten, optimum mixing time for the lentil protein-wheat flour blends should be shorter. Mixing was to distribute ingredients and develop gluten into continuous phase capable of retaining gas. Dough resistance to extension increased with mixing until a peak or "optimum mixing time" was reached. In this study, mixing was optimum for the control dough but too long for the dough supplemented with lentil proteins. Continued mixing increased the dough mobility and extensibility and weakened dough consistency, that is, the dough broke down. The dough was sticky probably due to release of water as a result of protein-protein interaction.

Effect on Specific Volume

Figure 2 shows the effect of lentil protein supplementation on specific volume of bread. Specific volume decreased as supplementation level increased. This effect was similar to the findings of previous workers who studied bread supplementation with various legumes (Appolonia,

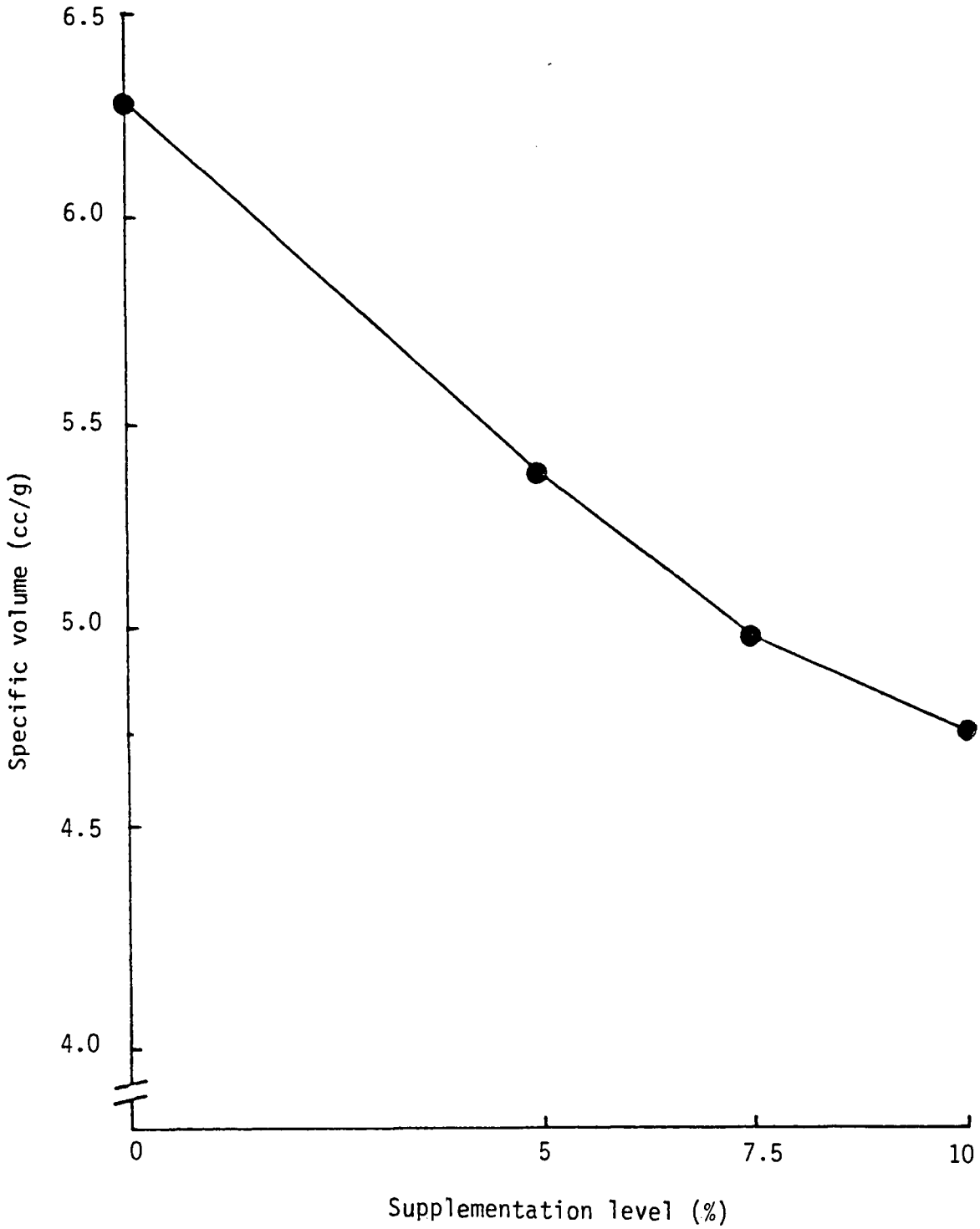

Figure 2. The effect of lentil protein supplementation on specific volume of bread.

1977, 1978; Fleming and Sosulski, 1977, 1979; Lorenz et al., 1979; Repetsky and Klein, 1981). The control bread (0% supplementation) had the highest specific volume (6.25) and was significantly higher ($p < 0.05$) than the other three treatments (Table 6). Five and 10% lentil protein breads gave specific volumes of 5.37 and 4.75 respectively, which were higher than the values of 4.53 and 4.59 reported by Lorenz et al. (1979) using 5% and 10% faba bean protein concentrate in place of wheat flour. Repetsky and Klein (1981) obtained specific volumes of 4.7, 4.1 and 3.8 using pea flour replacement at 0, 5, and 10% levels respectively. Ten percent lentil flour replacement gave a specific volume of 6.08 (Appolonia, 1977) while 15% supplementation with soy protein concentrate gave a specific volume of 4.7 (Fleming and Sosulski, 1977). In this study, specific volumes of 5% and 10% lentil breads were significantly different ($p < 0.05$) but the 7.5% lentil supplemented bread did not differ significantly from either of the two.

Volume of bread is affected by factors such as quantity and quality of gluten in the flour blends, quantity and quality of other ingredients such as yeast, amount of moisture, length of mixing and fermentation. The mixing time employed was too long for the doughs other than the control. Overmixing weakened the gluten resulting in low bread volume. However, the dough conditioner, SSL, did help improve the volume of the breads in preliminary studies because the specific volume of breads with SSL added was higher than those made without the addition of SSL.

Table 6. Effect of lentil protein supplementation on specific volume of bread.

Supplementation Level, % (on dry wt basis)	Specific Volume[a] (cc/g) Mean[b]	S.D.
0	6.25 [c]	± 0.10
5.0	5.37[d]	± 0.07
7.5	4.98[d,e]	± 0.48
10.0	4.75[e]	± 0.30

[a]Mean of four replicate bakes.

[b]Means not followed by the same letter are different at the 5% level of significance based on Duncan's Multiple Range Test.

Effect on Color

The lentil protein extract was creamy in color. When mixed with the other bread ingredients and baked, there were color changes. Figure 3 shows the color of the four bread samples. The higher the lentil proteins in the breads, the darker the color became. Without lentil protein, crumb color of the control bread was creamy white. Table 7 shows the Hunter values of the crumb and crust color of the breads.

It was interesting to compare the bread color obtained in this study with color of breads available commercially. Three different breads namely; white bread, crushed wheat bread, and whole wheat bread were obtained from the local market and color measurements were taken with the Hunter D-25 Color Difference Meter. Crushed wheat bread contained a mixture of white wheat flour and whole wheat flour. Figure 4 shows the effect of lentil protein supplementation on crumb color and comparison of lentil breads with the three commercial breads. There was a decrease in Hunter L values of the crumb color as level of lentil protein supplementation increased. This meant that the bread crumb became darker when lentil protein were added. The control bread was significantly lighter ($p < 0.05$) than breads from the other treatments. The bread supplemented with 5% lentil protein was significantly lighter ($p < 0.05$) than the 7.5 and 10.0% lentil supplemented breads. There was no significant difference between crumb color of breads supplemented with 7.5% and 10% lentil protein (Figures 3 and 4). Hunter L values of the commercial breads showed that crushed wheat bread was darker than white bread and whole wheat bread darker than crushed wheat bread (Table 8 and Figure 4).

1 2 3 4

Figure 3. Color photograph of breads
1. Control bread
2. 5.0% lentil bread
3. 7.5% lentil bread
4. 10.0% lentil bread

Table 7. Effect of lentil protein supplementation on crumb and crust color of breads.

Supplementation Level, % (on dry wt basis)	Crumb Color[a] Hunter values*			Crust Color[a] Hunter values*		
	L	a	b	L	a	b
0	+60.03[b] ± 1.49	-1.50[b] ± 0.09	+12.88[b] ± 0.76	+41.70[b] ± 4.30	+10.93[b] ± 0.46	+17.30[b] ± 2.14
5	+47.31[c] ± 2.79	-0.05[c] ± 0.28	+12.00[b] ± 0.71	+38.75[b] ± 1.81	+10.08[b] ± 0.48	+15.62[b] ± 1.83
7.5	+43.31[d] ± 3.17	+0.45[d] ± 0.35	+11.99[b] ± 0.95	+39.13[b] ± 2.56	+9.31[b] ± 1.37	+15.01[b] ± 0.93
10.0	+41.40[d] ± 2.58	+0.75[d] ± 0.20	+12.34[b] ± 0.73	+37.36[b] ± 2.46	+9.80[b] ± 1.41	+15.24[b] ± 1.66

[a]Mean of four replicate bakes.

[b,c,d]Means in the same column not followed by the same letter are different at the 5% level of significance on Duncan's Multiple Range Test.

*Hunter values: L - Total light reflectance.

a - Positive values indicate redness; negative values indicate greeness.

b - Indicates yellowness.

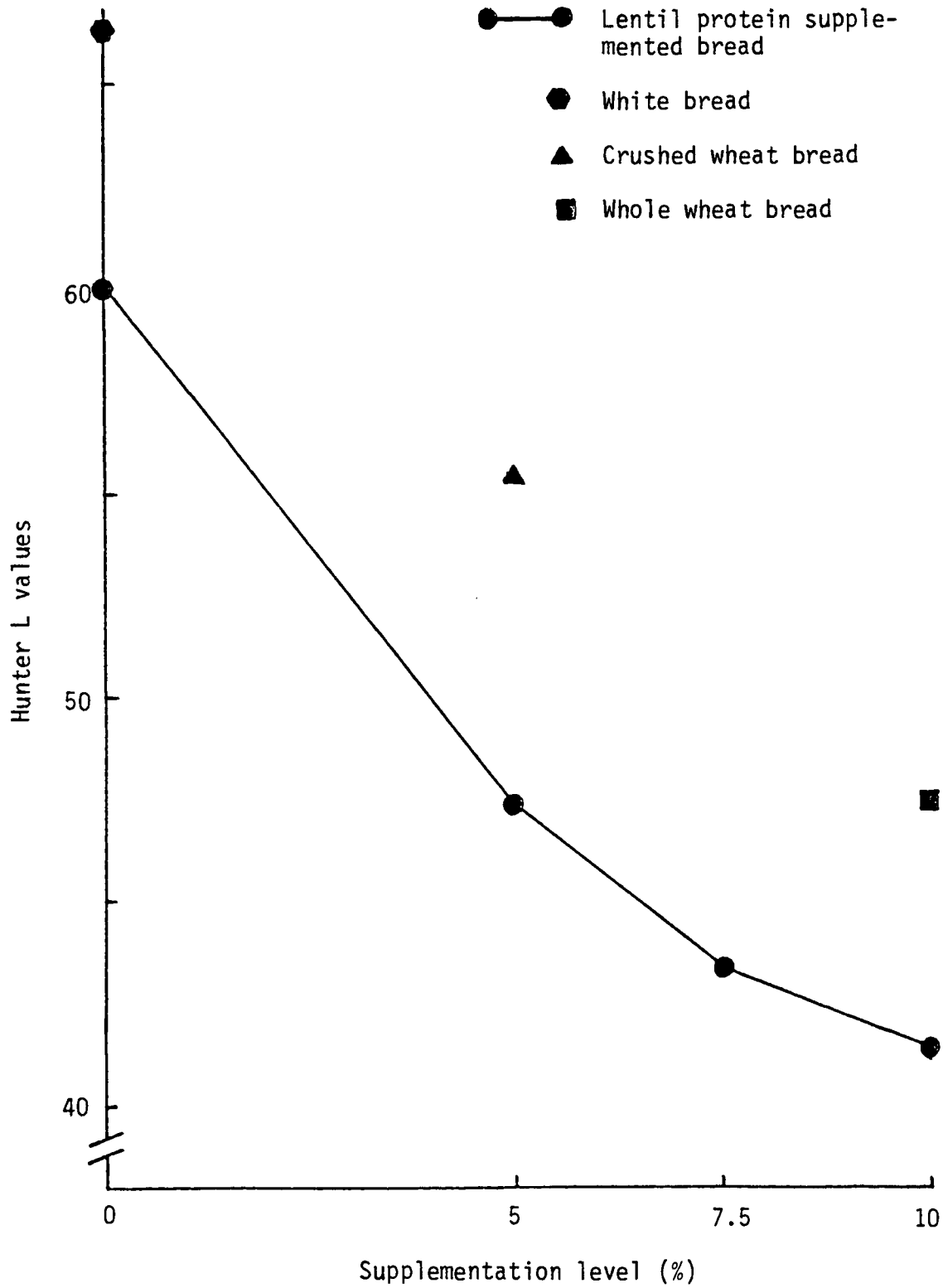

Figure 4. The effect of lentil protein supplementation on crumb color of bread and comparison with commercial breads.

Table 8. Hunter color values of commercial breads.

Hunter Values[a]	White bread	Crushed wheat bread	Whole wheat bread
L	+ 66.35	+ 55.30	+ 47.38
a	- 2.25	2.30	+ 4.45
b	+ 13.00	+ 13.20	+ 13.50

[a]Mean of triplicate determinations.

This pattern was similar to the effect of lentil protein supplementation. In terms of color lightness/darkness, 5% lentil protein bread was similar to whole wheat bread while 7.5% and 10% lentil protein breads were a little darker than the whole wheat bread.

Hunter 'a' and 'b' values measure basic colors. Hunter 'b' values did not differ very much but there was marked increase in Hunter 'a' values with increase in lentil protein or whole wheat flour levels. Crushed wheat bread was beige and whole wheat bread was brownish. The 5% lentil bread was light greyish yellow while 7.5% and 10% lentil breads were brownish yellow.

Crust color was not significantly different among the four bread treatments in this study (Table 7 and Appendix Table C.5).

The effect of lentil protein supplementation in color score by sensory evaluation is shown in Figure 5. There was significant difference ($p < 0.05$) in mean color scores between the control bread and protein supplemented breads but there was no significant difference among the supplemented breads. Objective evaluation using Hunter D-25 Color Difference Meter, however, showed that 5% lentil bread was significantly lighter ($p < 0.05$) than the 7.5% and 10% lentil breads.

Effect on Texture

Objective evaluation of bread texture was evaluated by crumb compressibility. Figure 6 shows the crumb compressibility curves using the Universal Testing Instrument (Instron Inc.). The peak heights and areas under the curves formed on the chart were related to the amount of force needed to compress the bread to 25% depression. Small force is needed

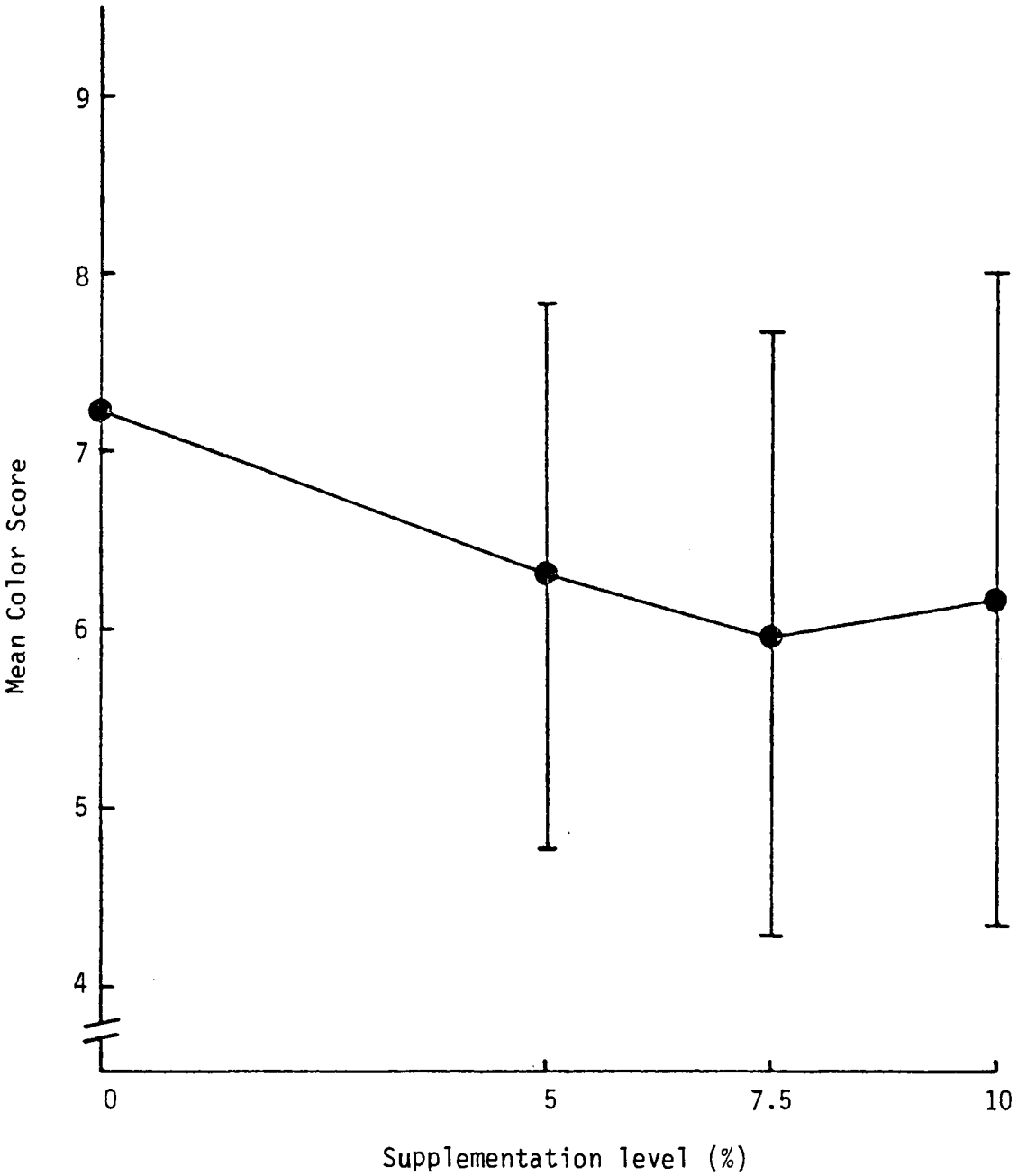

Figure 5. The effect of lentil protein supplementation on sensory evaluation color scores (Standard deviation shown by vertical lines).

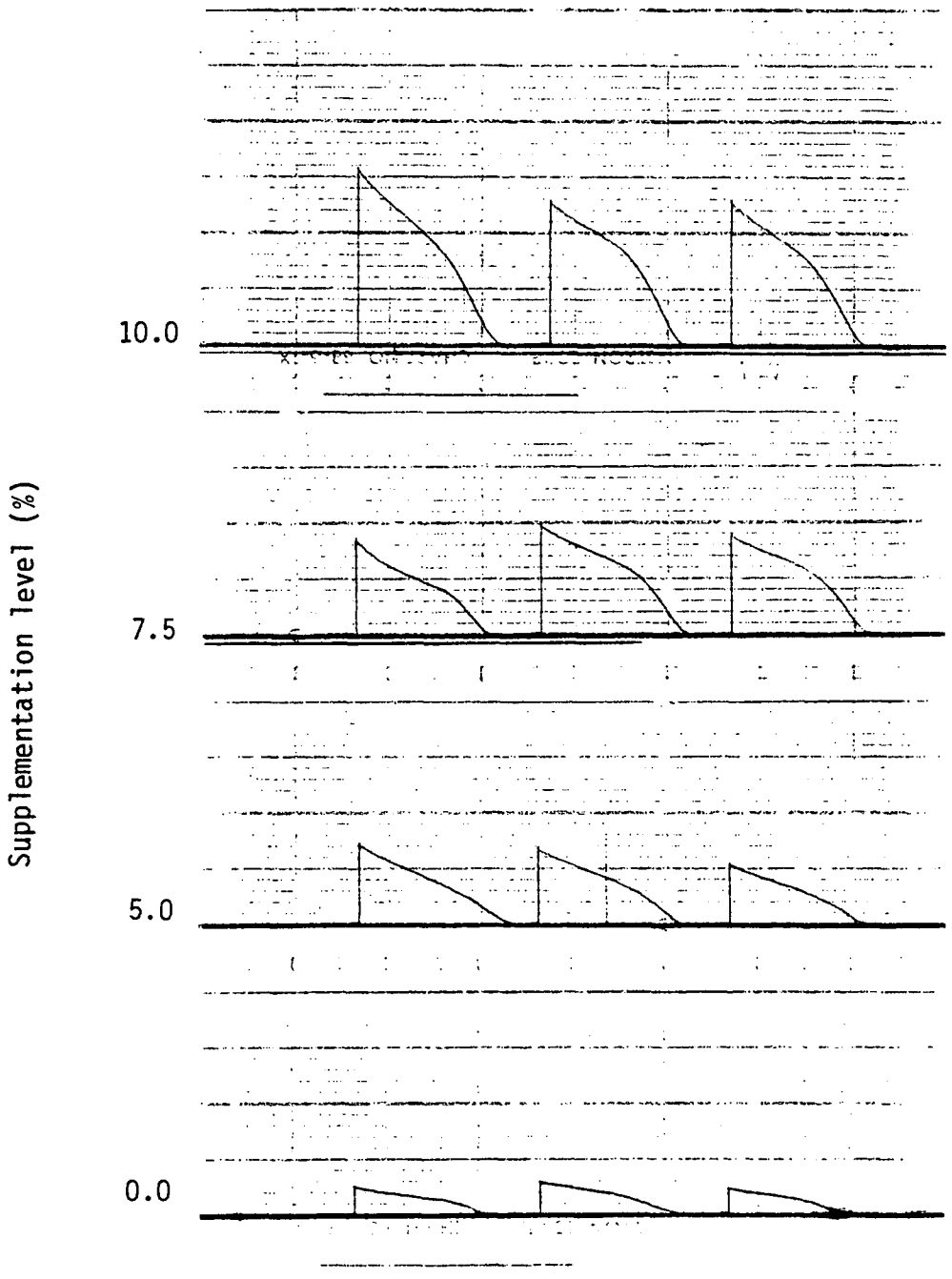

Figure 6. The effect of lentil protein supplementation on crumb compressibility curves.

to compress a slice of soft bread but greater force is needed to compress a firm slice. Figure 6 shows that peak heights and areas under the curves became greater as level of protein in the bread increased. Measurements of peak heights and areas under the curves are summarized in Table 9. The results indicated that more force was needed to compress breads that had greater amount of protein (Figure 7). Bread supplemented with 10% lentil protein was significantly different ($p < 0.05$) from the other treatments.

The effect of lentil protein supplementation on texture score is shown in Figure 8. The control bread received a significantly higher ($p < 0.05$) score (7.32) than the supplemented breads (6.82 - 6.52). Differences in mean texture scores among the supplemented breads were not significant. The judges did not detect the difference in the 10% lentil bread as was shown by crumb compressibility data. Texture of lentil breads was judged as acceptable by the panelists although they were not scored as high as the control bread.

Sensory evaluation scores for color, texture, moistness, flavor, and overall desirability are summarized in Table 10. In terms of flavor, 5% lentil bread received significantly higher ($p < 0.05$) score than 10% lentil bread. Increasing supplementation levels caused a decrease in flavor score (Figure 9).

Effect on Product Acceptability

Color, texture, moistness, and flavor influenced the overall desirability score. In general, the control bread received a significantly higher ($p < 0.05$) desirability score (7.28) than the lentil supplemented

Table 9. Effect of lentil protein supplementation on crumb compressibility of bread.

Supplementation Level, % (on dry wt basis)	Crumb Compressibility[a] Peak[b] (lb force)	Area[b] (cm^2)
0	0.59[c] ± 0.07	0.74[c] ± 0.05
5.0	1.03[c] ± 0.18	1.56[c] ± 0.27
7.5	1.45[c] ± 0.35	1.74[c] ± 0.54
10.0	2.20[d] ± 1.09	3.10[d] ± 1.35

[a]Mean of four replicate bakes.

[b]Values in the same column not followed by the same letter are significantly different at $p < 0.05$.

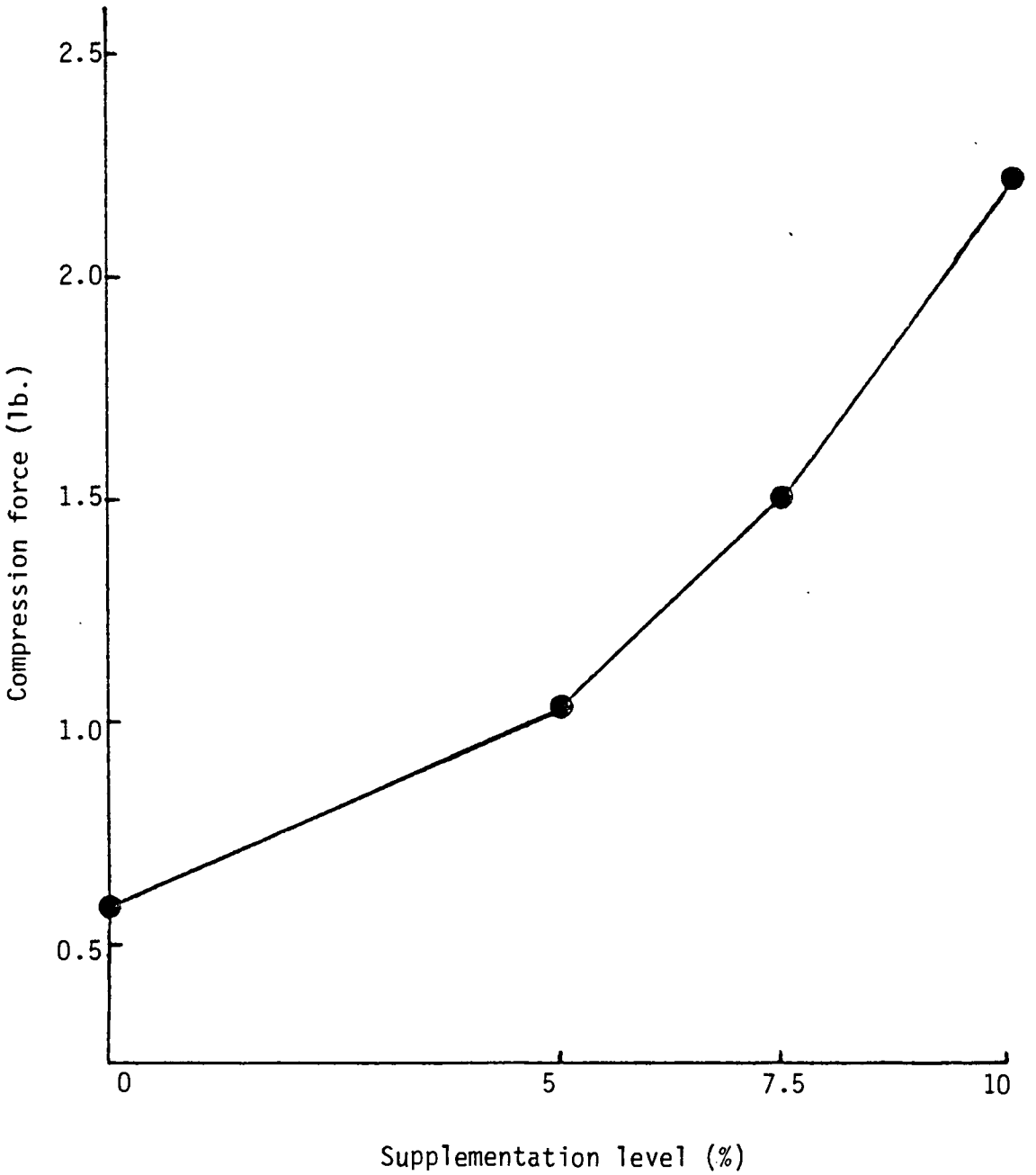

Figure 7. The effect of lentil protein supplementation on compression force on crumb.

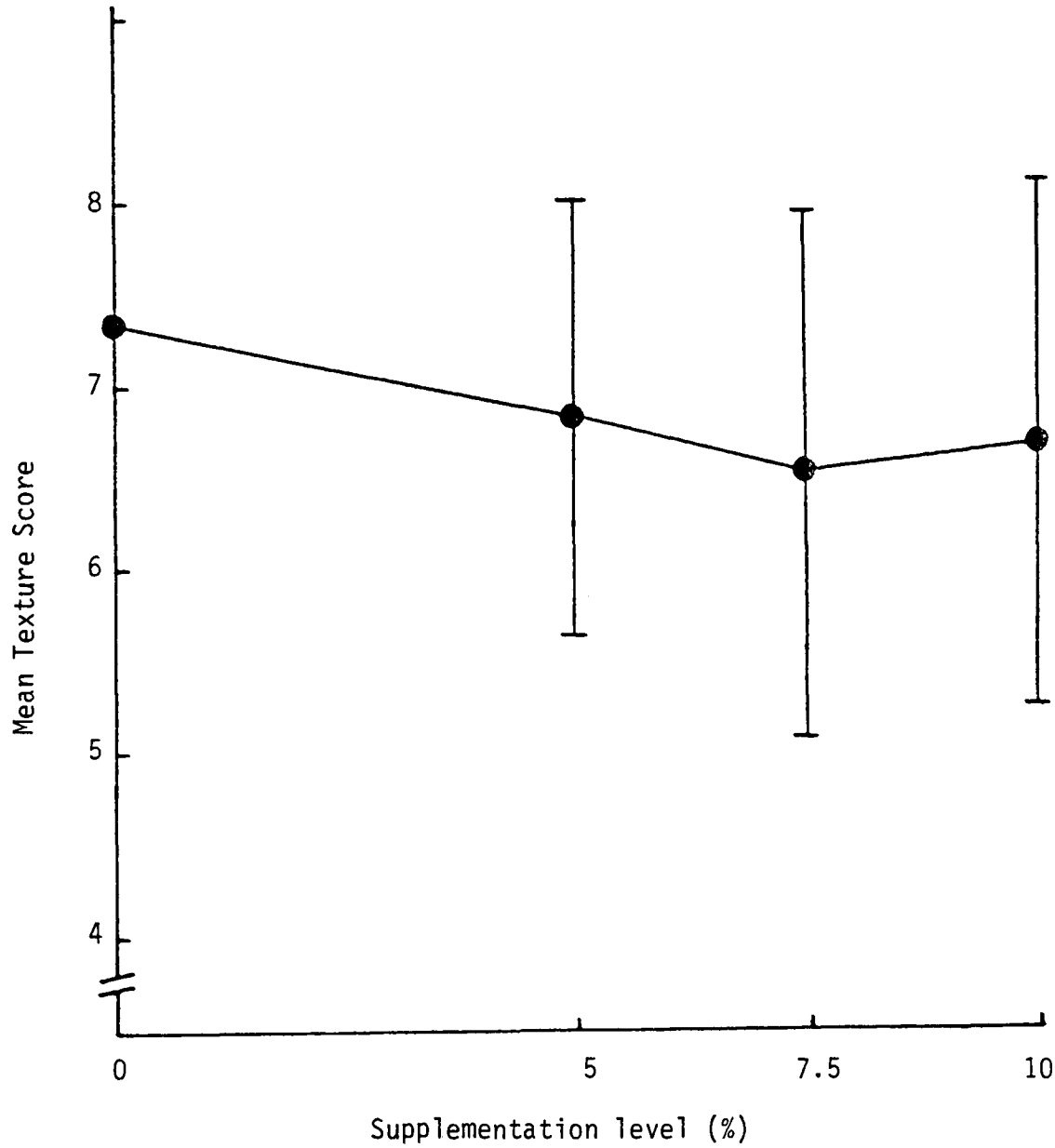

Figure 8. The effect of lentil protein supplementation on sensory evaluation texture scores (Standard deviation shown by vertical lines).

Table 10. Sensory evaluation mean scores[a] for color, texture, moistness, flavor and overall desirability of bread samples containing different supplementation levels of lentil protein.

Supplementation Level, % (on dry wt basis)	Color		Texture		Moistness		Flavor		Overall desirability	
	Mean[b]	S.D.	Mean[b]	S.D.	Mean[b]	S.D.	Mean[b]	S.D.	Mean[b]	S.D.
0.0	7.36[c]	± 1.33	7.32[c]	± 0.98	7.16[c]	± 0.98	7.20[c]	± 1.20	7.28[c]	± 0.93
5.0	6.30[d]	± 1.52	6.82[d]	± 1.19	6.80[c]	± 1.12	6.48[d]	± 1.47	6.48[d]	± 1.27
7.5	5.96[d]	± 1.69	6.52[d]	± 1.43	6.36[d]	± 1.32	5.98[d,e]	± 1.71	6.02[e]	± 1.46
10.0	6.16[d]	± 1.81	6.52[d]	± 1.42	6.82[c]	± 1.14	5.78[e]	± 1.82	6.10[d,e]	± 1.53

[a]Scores ranged from 1 to 9 with 9 being the maximum score.

[b]Means not followed by the same letter are different at the 5% level of significance based on Duncan's Multiple Range Test.

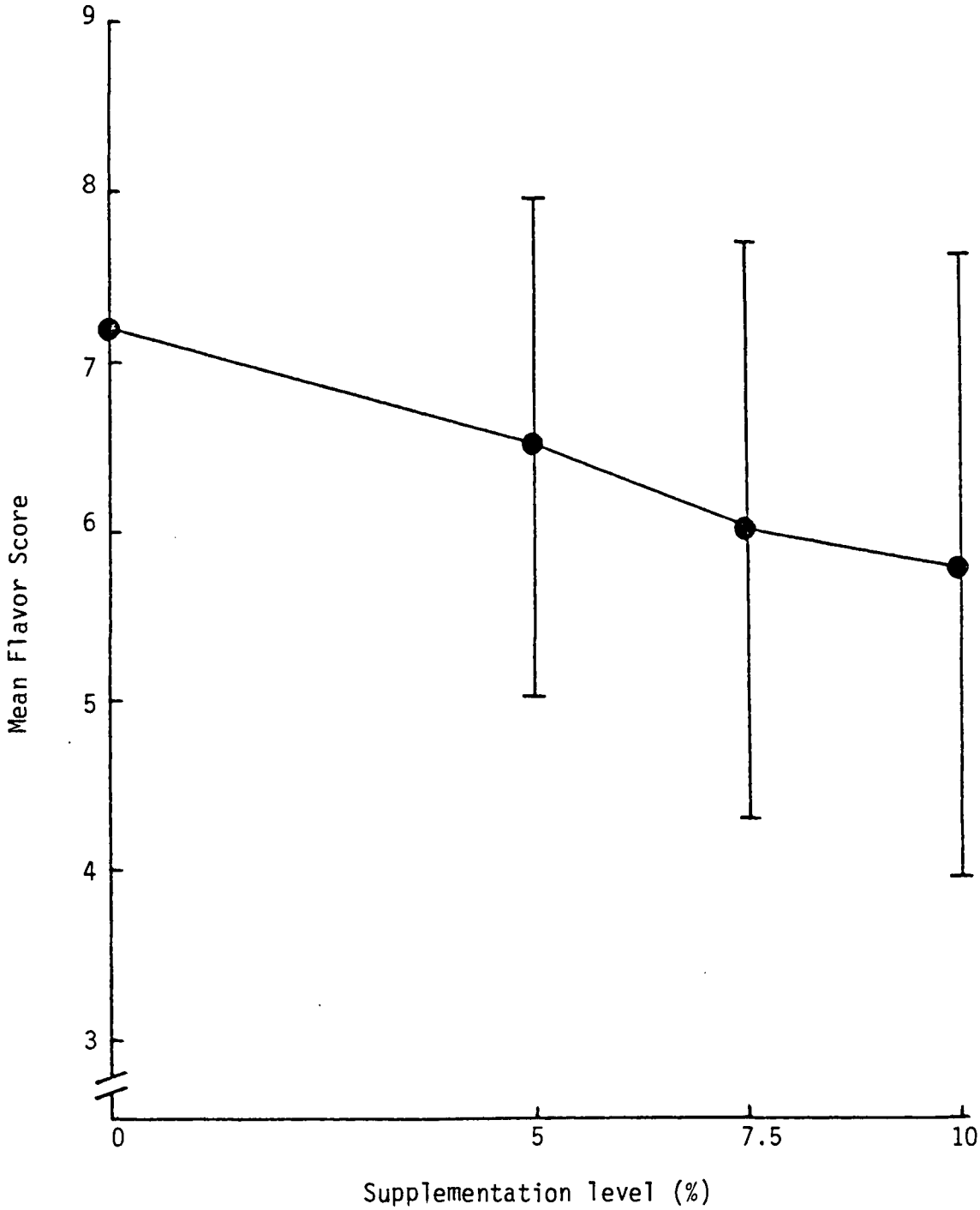

Figure 9. The effect of lentil protein supplementation on sensory evaluation flavor scores (Standard deviation shown by vertical lines).

breads (6.48 - 6.02). A score of 6.00 or higher is considered as acceptable (McGill, 1982). Even though overall desirability score for the supplemented breads was lower than the control bread, they were judged as acceptable at the 5%, 7.5% and 10% supplementation levels by the panelists (Figure 10).

Protein Content of Bread

Table 11 shows the protein content of breads at the different supplementation levels. Increase in supplementation levels resulted in higher protein content of the breads; the relation appeared to be linear (Figure 11). Products in this study contained more protein compared to products of other studies previously completed. Fleming and Sosulski (1979) reported protein contents of 10.6% and 11.4% using 5% and 10% soy replacement, respectively; whereas 13.0% and 14.6% were obtained in this study using 5% and 10% lentil protein replacement. It was reported by Sosulski and Fleming (1979) that 6% soy supplementation increased PER value of bread by 30% from 1 to 1.3 and 12% soy increased the PER value by almost 100% to 1.9 because the lysine content was increased. PER value of lentil supplemented breads in this study would be expected to increase by 30 to 50%. This is of significance because high protein breads could be produced at low cost since lentils are relatively cheap. This would help reduce deficiency problems especially in areas where animal proteins are expensive or scarce.

Limitations of the Study

In making the breads, mixing time was too long for the lentil

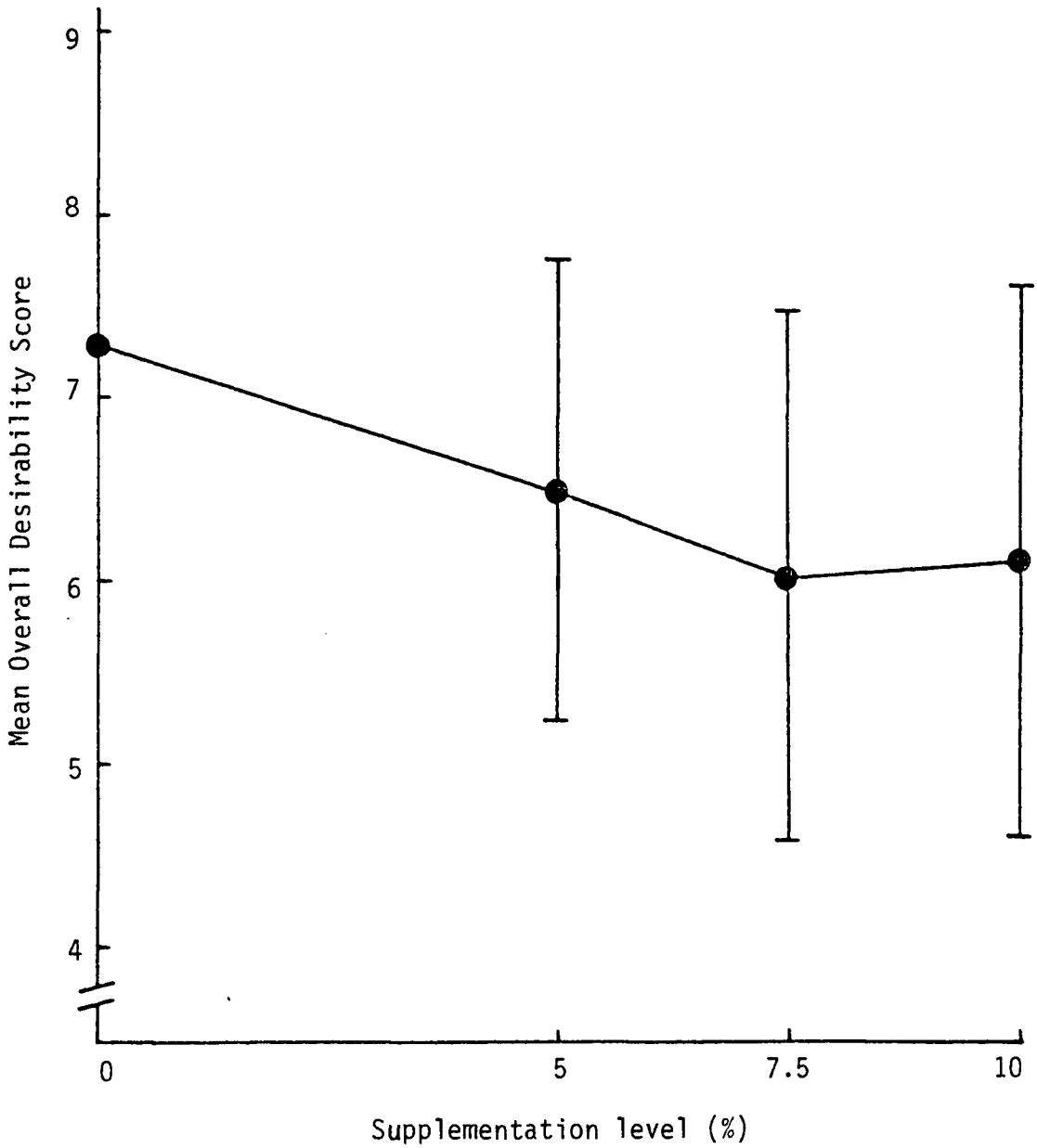

Figure 10. The effect of lentil protein supplementation on sensory evaluation overall desirability scores (Standard deviation shown by vertical lines).

Table 11. Protein content of breads supplemented with different levels of lentil protein.

Supplementation Level, % (on dry wt basis)	Protein Content (%)[a] Mean[b]		S.D.
0.0	11.44[c]	±	0.40
5.0	13.02[d]	±	0.80
7.5	13.80[d,e]	±	0.80
10.0	14.59[e]	±	0.35

[a]Mean of four determinations.

[b]Means not followed by the same letter are different at the 5% level of significance based on Duncan's Multiple Range Test.

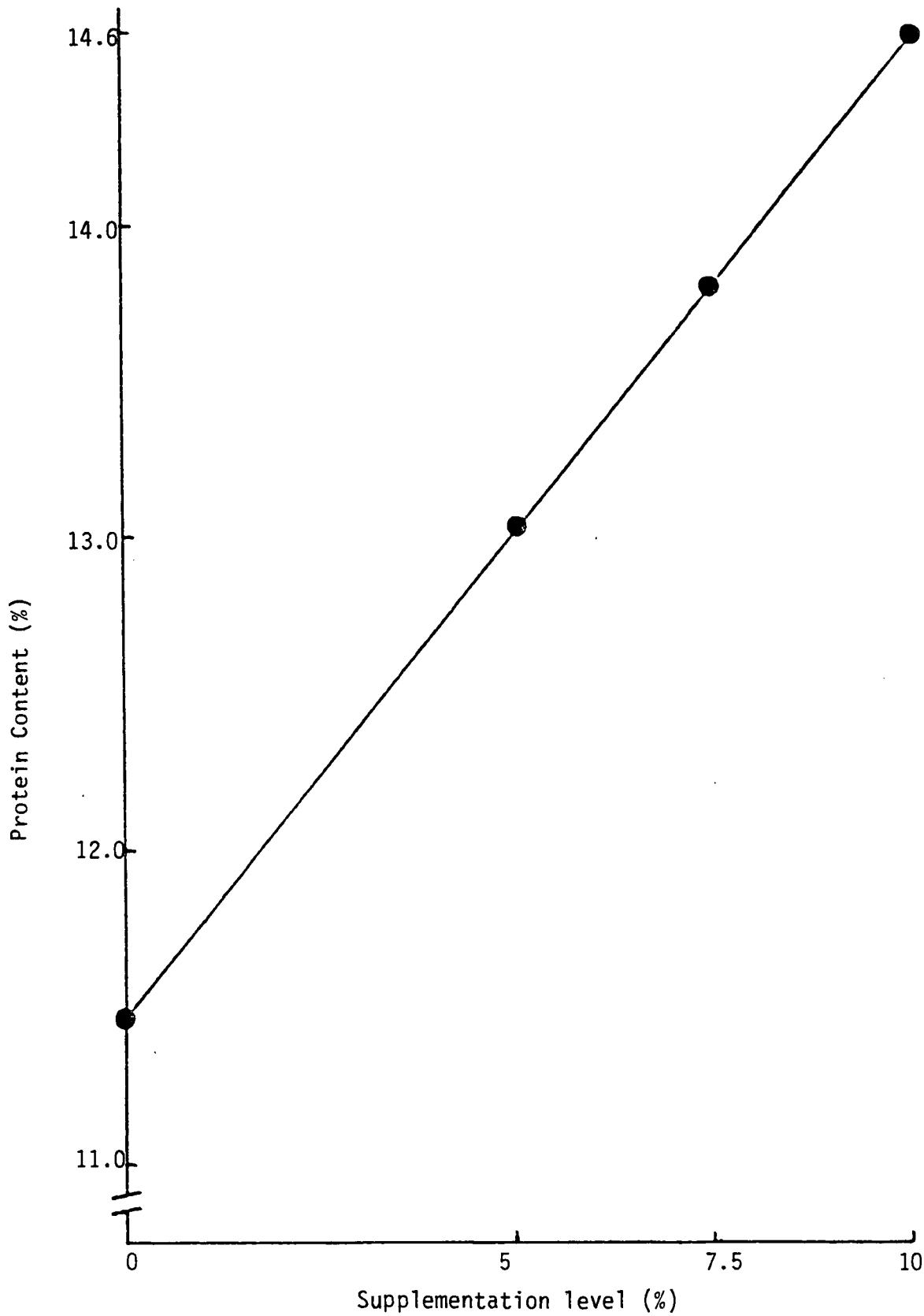

Figure 11. The effect of lentil protein supplementation on protein content of bread.

protein-wheat flour blends which weakened the gluten and resulted in low loaf volume. Another limitation was that a few panelists found the color undesirable.

Suggestions for Future Research

Future research should determine optimum mixing time for lentil protein-wheat flour blends so that loaf volume could be improved. Ways to reduce the color problem should be investigated. Nevertheless, the trend now is that people associate darker colored bread as more nutritious than white bread and consumption of this kind of bread has increased. Using whole wheat flour instead of white flour would probably be better because the effect of lentil protein on crumb color could probably be masked. Effect of lentil protein supplementation on PER value should be investigated. It would also be interesting to add lentil protein at various levels while keeping amount of wheat flour constant and see how this affects bread characteristics.

CONCLUSIONS

Lentil protein extraction studies indicated that:

1. Extraction was effective using either distilled water with the slurry adjusted and maintained at about pH 10.0 or using 0.05 M NaOH.
2. Protein recoveries obtained using the two methods ranged from 66.01 to 68.79% and 64.01 to 66.62% respectively.
3. The methods are practical and economical for application in areas where dietary protein is lacking. They are simple and do not require expensive or sophisticated instruments.

Bread supplementation studies indicated that:

1. As the level of lentil protein supplementation increased, specific volume of bread decreased.
2. Lentil protein had a significant effect on crumb color of bread. The color became darker with increasing levels of lentil protein.
3. Crumb compressibility decreased as lentil protein supplementation levels increased, indicating that the texture became firmer.
4. Lentil protein supplementation increased the protein content of the breads. High-protein breads of better nutritional quality could be produced using lentil proteins.

5. Sensory evaluation indicated that the overall desirability of the breads supplemented with 5%, 7.5% and 10% lentil proteins were judged as acceptable. This is of significance because lentil protein has potential as a source of dietary protein.

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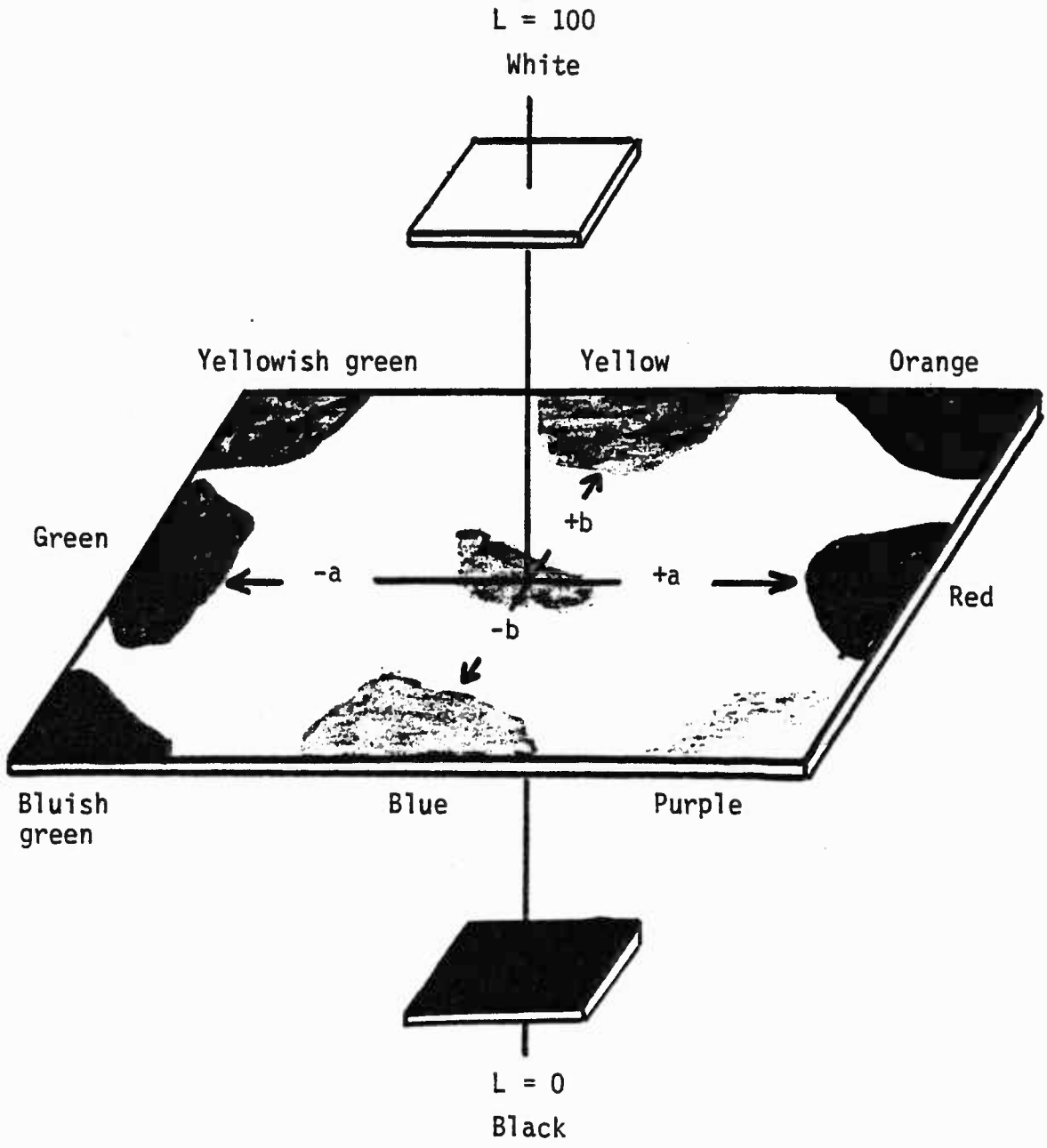
APPENDICES

Appendix A



Appendix Figure A.1. The Hunter Color Coordinates

Appendix B

Sensory Evaluation Sheet

Department of Food Science and Technology
Oregon State University

Product: Name:_____

Date:_____

Please write the sample number in the space following the statement which best describes your opinion of the sample.

	COLOR	TEXTURE	MOISTNESS	FLAVOR	OVER-ALL DESIRABILITY
9- extremely desirable					
8- very desirable					
7- moderately desirable					
6- slightly desirable					
5- neither					
4- slightly undesirable					
3- moderately undesirable					
2- very undesirable					
1- extremely undesirable					

Which sample did you prefer?_____

Why?

Appendix C.

Analysis of Variance

Appendix Table C.1. Analysis of variance for yield extract (g/100 g powder).

Source	d.f.	Mean square	F-value
pH	1	15.84	.38
Treatments	2	1267.56	30.38*
Interaction	2	8.84	.21
Error	24	41.73	

*Significant at ($p < 0.05$).

Appendix Table C.2. Analysis of variance for protein in extracts (%).

Source	d.f.	Mean square	F-value
pH	1	.16	.16
Treatments	2	.89	.89
Interaction	2	.3	.3
Error	24	1	

Appendix Table C.3. Analysis of variance for protein recovered (%).

Source	d.f.	Mean square	F-value
pH	1	11.88	.36
Treatments	2	1828.95	55.48*
Interaction	2	15.5	.47
Error	24	32.96	

*Significant at ($p < 0.05$).

Analysis of Variance (continued)

Appendix Table C.4. Analysis of variance for specific volume (cc/g).

Source	d.f.	Mean square	F-value
Treatments	3	1.75	20.95*
Error	12	.08	

*Significant at ($p < 0.05$).

Appendix Table C.5. Analysis of variance for Hunter L, a, and b color values of bread crumb and bread crust.

Source	d.f.	Mean square	F-value
Hunter L, crumb			
Treatments	3	277.32	43.63*
Error	12	6.36	
Hunter L, crust			
Treatments	3	13.10	1.53
Error	12	8.59	
Hunter a, crumb			
Treatments	3	14.07	91.36*
Error	12	0.04	
Hunter a, crust			
Treatments	3	1.83	1.71
Error	12	1.07	
Hunter b, crumb			
Treatments	3	.69	1.10
Error	12	.63	
Hunter b, crust			
Treatments	3	3.24	1.12
Error	12	2.88	

*Significant at ($p < 0.05$).

Analysis of Variance (continued)

Appendix Table C.6. Analysis of variance for crumb compressibility of bread based on peak and area of compressibility curves.

Source	d.f.	Mean square	F-value
Peak			
Treatments	3	1.88	5.53*
Error	12	0.34	
Area			
Treatments	3	3.83	7.03*
Error	12	0.55	

*Significant at ($p < 0.05$).

Appendix Table C.7. Analysis of variance for sensory evaluation on color, texture, moistness, flavor and overall desirability scores.

Source	d.f.	Mean square	F-value
Color score			
Panelist	49	4.56	2.44*
Treatments	3	19.58	10.46*
Error	147	1.87	
Texture score			
Panelist	49	2.81	2.32*
Treatments	3	5.98	4.94*
Error	147	1.21	
Moistness score			
Panelist	49	2.82	3.46*
Treatments	3	5.38	6.61*
Error	147	.81	
Flavor score			
Panelist	49	4.71	2.77
Treatments	3	20.01	11.79*
Error	147	1.7	
Overall desirability score			
Panelist	49	3.27	2.68
Treatments	3	16.59	13.57*
Error	147	1.22	

*Significant at ($p < 0.05$).

Analysis of Variance (continued)

Appendix Table C.8. Analysis of variance for protein content of breads (%).

Source	d.f.	Mean square	F-value
Treatments	3	6.67	20.29*
Error	12	.33	

*Significant at ($p < 0.05$).