THE EFFECTS OF PRETREATMENT AND MICRONIZATION ON THE QUALITY AND COOKABILITY OF LENTILS

By
Yinghua Zhao

A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Food Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of Master of Science

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ABSTRACT

The potential for using micronization (infrared heat processing) to add value to lentils by reducing the cooking requirements was investigated in this study. Canada No. 2 Laird and Eston lentils were subjected to a range of tempering solutions and micronization, and then assessed for the final moisture content, degree of starch gelatinization, protein solubility, color, cooking quality and the antinutritional factors. The results showed that the cooking time of Laird and Eston lentils could be significantly reduced to 16 and 18 min respectively, if treated under the appropriate tempering and micronization conditions. Under the same heat treatment condition, different pretreatment conditions (tempering level, tempering time and tempering solution) had different effects on the quality of the end products. The final moisture content was mainly determined by the tempering level ($p<0.0001$), as about 10-15% moisture was lost during the heat treatment, regardless of moisture level after tempering. Tempering level was also the most important factor contributing to the degree of starch gelatinization ($p<0.001$), the protein solubility ($p<0.001$) and the cooking quality ($p<0.001$) of the micronized seeds. Tempering time also affected these characteristics but only at high tempering levels. The contents of two antinutritional factors, phytic acid and the phenolic compounds were significantly reduced after tempering and micronization treatments. Among the four tempering solutions examined (distilled water, 150 ppm disodium EDTA, 2% sodium tripolyphosphate and the mixture of 1% citric acid and 2% ascorbic acid), the distilled water, disodium EDTA and sodium tripolyphosphate produced seeds with good cooking quality and little color change. The two infrared heating systems were used to process lentils (the laboratory scale infrared lamp and the pilot scale micronizer), both improved

the quality of the lentils. The lentils produced by the pilot scale micronizer appeared to be more stable and had a better appearance due to its efficient and uniform heating conditions.

ACKNOWLEDGMENTS

I would first like to express my great appreciation to my advisor, Dr. S. D. Arntfield for all of her expertise, guidance, patience and continuous support during my study. I would also like to thank Dr. B. Watts and Dr. S. Cenkowski for their advice and participation on my thesis examining committee.

I am also deeply grateful to all the people who provided me with technical support: Aniko Bernatsky, Donna Ryland and Jim Rogers. Also, thank you to Ted Russin and Crystal Cinq-Mars for their invaluable technical assistance. Financial support from the Saskatchewan Wheat Pool and the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

A special thank you to my parents and my sister for their endless love and encouragement, it was you who gave me the strength and confidence to pursue my goals. I would also like to extend my thanks to all my friends, whose friendship supported me over the past two years.

I would also like to thank my husband Guopeng for his encouragement and assistance during my study.

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CHAPTER 1

INTRODUCTION

Lentil (*lens esculenta*) is one of the most commonly grown food legumes in the world and it has been widely used in the human diet for quite a long time. Lentil contains 20-30% protein, and has excellent nutritional value due to the low concentration of antinutritonal factors compared with other food legumes (Adsule et al., 1989). Lentil is a good source of starch, lipid, minerals and B-complex vitamins. The starch content of lentils varies from 35-53%; the digestibility of lentil starch is nearly 100% (Bhatty, 1988).

Since lentil introduction in the 1970`s, Canada has recently become one of the largest producers and exporters of lentils in the world. There are two species of lentils widely cultivated in western Canada, Laird and Eston lentils. Laird is an extra large-seeded, yellow cotyledon, Chilean type of lentil, and Eston is a small-seeded, yellow cotyledon, Persian type of lentil (Bhatty, 1988). Lentils are primarily used in human foods, and as a result, cooking quality is a foremost quality criterion. The long cooking time, about 30-40 minutes, limits the use of lentils since people like to select food on the basis of ease and convenience of preparation (Adsule et al., 1989). Soaking is a traditional pretreatment method to reduce the cooking time of dry legumes, however, soaking is not suitable for the large scale production and distribution due to difficulty in handling of the wet seeds. In recent years, the interest in processing legumes by micronization (infrared processing) has increased due to its rapid heat transfer and deep penetration (Driscoll, 1992).

Infrared radiation is a form of electromagnetic energy and it is transmitted as a wave. Infrared heating involves the exposure of a material to electromagnetic radiation in the infrared wavelength. It will cause rapid internal heating and a rise in water vapor pressure inside the heated sample (Fasina et al., 1997). Micronization can be used to increase the digestibility and nutritional quality of cereals and legumes for human food (Kadam et al., 1987). Recent studies found that micronization has the potential for reducing cooking time of lentils (Arntfiled et al., 1997). The micronized Canada No. 1 Laird lentils could be softened to an acceptable degree after only 15 min cooking. It was also found that the effect of micronization varied greatly with the pretreatment conditions and the quality of the seeds.

The objective of this study was twofold. First, the optimization of the pretreatment condition time of Laird and Eston lentils before micronization to reduce their cooking times. Variables including tempering level, tempering time and the composition of tempering solutions. Secondly, the characterization of the micronized products with respect to the color, cooking texture, solubility of dietary fiber and the reduction of antinutritional factors.

Canada No.2 Laird and Eston lentils were used to investigate the effects of micronization under different pretreatment conditions. The micronizing equipment includes a laboratory scale infrared lamp and a pilot scale gas-generated infrared heating system (Micronizing Company, UK). With a small amount of sample, about 60g for each run, the laboratory scale infrared lamp provided initial results for further application with the pilot scale infrared heating system. The cooking quality of the resulting micronized products was determined with texture, degree of starch gelatinization, protein solubility

and the color. The solubility of dietary fiber and the reduction of some antinutritional factors of the final products, including phytic acid, phenolic compounds and oligosaccharides were tested as well.

CHAPTER 2

LITERATURE REVIEW

2.1. Characteristics and Quality of Lentils

2.1.1. Seed Characteristics

The structure and characteristic of the lentil seed are similar to those of other legumes, although the lentil seed coat is much thinner than seed coats of many other legumes (Hughes and Swanson, 1986; Salunkhe, et al., 1985). There are three main components in legume seed: the seed coat, the cotyledon and the embryo. These components of lentil seed form about 8, 90 and 2% of the total weight, respectively. (Singh et al., 1968). As with the other legumes, the cotyledons are the major component of lentil seeds, and this is the component that contains almost all the proteins, starch and lipids of the whole seeds (Bhatty, 1995b).

The size, color and shape of lentil seeds are cultivar characteristics. The Canadian Laird is large seeded (13-14 seeds per gram), olive green colored seed, and Canadian Eston is small seeded (26-28 seeds per gram), green to light brown colored seed (Bhatty, 1995b). Other cultivars, Rose and CDC Richlea are medium seeded, olive green colored seeds. Turkish lentil has similar seed size to Eston lentil, but the seed color of Turkish lentil is red. The appearance of the seeds can be influenced by their growth conditions. Since the appearance of the seeds is the first test of acceptance of lentils for foods, the Canadian Grain Commission has established four main grades (grade 1 to grade 4) of Canadian lentil (Table 1). Generally, the seeds of high quality should have the uniform size, natural color, proper shape and smooth surface.

Table 2.1. Primary grade determinations in lentils.

Grade Name	Color	Damaged					Foreign material		
		Heated	Peeled split and broken	Other damage	Total	Stones	Ergot	Sclerotinia	Total foreign material
No. 1 Canada	Good natural color	0.2%	2.0%	1.0%	2.0%	0.1%	0.05%	0.10%	0.2%
No. 2 Canada	Reasonably good natural color	0.5%	3.5%	2.0%	3.5%	0.2%	0.05%	0.10%	0.5%
No. 3 Canada	Fair color	1.0%	5.0%	5.0%	5.0%	0.2%	0.05%	0.10%	1.0%
No. 4 Canada	Fair color	1.0%	10.0%	10.0%	10.0%	0.2%	0.05%	0.10%	1.0%

(Bhatty, R. S., 1995b).

2.1.2. Nutritional Quality of Lentils

Lentils have been called "poor man's meat" for quite a long time (Bhatty, 1995b). They have excellent nutritional quality because of their high protein content and relatively low level of antinutritional factors. Although lentil protein is low in methionine, the combination of cereals and lentils can provide a good balance of amino acids, which is especially important in many developing countries where the animal proteins are deficient and expensive (Deshpande and Deshpande, 1991). Partial replacement of meat with lentils in Western diets would increase the intake of complex carbohydrates and dietary fiber and decrease the intake of saturated fat and cholesterol.

2.1.2.1. Proteins

The protein content of lentils is about 22-31%, which is comparable with most food legumes (Swaminathan and Jain, 1975). The average protein content of wheat is only about 12% (Deshpande and Deshpande, 1991). Like the other legume proteins, lentil protein has poor quality when compared to animal protein because of the deficiency in methionine and cystine and low protein digestibility. The low biological value of legume proteins is a critical nutritional factor. However, the protein digestibility of lentils is much higher than that of soybeans, lima beans, and some other common legumes (Adsule et al., 1989). It was found that the methionine-enriched lentil diets could markedly improve the growth response of animals, and diets containing 40% raw lentils and 0.5% methionine exhibited the highest protein efficiency ratio (PER) (Adsule et al., 1989).

Protein solubility is another important functional property that affects the utilization and nutritional value of food legumes (Sosulski, 1977). Generally, heat

processing can reduce the protein solubility due to the denaturation of proteins. Studies found that the net protein utilization (NPU) of cooked lentils was greatly improved over raw lentils (Habibullah, 1974). Lentil protein has a broad apparent isoelectric point and it is relatively insoluble in acid solutions (Hsu et al., 1982). The nitrogen solubility of raw lentils with 0.5M NaCl (pH7) is about 53.2%, with deionized water (pH 6) it is about 23.7%, and with 70% EtOH it is about 2.7% (Zheng et al., 1998).

2.1.2.2. Carbohydrates

Lentils contain up to 69% carbohydrate. Starch is the major component of lentil carbohydrates. It was reported that the starch content of lentils is in the range of 35 to 53% (Reddy et al., 1984). Lentil starch contains about 20.7 to 45.5% amylose, and the amylopectin content of lentil starch varies inversely with the amylose content (Reddy et al., 1984). The digestibility of cooked lentil starch is nearly 100%, shows no adverse effect on animal growth and is comparable to that of wheat starch (Fleming and Vose, 1979).

Lentil starch granules are oval in shape and about 10 to 28 μm in size (Sosulski and Youngs, 1979). The water-binding capacity of lentil starch is 92.4-98.0%, higher than that of faba bean, pea and phaseolus bean starches, but similar to that of wheat starch (Bhatty, 1988). The degree of starch gelatinization can affect food texture by absorbing water, forming a gel or increasing the viscosity of solutions. Therefore, the proportion of raw and gelatinzed starch in food products is critical in determining the product acceptability (Guraya and Toledo, 1993). In addition, raw lentil starch has relatively low

digestibility and the characteristic beany taste. The extent of starch gelatinization is dictated by moisture content, heating temperature and time (Guraya and Toledo, 1993).

Polarized microscopy, X-ray diffraction and differential scanning calorimetry have been used to study the degree of starch gelatinization, however, those techniques are quite expensive and only suitable for purified fractions of starch (Lund, 1984). Chiang and Johnson (1977) developed a method to measure the gelatinized starch in foods by measuring glucose released by digesting the product with glucoamylase with o-toluidine color reaction. This method is quite applicable to dry, processed starch mixtures.

The other carbohydrates in lentils are mostly sugars and dietary fiber (DF). The importance of dietary fiber in the diet has been acknowledged recently. Anderson et al. (1984) reported that dietary fiber has important therapeutic implications for certain conditions, such as diabetes and hyperlipidemia. It may also have preventive implications for other diseases, such as hypertension, coronary disease, and intestinal disorders (Anderson, 1983 and Jenkins et al., 1986). Legumes have been used successfully as part of the dietary treatment of diabetes (Brand et al., 1990 and Thorne et al., 1983).

Lentil seeds contain a significant amount of dietary fibers. The main components of dietary fiber in lentils are cellulose, hemicellulose and lignin. Most components are present in the seed coat; therefore, dehullled lentils contain very low amounts of fiber (Adsule et al., 1989). Cellulose has a chain structure and is insoluble in water, hemicellulose has low solubility in water and lignin is a hydrophobic polymer (Theander et al., 1993). Heat treatment can lead to an increase of soluble dietary fiber (SDF) because of the redistribution from insoluble to soluble components (Theander et al., 1993). The ratio between the water-soluble dietary fiber and the water-insoluble dietary

fiber (IDF) may also depend on the pretreatment and solubilization procedure of the analysis method.

The content of total dietary fiber (TDF) in raw lentils is about 15 to 22% on a dry basis (Vidal-Valverde and Frias, 1991). However, the values of fiber content may vary greatly with different analysis methods. There are several methods available for the determination of dietary fiber in food products: the enzymatic-gravimetric methods, the enzymatic-LC, - GLC, and –colorimetric methods and the near-infrared reflectance spectroscopy method. The enzymatic-gravimetric method is considered most appropriate method for nutritional research and quality control purposes (Lee and Prosky, 1995). This method can determine the contents of TDF, SDF and IDF, respectively, in food products.

A number of recent studies have also been conducted on free sugars in lentils because of the flatulence potential of oligosaccharides in humans and animals (Ruperez, 1998). They found that a certain amount of those sugars could be removed by common processing methods, such as cooking, dry roasting and pressure-cooking.

2.1.2.3. Lipids and Other Nutritional Components

Lentil seeds contain 0.6 to 3.8% lipids, 2.4 to 4.2% minerals and a fair amount of Vitamin B (Adsule et al., 1989). Lentil lipids are probably less susceptible to rancidity than those of other food legumes because of the high content of linoleic acids (Exler et al., 1977). Lentils are rich in potassium, iron, zinc, magnesium and calcium. While almost all of the minerals are in the cotyledons, but 40% of calcium is in the seed coat (Singh et al., 1968). As with most food legumes, the nutritional composition of lentils may vary widely depending on cultivars, growth conditions and agricultural practices.

2.1.3. Antinutritional Factors

Compared with other food legumes, lentil seeds are almost free of antinutritional factors. It was found that the trypsin inhibitor activity of raw lentil is about one-tenth that of soybean (Bhatty, 1977). Studies also found that the concentration of lectins in lentils is too low to produce an adverse effect on protein digestion (Bhatty and Christion, 1984 and Jaffe, 1980). However, there are still some undesirable components, such as the polyphenols, the phytates and the raffinose sugars, existing in appreciable amounts in lentils that could limit their acceptance and consumption.

2.1.3.1. Polyphenols

It is well known that polyphenols of food legumes can interact with proteins resulting in inactivation of the enzymes or insolubility of the proteins (Deshpande and Deshpande, 1991). Feeding experiments showed that 0.5% tannic acid in the diet resulted in growth depression with chickens. Rats could tolerate up to 5% tannic acid in the diet but high levels caused a marked growth depression (Jadhav et al., 1989). The maximum intake of dietary legume tannins for humans is still unknown. However, scientists have assumed the consumption of plant tannins is a possible factor in the incidence of esophageal cancer in many areas of the world (Jadhav et al., 1989).

The polyphenols are mainly distributed in the seed coat of lentil. Therefore, they also contribute to the discoloration and the loss of germination capacity of the seeds after prolonged storage (Bhatty, 1988). The polyphenols are heat-stable. A major portion of polyphenols can be removed by dehulling. Other processing methods, such as soaking,

cooking, germination and alkaline treatment can also reduce the polyphenols to a certain extent (Vidal-Valverde et al., 1994).

2.1.3.2. Phytic Acid

Phytic acid commonly exists in most legume seeds, and it is the major source of total phosphorus in legumes. The adverse effects of phytic acid are due to the interaction between phytic acid and dietary proteins or some essential minerals. The phytate-protein complex decreases the solubility of proteins and thus affects their functional properties and the phytate-mineral complex causes the minerals to be biologically unavailable for absorption (O'Dell, 1979). It was reported that the phytate content of whole lentils is about 0.5% (Davies and Warrington, 1986).

The effects of several processing methods on the removal of phytic acid have been investigated extensively. It was found that heat treatment was not effective in breaking down the phytic acid in legumes (Reddy et al., 1978). However, soaking, cooking and germination can remove a large portion of phytic acid from legumes (Vidal-Valverde et al., 1994).

2.1.3.3. Raffinose Sugars

Lentils contain about 5 to 9% sugars, including mono-, di-, and oligosaccharides of the raffinose family (Bhatty, 1988). The raffinose sugars (raffinose, stachyose and verbascose) were found to be responsible for the formation of intestinal gas following ingestion of legumes by human (Reddy et al., 1989). Stachyose is the major raffinose sugar in lentils, contributing about 35 – 64% of the total free sugars. The next highest is

raffinose, while the concentration of verbascose in lentils is too low to be detected (Ruperez, 1998). The degree of seed maturity also influences the flatulence activity, in general, the immature green seeds are nonflatulent compared to dry mature seeds.

Various approaches have been studied in order to decrease the flatulence-causing factors of food legumes. The raffinose sugars are heat stable and soluble in water. Therefore, soaking beans in water and then discarding the water will certainly remove most of these sugars. Discarding cooking water also reduces the raffinose sugars in beans. It was reported that boiling soybeans in a 1:10 bean to water ratio removed 33 to 59% of the oligosaccharides, and germination can remove over 70% of the raffinose sugars from dry beans (Galloway et al., 1971 and Reddy et al., 1980). Several researchers have developed an enzymatic processes using α-galactosidases from exogenous microbial sources to degrade the raffinose sugars in bean products (Sugimoto and VanBuren, 1970 and Delente et al., 1974). It was found that about 38-96% hydrolysis of those sugars occurred when the beans were incubated in water for 24-48 h at 45°C (Kon et al., 1973 and Olson et al., 1975).

2.1.4. Cooking Quality of Lentils

Lentils are mostly used in human foods, therefore, the cooking quality is a primary requirement for its production and consumption. The cooking process improves the plasticity of the cell, thereby softening the seeds to an acceptable extent. Cooking can almost inactivate the heat sensitive antinutrients completely and remove the other antinutritional factors to a certain degree. Dry, unprocessed legume seeds usually require a long time to cook. Efforts to improve cooking quality of dry beans have been

investigated by many researchers (Bhatty, 1984; Esaka et al., 1987; Molina et al., 1976; and Plhak et al., 1987). Cooking time, uniformity in cooking and hull adhesion are considered as three major factors determining the cooking quality of dry legumes. Lentils usually take shorter cooking time than other legumes due to their smaller seed size and thinner seed coats. However, dry Laird and Eston lentils still need about 30 - 70 min of boiling to achieve an acceptable degree of tenderness (Bhatty, 1995a). A good-cooking lentil should have a short cooking time, uniformity of cooking, pleasant appearance and have the required flavor and taste.

2.1.4.1. Factors Affect the Cooking Quality

The cookability of raw, intact lentils is closely related to the cultivars, the growth conditions, the stage of seed maturity and the storage conditions. It was found that the cooking time for lentils was decreased with the increase in seed maturity. Cooking time was also significantly influenced by mineral composition of the seeds. Studies found that plants watered with adequate levels of N, P, Ca, Mg, S, B, Cu, Fe, Mn, Mo, Zn, and a high level of K (210 ppm) produced fast cooking lentils (Shakra and Tannous, 1981). If the seeds are stored under conditions of high temperature and moisture, a hard-to-cook (HTC) phenomenon of lentils may develop (Bhatty and Slinkard, 1989).

Muller (1967) and Bhatty (1984) reported that the composition of legumes, especially the content of pectin, phytate, calcium and magnesium, and the hardness of the seed determine the cooking quality of legumes. During cooking, large amounts of insoluble calcium and magnesium pectates are formed and with plenty of cooking water, the presence of insoluble pectins in the cell wall increases the cooking difficulty of

legume seeds (Shakra and Tannous, 1981). Muller (1967) introduced two formulae that correlate with cooking time of legumes. Formula A: Cooking time = Pectin (Ca + ½ Mg)/Phytin, and Formula B: Cooking time = Pectin (Ca + ½ Mg) – Phytin. These formulae indicated that the cookability of legume seeds is largely related to the concentration of pectin, calcium, magnesium and phytin. They also found that seeds with higher levels of sodium or high levels of sodium plus potassium (monovalent elements) have better cooking quality.

2.1.4.2. Determination of Cooking Quality

In the early 1970s, the cooking quality of lentil seeds was assessed by touching after pressing the individual seeds with the forefinger or the back of a spoon. Well cooked seeds were supposed to produce a smooth uniform paste, and poorly cooked seeds couldn't form a paste (Narasimha and Desikachar, 1973 and Wassimi et al., 1978). However, those methods are quite subjective and hardly can be reproduced. Bhatty et al. (1983) investigated an objective instrumental method to measure the cooking quality of lentils with a Kramer shear press. In their method: ten grams of lentil (dry weight) were added to 50 mL of distilled water in a 250 mL Erlenmeyer flask covered with a glass marble, then the flask was immersed in a boiling water bath (98°C), and cooked for 15 to 60 min. The cooking water was discarded, the lentils were cooled to room temperature. The texture of the cooked lentils was measured with the Kramer shear press, which is fitted with a TG-300 ring and a thin multi-blade shear compression cell, using a ram speed of 0.7 cm/s and a ram force of 45 Kg. Fig. 2.1 shows the typical graphs of good- and poor-cooking lentils obtained from the shear press. The shear force is the strength

needed to compress the cooked lentils at a certain defomation. Shear force is now used as well as the cooking time as an index of the cooking quality of dry beans. With the same sample, the shear force decreased as the cooking time increased. While other instruments have been used to measure the texture of cooked legume seeds since then, they all have a similar function to that of the Kramer shear press.

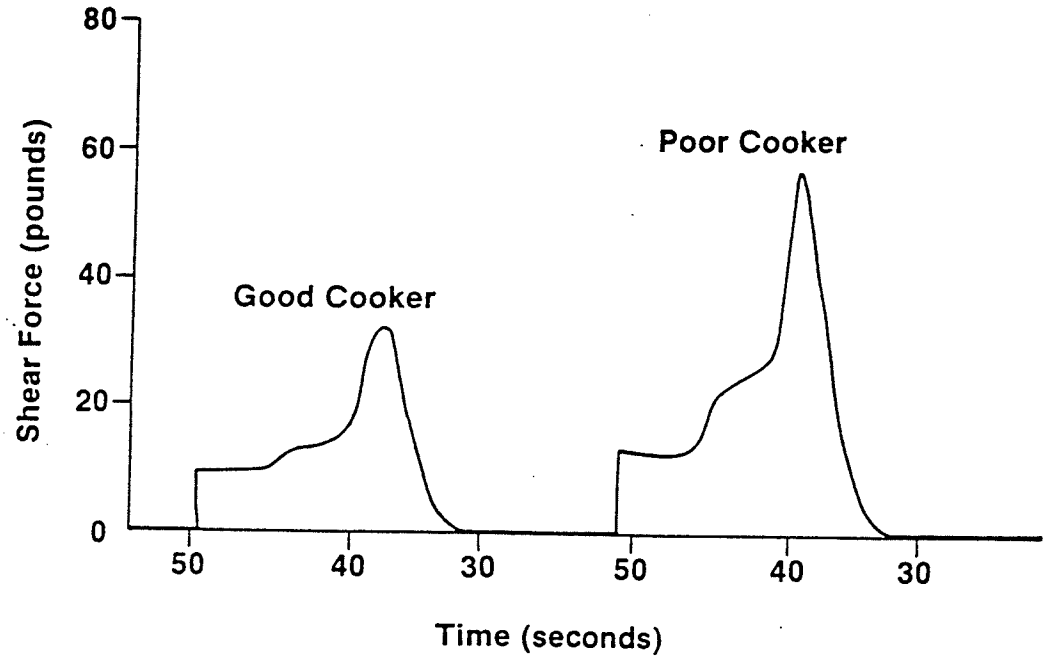


Fig. 2.1. Good (left) and poor-cooking (right) Eston lentils (Bhatty 1988).

2.2. Micronization (Infrared Heating)

Heating methods which thirty years ago were seldom used are now beginning to appear in large-scale applications. The electrothermal methods, such as infrared heating, laser heating etc., can provide very high power densities. Infrared heating is mainly used in the food, paper and textiles industries. With infrared heating, the energy is transferred to the objects from the heating source by radiation, not like the conventional oven, the air in the equipment is not heated and consequently the ambient temperature may be kept at normal levels. The most important advantage of this heating method is its high efficiency, which could reduce the processing time and energy cost.

2.2.1. The Electromagnetic Spectrum

As we all know, the electromagnetic spectrum consists of audio frequency waves, radio waves, microwaves, infrared waves, visible light, ultraviolet, x rays, γ rays and cosmic rays (Fig. 2.2). The infrared radiation (IR) is classified as the region of wavelength between visible light (380 nm to 780 nm) and microwaves (10^6 nm to 10^9nm) (Vanzetti, 1972). From Fig. 2.2, we can see that infrared radiation has shorter wavelength, but higher frequency and more energy content than those of microwave radiation. However, in spite of the difference in frequency, wavelength and energy content, all of the electromagnetic waves behave in a similar fashion in that, they all travel with the same speed (the speed of light) in air, and obey the laws of reflection, refraction, diffraction and polarization.

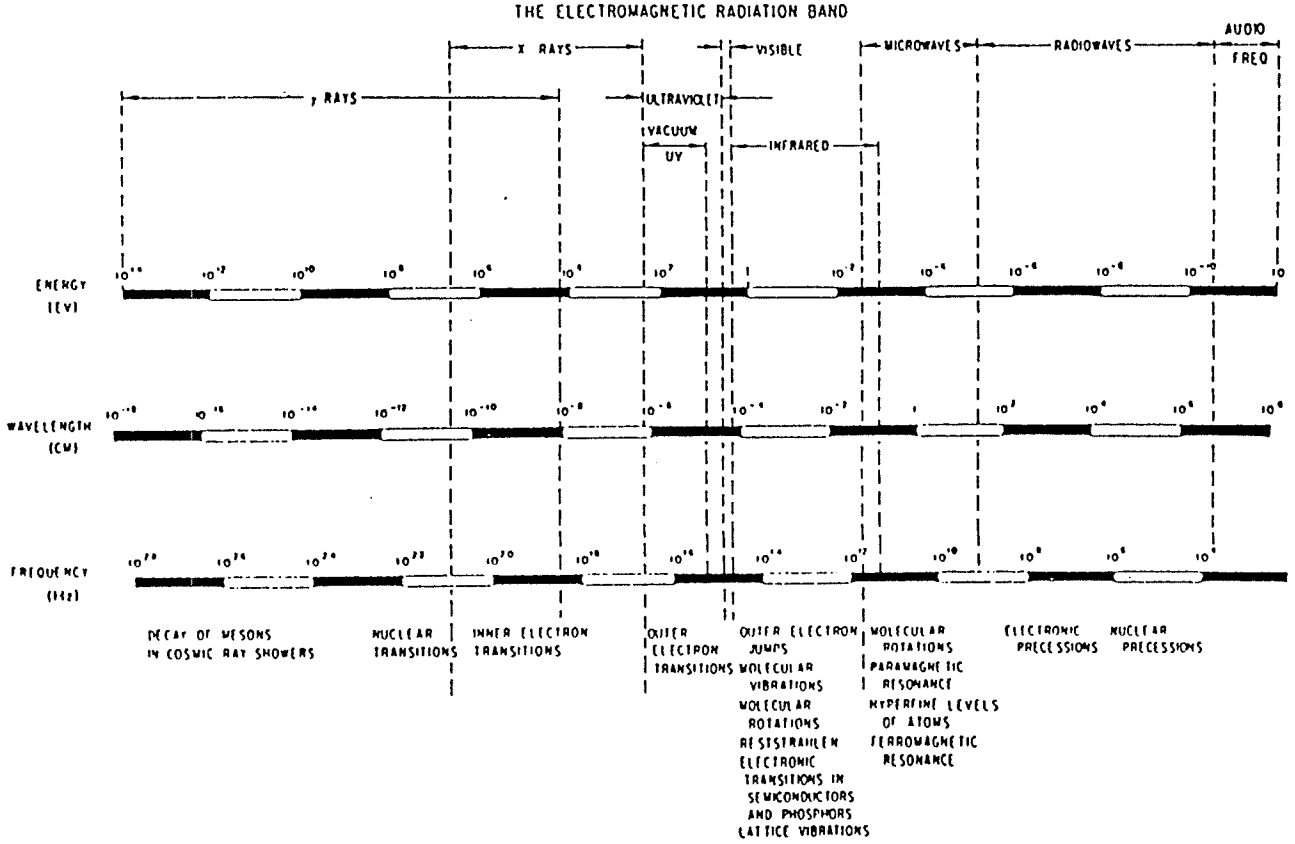


Fig. 2.2. The electromagnetic spectrum (Vanzetti 1972)

The infrared spectrum was discovered in 1800 by Sir Frederick Wm. Herschel when he was using a prism and a thermometer to detect the energy beyond the wavelength of red light. Generally, the infrared region is regarded as the heat region of the electromagnetic spectrum because the heated objects radiate energy in this range. Infrared radiation can be further divided into three classes according to wavelength, near infrared (780 nm to 1,400nm); middle infrared (1,400 nm to 3,000 nm) and far infrared (3,000 nm to 10^6 nm).

2.2.2. Infrared Radiation

Infrared radiation is a form of electromagnetic radiation. Here, it is noted that the term "radiation" refers purely to the physical method of heat transfer and has no relevance to nuclear radiation. Some other radiation, such as ultraviolet, may have photochemical effects on humans, but the effect of infrared radiation is identical to the thermal effect of the sun on humans or nature, therefore, infrared radiation is very safe (Driscoll, 1992). Infrared energy is transmitted as a wave in the air and there is very little energy loss before reaching the objective since air is largely transparent to infrared radiation. The distribution and absorption of infrared radiation are based on the following fundamental laws: Planck and Wien displacement law, the absorption and transmission of infrared radiation.

2.2.1. Distribution of Emitted Energy

The emitted energy follows the radiation law as given by Planck and Wien displacement law (Orfeuii, 1987). Planck's law defines the monochromatic emissivity M_λ of a black body for wavelength λ (Fig. 2.3).

$$M_\lambda = C_1/[\lambda^5(e^{C_2/\lambda T} - 1)] \qquad (2.1)$$

Where M_λ = emitted radiation, $W/(cm^2 \cdot \mu m)$

$C_1 = 3.74 \times 10^4\ W \cdot \mu m^4/cm^2$

$C_2 = 1.438\ \mu m \cdot K$

T = absolute temperature, K

λ = wavelength, μm

Planck's law illustrates the rate of energy emitted at particular wavelength and temperature. As we can see from Fig. 2.3, there is a radiation peak at each temperature.

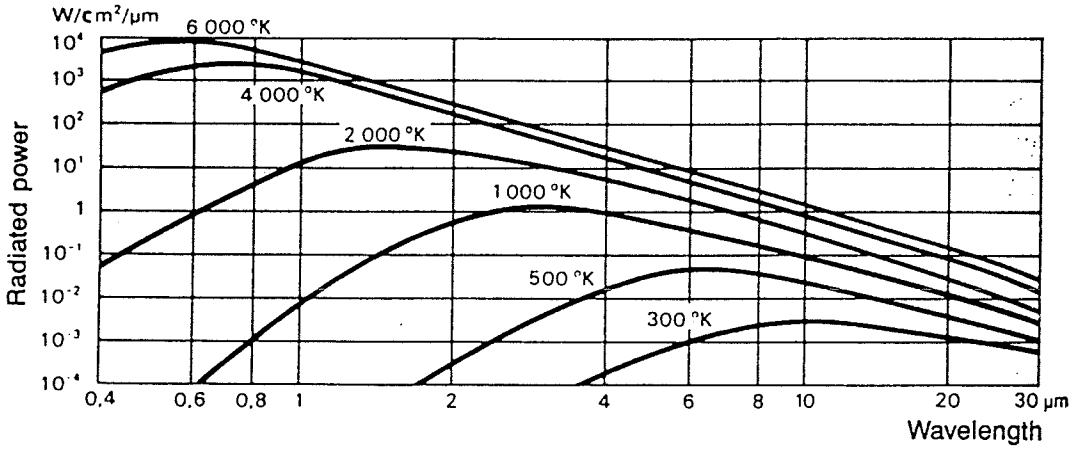


Fig. 2.3. Planck's law (Orfeuii 1987)

Wien's law describes the relation between the absolute temperature T and the wavelength λ at the maximum monochromatic emissivity (Fig. 2.4).

$$\lambda m \cdot T = C \qquad (2.2)$$

Where λm = maximum wavelength, μm

T = absolute temperature, (E)K

C = 2,898

The Wien displacement shows that with an increase in the source temperature, the emitted energy (area under the curve) is increased proportionally, and the maximum wavelength becomes shorter. Therefore the hotter the emitter, the more it radiates in the short wavelength.

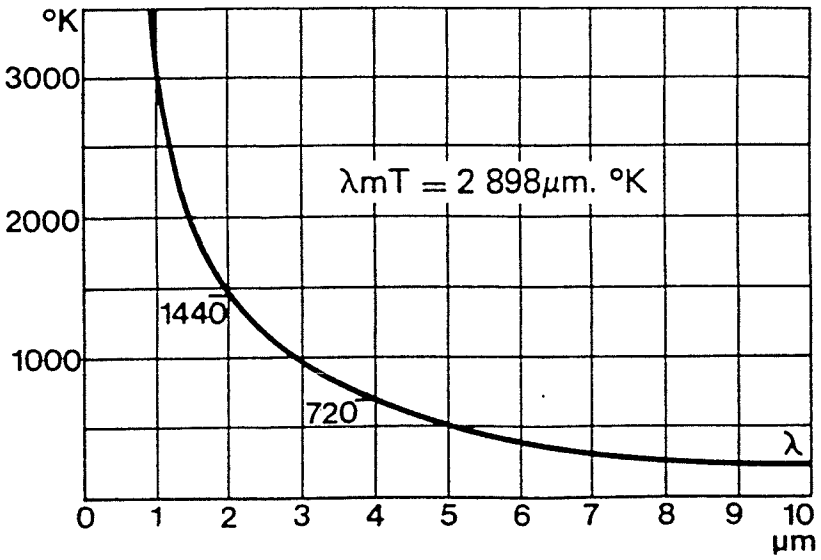


Fig. 2.4. Wien's law (Orfeuii 1987)

2.2.2.2. Absorption

For all kinds of receivers, the emitted infrared radiation cannot be completely absorbed because a part of the radiation is reflected and, if the body is not opaque, transmitted (Fig. 2.5). The relation between absorption, reflection and transmission is:

$$\alpha + \rho + \tau = 1 \qquad (2.3)$$

Where α is the absorption factor, ρ is the reflection factor and τ is the transmission factor.

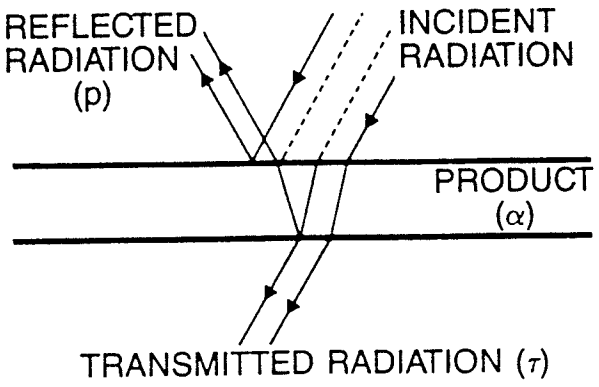


Fig. 2.5. Absorption of infrared radiation (Orfeuii 1987)

For a given body, the absorption factor at a point is the ratio between the flux absorbed and the incident flux. It depends on the wavelength and direction of the incident radiation, the distance between the emitter and the receiver, and the characteristics of the receiver, which include surface condition, chemical nature, color, thickness and temperature (Orfeuii, 1987). Different receivers have their best absorption factors at different wavelengths. For example, water absorbs large quantities of infrared energy at wavelengths of 2.5 and 2.6 μm, organic compounds such as proteins and starches absorb

more infrared energy at wavelengths longer than 2.5 μm (Orfeuii, 1987; Sakai and Hanzawa, 1994). The thickness of the object also significantly affects the absorption factor. It was found that at a wavelength of 1.5 μm, the α value of water could be greatly increased by increasing the thickness of the water layer.

As we discussed above, the infrared radiation does not release its energy until it penetrates an absorbent surface. The absorbed energy is then converted into heat immediately by interaction with molecules of the object. The absorption of radiation by an object usually takes place within several millimeters under the surface of the objective (Orfeuii, 1987). The penetration capacity of infrared radiation is greatly dependent on the wavelength of the incident radiation.

2.2.2.3. Transmitted Power

Based on the laws of radiation, the power transmitted by infrared radiation emitters to a black body can be described as follows:

$$\Phi = \sigma S_1 F_{12} (T_1^4 - T_2^4) \qquad (2.4)$$

Where Φ = transmitted heat flux, W

$\sigma = 5.73 \times 10^{-8}$ W/ m^2K^4

T_1 = emission temperature, K

T_2 = receiving surface temperature, K

F_{12} = surface angle factor S_2 as seen from S_1

S_1 = emitter surface, m^2

Therefore, the transmitted power is a function of temperature, shape, relative position of surfaces, and emission coefficients. This is the main reason why the infrared

heating is much more efficient than the convection heating: the transmitted power of infrared radiation is proportional to (T_1^4 - T_2^4), where that of convection is proportional to (T_1 - T_2). Therefore, the power density is much higher with infrared heating.

2.2.3. Emitters of Infrared Radiation

Infrared radiation can be produced either by specially engineered gas-fired sources or by electric sources. The electrical generators of infrared radiation include: reflector type infrared incandescent lamps; radiators with quartz tubes and resistance elements. The gas generators of infrared radiation may comprise: panels that are not permeable to gas, porous ceramic panels or metallic perforated surfaces in which flameless combustion of gas takes place (Ginzburg, 1969). The surface temperature of the emitter can be controlled by the gas input to the burner and the type of gas used. The emitters can also be classified according to the wavelength of maximum radiation: the short-wave radiator (temperature above 1773 - 2073 K), and the long wave radiator (temperature below 623 – 673 K). Today, there are lots of infrared equipment manufacturers around the world, and some have been working with the food industries for quite a long time.

2.2.4. Applications of Infrared Heating in Food Industry

With properly designed systems, infrared heating is a precise, efficient, controllable heating method that provides heating effects not available by other techniques. Conventional heating technology is effective in sealed containers at high moisture levels due to its slow temperature build up. Infrared heating is best suited to

products in layers or sheets due to its rapid temperature rise. The infrared radiation can increase the temperature of the legumes to above 100°C in 60 second (Kouzeh-Kanani et al., 1981). In comparision with the long processing time needed for the conventional oven, infrared heating has the advantage of being able to control the final moisture, as well as modifying the nutritional characteristics of the products. In recent years, infrared heating has been successfully used in baking, drying, thawing, cooking and pasteurization applications. In the baking processes, the use of rapid infrared heating can produce the products with highly acceptable sensory characteristics without burning the surface black because of the chemical reactions involving flavor and aroma compounds. Infrared radiation is also used in roasting of coffee, green tea, sweet potatoes and nuts. Table 2.2 compares the performance of infrared and conventional ovens. With much lower energy cost, infrared ovens provide higher production rate and efficiency (Sakai and Hanzawa, 1994).

Infrared drying operations are mainly used for vegetables and marine products (Fu and Lien, 1998). It was found that infrared drying gave the better rehydration rate and nutrient retention with less discoloration compared to the hot-air dryer (Itoh, 1986). The infrared thawing process is suitable for office kitchens and fast-food restaurants. The infrared pasteurization technology has been applied to milk and packaged cooks, short wave infrared heating can pass through the plastic packaging without overheating it.

Table 2.2. Comparative performance characteristics of infrared and conventional heating ovens.

	Criterion for Comparison	Type of Oven		I (Infrared /Conventional) x 100%
		Infrared oven	Conventional oven	
Baking of rice crackers	Calorie consumption	223,200 KJ/h	836,000 KJ/h	26.7
	Production rate	10,000 pieces/h	10,000 pieces/h	100
	Baking time	10 min	15 min	66.7
	Energy cost	US$13.70/h	US$25.10/h	54.5
Roasting of fish paste	Length of furnace	9 m	19 m	47.4
	Production rate	1,470 Kg/day	500 Kg/day	294
	Electric power consumption	0.06 Kw/Kg	0.23 Kw/Kg	26.1

(Sakai and Hanzawa, 1994)

2.3. The Effects of Micronization on Legumes

Micronization is a process used for treating food, to affect the cookability of cereals, legumes or oil seeds through the use of infrared radiation, it results in starch gelatinization, thereby giving greater starch availability and digestibility, as well as the removal of enzymatic and other inhibitory substances (Blenford, 1980). This definition describes the technology of micronization and the benefits in processing food materials by this technique. To date, micronization technology in legume processing is still in its early stage of development, but previous studies have indicated the wide potential that exists and that may be developed in the future (Abdul-Kada et al., 1990; Igbasan and Guenter, 1996; Mulimani and Supriya, 1994 and McCurdy, 1992).

2.3.1. The Effects of Micronization on the Cooking Quality of Legumes

Preparation of legumes with infrared heat to reduce their cooking time has been the subject of a number of recent studies. However, most research was performed with laboratory-scale infrared lamps. There are very few published papers investigating the potential effects of infrared heating on the cooking quality of legumes with pilot or factory-scale micronizers.

Infrared heating in the processing of legume seeds has been reported to increase the starch gelatinization and decrease the protein solubility of the products (Blenford, 1980; Zheng et al., 1998). The transition enthalpies of micronized lentils obtained with differential scanning calorimetry (DSC) were greatly reduced and the degree of reduction depended on the initial moisture content of the seeds before micronization (Cenkowski and Sosulski, 1997). The peak gelatinization temperature (Tp) of raw lentil starch was

65.9°C. The total heat absorbed by the sample during the transition period (ΔH) was 9.2 J/g. When micronized with a quartz infrared lamp at 25.8% or 38.6% initial moisture contents, the gelatinization peaks of lentil starch were replaced by multiple small peaks in the range of 70-80 °C, and the ΔH values were reduced to 2.6 J/g and 0.9 J/g, respectively. Arntfield et al. (1997) reported that higher moisture content in the seed during micronization resulted in a higher degree of starch gelatinization. The gelatinized starch softens the texture of the seeds and thus reduces their cooking time. They found that micronization could reduce the cooking time of Canada No. 1 Laird lentils from 30 to 15 min if the seeds were tempered to 25% moisture content before micronization.

During the micronization process, there is usually a loss in moisture from the product. Zheng et al. (1998) reported that the moisture content of green peas could be reduced by about 7% by heating to 140°C with a laboratory-scale, propane-fired micronization system. It was found that the tempering level, the tempering time before micronization, the final temperature and the final moisture content after micronization could significantly affect the cooking quality of the micronized lentils (Arntfield et al., 1997).

The decrease in protein solubility by micronization is due to the heat-induced denaturation of proteins. The proteins in micronized peas were seriously denatured by about 70% at pH8 (Cenkowski and Sosulski, 1998). Zheng et al (1998) found that the protein denaturation of legumes was more pronounced in water- and salt-soluble proteins (albumins and globulins), and the micronization-induced loss of solubility in legumes was mainly due to hydrophobic aggregation of polypeptide chains. Protein denaturation

increases the digestibility of legume proteins and also helps to produce a soft texture in the seeds.

Abdul-Kadir et al. (1990) reported a significant increase in the rehydration rate of pinto beans after micronization to about 100°C. However, they also found that the infrared process increased the cooking time by about 25-50%. The increased hardness of the seeds was due to the low initial moisture content (17%). Other studies also reported that infrared treatment might cause the cracking, toughness or higher leaching losses of the products if the seeds were heated to a high temperature (above 140°C) with low initial moisture content (Fasina et al., 1997). Therefore, the moisture content of the seeds before micronization is critical to the quality of the end products. The initial moisture content of the seeds is mainly dependent on the tempering level of the seeds, and the tempering time may determine the distribution of the water inside the seeds (Arntfield, et al., 1997).

As mentioned before, the cooking time of legumes is also related to the content of certain elements in the seeds. It was found that dry beans became softer if soaked in alkaline solutions rather than water, and cooking times were greatly reduced (Uzogara et al., 1988; Buckle and Sambudi, 1990). This is probably due to the replacement of divalent cations with monovalent sodium. The sodium salts of carbonate, bicarbonate, phosphate, tripolyphosphate and ethylenediaminetetraacetic acid (EDTA) have been used as tempering solution before micronization to investigate their effects on the cooking quality of lentils (Scanlon et al., 1998). The reagents identified as having potential for tempering solutions used as successful micronization pretreatment were 2% sodium tripolyphosphate, a mixture of 1% citric and 2% ascorbic acid and 150 ppm disodium

EDTA. However, it was found that only at an initial moisture level above 40%, were those reagents effective in reducing the cooking time of lentils.

2.3.2. Effects of Micronization on The Other Properties of Legumes

The first application of infrared heating to legumes was to dry the seeds for storage. Infrared drying was found to be a viable method for the destruction of microorganism, storage insects and fungal infections on legume seeds (Deshpande and Deshpande, 1991; Sarantinos and Blank, 1996).

The gelatinization of starch and the denaturation of proteins can improve the nutrient availability of legumes. Kadam et al. (1987) reported significant improvements in biological values and net protein utilization values for diets containing winged beans treated with infrared heat compared to untreated or oven heated samples. The effects of several processing methods were compared in their experiments, including dry-air heating with a hot air oven, infrared radiation with a gas burning infrared heater, and cooking in boiling water. The treatment times for each process were 60 min, 1 min and 30 min, respectively. For cooking, the seeds were presoaked in distilled water for 48 hour at room temperature. The results indicated that the digestibility of proteins in infrared treated bean meals was improved from 50 to 84%, whereas a noticeable decrease in protein digestibility was observed in oven-heated meals. Both the infrared treatment and cooking could almost completely inactivate the trypsin inhibitors and the lectins. The highest reduction of tannins was obtained by infrared heating. Infrared radiation may also affect the removal of oligosaccharides in legumes (Sarantinos and Blank, 1996).

Infrared heat treatment can also decrease the off-flavors of legumes (McCurdy, 1992). Metussin et al. (1992) reported the improved digestibility of soymilk protein after infrared radiation. However, they also pointed out that overheating with infrared could cause unpleasant odor and flavor development and decrease the quality of the end-products. Therefore, careful selection of the optimal processing conditions for different sample is essential to the quality of the end products.

2.3.3. Potential for Further Application of Micronization on Legumes

As stated above, lentils are high in protein and starch contents. The utilization of lentil protein and the digestibility of lentil starch are higher than most other food legumes due to lower antinutritional contents. As with other food legumes, lentils have less than ideal cooking quality, in that it takes about 30-70 minute to cook dry lentils. The long cooking time has limited the consumption of lentils. Micronization is a newly developed large-scale food processing technology which may reduce the cooking time of lentils.

It is very important to control the processing conditions during micronization because of its rapid heat transfer. Otherwise, the seeds may be overcooked or burned. The main factors affecting the quality of end products include: the surface temperature of the emitter; the distance between the emitter and the legumes; the initial moisture content, the shape and the size of the legumes and the processing time. The temperature of the emitter depends on the micronization system, it may be controlled by the input of the gas. The size and shape are cultivar characteristics. The controllable processing conditions are the distance between the emitter and the processed product, the initial moisture content of the seeds and the processing time. Therefore, with selected distance and processing time, the

pretreament conditions, including the amount of water used to temper the seeds, the treatment duration and the type of tempering solution, can control the quality of the end of products. This technology may also be applied to other food legumes, such as peas, soybeans and broad beans to improve their attractiveness in the market.

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

3.1.1. Lentil Samples

The Laird and Eston lentils in this study were from the Roy Legumex Inc., (St. Jean Baptiste, MB, Canada). The grades of Laird lentils and Eston lentils were Canada No. 2. The lentils used on the laboratory scale infrared lamp were from the a single batch, and those used for the pilot scale micronizer were from a second batch of the lentils obtained 10 months later. Upon the arrival of each batch of samples, the chemical composition of the seeds, including the contents of starch, protein, fat, ash and moisture and the Hunterlab color values of the seeds were measured. During the study, the samples were stored in the storage room at 2-8°C.

3.1.2. Chemicals and Reagents

The chemicals and reagents used in this study are listed in Table 3.1.

3.1.3. Supplies and Instruments

The supplies and instruments used in this study are listed in Table 3.2.

Table 3.1. Chemicals and reagents

Chemicals	Manufacturers
Acetic acid glacial	Fisher Scientific Inc., Nepean, ON
Acetone	Fisher Scientific Inc., Nepean, ON
AG 1-X8 resin	Bio-Rad Laboratories
α-Amylase	Sigma Chemical Co., St. Louis, MO
Amyloglucosidase	Sigma Chemical Co., St. Louis, MO
Ascorbic acid	Canada Colors and Chemicals Ltd.
Celite	Sigma Chemical Co., St. Louis, MO
Coomassie protein assay reagent	Pierce, Rockford, Illinois
Disodium EDTA	Fisher Scientific Inc., Nepean, ON
D-(+)-Fructose	Sigma Chemical Co., St. Louis, MO
D-(+)-Glucose	Sigma Chemical Co., St. Louis, MO
Ferric chloride	Fisher Scientific Inc., Nepean, ON
Folin Ciocalteau reagent	Sigma Chemical Co., St. Louis, MO
Hexane	Fisher Scientific Inc., Nepean, ON
Hydrochlorid acid	Fisher Scientific Inc., Nepean, ON
L-(+)-Arabinose	Sigma Chemical Co., St. Louis, MO
Methanol	Fisher Scientific Inc., Nepean, ON
MES	Sigma Chemical Co., St. Louis, MO
O-toluidien	Sigma Chemical Co., St. Louis, MO
Phytic acid	Sigma Chemical Co., St. Louis, MO
Protease	Sigma Chemical Co., St. Louis, MO

Table 3.1. *(Continued)*

Chemicals	Manufacturers
Raffinose	Sigma Chemical Co., St. Louis, MO
Sodium acetate anhydrous	BDH Chemical, Toronto
Sodium carbonate	Sigma Chemical Co., St. Louis, MO
Sodium chloride	Fisher Scientific Inc., Nepean, ON
Sodium hydroxide	Mallinckrodt Specialty Chemicals Co., Paris, KT
Sodium phosphate tripoly	Fisher Scientific Inc., Nepean, ON
Stachyose	Sigma Chemical Co., St. Louis, MO
Sucrose	Sigma Chemical Co., St. Louis, MO
Sulfuric acid	Fisher Scientific Inc., Nepean, ON
Tannic acid	Sigma Chemical Co., St. Louis, MO
Trichloroacetic acid	Fisher Scientific Inc., Nepean, ON
Trizma Base	Sigmal Chemical Co., St. Louis, MO

Table 3.2. Supplies and instruments

Supplies/Instruments	Manufacturers
Accumet® pH meter 925	Fisher Scientific Inc., Nepean, ON
Aminex® HPX-87N column	Bio-Rad Laboratories, Hercules, CA.
1~10, 1~100, 100~1000 Eppendorf pipetters	Fisher Scientific Inc., Nepean, ON
20 µl syringes and needles	Fisher Scientific Inc., Nepean, ON
F3 Digital water bath	Haake F3 Digital
440 Liquid Chromatograph	Water Associates Co.
CR601 Chromatopac recorder	Shimadzu Scientific Instruments Inc., Japan
Constant temperature cabinet	Blue M Electric Co., Illinois
Chromaflex column SZ 23	KONTES Class Co., Vineland, NJ.
Crucible	Corning Inc., Corning, New York
Glass wool	Supelco, Bellefonte, PA
Hunterlab color difference meter	Hunter Associates Laboratory, Inc., USA
Infrared thermometer	Cole-Parmer Instruments Co.
KJELTEC auto 1030 analyzer	Tecator Co.
L1000R Lloyd materials testing machine	Lloyd Instruments Ltd., UK
MR2 Micronizer	Micronizing Co. (U. K.), Framlingham, England

Table 3.2. *(Continued)*

Supplies/Instruments	Manufacturers
Muffle Furnace	Blue M Electric Co., Illinois
1009 Digester	Tecator Co.
8451A diode array sepectrophotometer	Hewlett Packard Co.
PC-351 Hotplate stirrer	Corning Inc., Corning, New York
Sep-Pak C_{18} cartridge	Waters Associates Co.
Sorvall superspeed RC2-B automatic refrigerated centrifuge	Ivan Sorvall Inc., Norwalk, CT
Ultraspec 2000 UV/visible spectrophotometer	Pharmacia Biotech AB, Uppsala, Sweden
Vortex mixter	Fisher Scientific Inc., Nepean, ON

3.2. Methods

3.2.1. Experimental Design

This study was divided into two stages. In the first stage, the Laird and Eston lentils were tempered with water or salt solutions, and the tempered seeds were micronized with a laboratory scale infrared lamp. The experiments conducted with the infrared lamp were further grouped into two sections. First, 6 treatment combinations, including three tempering levels (20, 30 and 40%) and two tempering times (12 and 24 hours) were prepared and evaluated using distilled water as tempering solution (Table 3.3). Each treatment combination was tested in triplicate.

Table 3.3. Experimental design for Laird and Eston lentils with the infrared lamp

Sample No.	Tempering Level (% moisture)	Tempering Time (h)
1	20	12
2	30	12
3	40	12
4	20	24
5	30	24
6	40	24

The tempered and micronized lentils were evaluated for end moisture content, Hunter lab color values, percent starch gelatinized and protein solubility. According to these results, two-dimentional model graphs were generated from the fitted model using Design-Expert 6.0 software (Stat-Ease, Minneapolis, MN, USA). These graphs gave a general idea about the effects of water tempering on the quality of micronized Canada No.2 Laird and Eston lentils.

In the second experiment of the first stage, three salt solutions were used to temper the lentils, 2% sodium tripolyphosphate, mixtures of 1% citric and 2% ascorbic acid, and 150 ppm disodium EDTA as suggested by Scanlon et al. (1998). Two tempering levels, 20, and 30% and one tempering time, 12 hours were tried with each salt solution. Processing of each combination was duplicated. The micronized seeds were evaluated for all the characteristics listed above and were compared to seeds tempered using distilled water only.

The tests in the second stage were based on the information from the first stage. In this stage, the lentils were processed with the pilot scale micronizer. The experimental was designed with the central composite model of the response surface methodology using the Design-Expert software. The selected range of tempering level was 20-30%, and the range of tempering time was 6-18 hours. Thirteen treatment combinations of both Laird and Eston lentils (Table 3.4) were generated including 5 replicates of the central points, 25% tempering level and 12 hour tempering time. An alpha of 1.6 was used to determine the axial points. The micronized lentils were assessed for cooked texture, end moisture content, color, percent starch gelatinized and protein solubility.

The effects of the three salt solutions were also tested, using 12 hours as tempering time and 25% as tempering level. The samples treated with the salt solutions were compared with the samples treated with distilled water only for cooking texture, color, solubility of dietary fiber and the reduction of antinutrtional factors.

Table 3.4. Experimental design for Laird and Eston lentils with the pilot scale micronizer

Sample No.	Tempering Level (% moisture)	Tempering Time (h)
1	20	6
2	30	6
3	25	12
4	25	12
5	25	3.5
6	25	20.5
7	17.93	12
8	32.07	12
9	25	12
10	25	12
11	25	12
12	20	18
13	30	18

3.2.2. Chemical Analysis of Samples

The starch contents of the samples were determined by the procedure of Fleming and Reichert (1980). Total protein based on nitrogen content was determined by the Kjeldahl procedure (AOAC, 1990). The total ash content was determined with the muffle furnace, and the crude fat of the seeds were determined with the Soxhlet extraction equipment (Joslyn, 1970).

3.2.3. Tempering

Tempering of lentils involved the addition of a certain amount of tempering solvent (distilled water or, a salt solution), to certain amount of lentils for specified times

(tempering time) to reach a specific moisture content (tempering level). The amount of solvent required was calculated as below (Arntfield et al., 1997):

Weight of solvent

$$= \text{lentil weight} \times [\%H_2O(\text{target}) - \%H_2O(\text{original})] / [100 - \%H_2O(\text{target})] \quad (3.1)$$

Where $\%H_2O$ (target) is the tempering level required in the lentils, and the $\%H_2O$ (original) is the original percentage moisture content of the lentils. For the small infrared lamp, approximately 60 g of lentils were tempered in a covered glass jar. The seeds and solvent mixture was shaken and rolled until the liquid was evenly distributed. Then samples were left at room temperature for a set period of time to equilibrate to the desired moisture contents. For the pilot scale micronizer, 8 kg of lentils were tempered in two big plastic containers (40.6x27.9x22.9 cm). During tempering, the seeds and liquid mixture were shaken every 30 min in the first two hours.

3.2.4. Micronization

3.2.4.1. Infrared Lamp

In the first stage of this study, a small propane-fired infrared lamp was used to micronize the tempered lentils. A reflector focused most of the output towards the sample. Samples of tempered lentils (approximately 20 g) were placed in one layer on a white tray (12x5x2 cm) under the lamp. During micronization, the distance between the tray and the lamp was maintained at 85 mm. The lentils were heated for 1 min 45 seconds with continuous shaking of the tray by hand. The sample temperature during heating was

monitored by an infrared thermometer (Cole-parmer Instrument Co., Vernon Hills, IL). The surface temperature of the infrared lamp was around 300°C, and the final temperature of the micronized lentils at the end of micronization was around 120°C. This protocol was chosen to be the most effective way to heat the lentils without burning. Each sample was micronized in duplicate. After micronization, samples were left exposed for one hour to cool down to room temperature. At this point, the moisture content of the seeds was determined. The samples were then placed in sealed containers at 2-8°C until used for further analysis.

3.2.4.2. Micronizer

In the second stage, the pilot scale gas-fired MR2 micronizer, manufactured by the Micronizing Company (Framlingham, U. K.) was used. During micronization, the feed speed at which sample enters the vibratory belt can be adjusted by changing the setting on the rheostat. 60 was used both Laird and Eston lentils. The processing rate can be adjusted by changing the slope on the vibratory conveyor, the setting was at level –1 for Laird lentils, and level 2 for Eston lentils. Different final temperatures of the processed samples in the range of 60-120°C were tested. The final temperature of Laird lentils was determined to be 80-85°C. Below this temperature the seeds were undercooked, based on texture measurements, and the final moisture contents were too high (about 15-20%). Above this temperature, the seeds easily cracked and decorticated. The final temperature of Eston lentils was determined to be 95-100°C for the same reason. When the micronized seeds were cooled to room temperature for 3 hours, the final moisture contents and the color of the sample were determined. The rest of the

sample was placed in a sealed container and stored at 2-8°C prior to further analysis and texture evaluation.

3.2.5. Moisture Determination

The moisture determination followed the oven drying procedure (Joslyn, 1970) (Appendix 1). The moisture content of raw sample was measured every two weeks. The final moisture content of micronized lentils was determined once the samples were cooled down.

3.2.6. Color Testing

As stated above, color is one of the criteria used to determine the grade of lentils. The Hunterlab Color Difference Meter D25.2 (Hunter Associates Laboratory, Inc., USA) was used to measure the color of the uncooked micronized lentils. The Hunter L, a, b scale was designed to give measurements of color in units of approximate visual uniformity. L measures lightness and varies from 100 for perfect white to zero for black, approximately as the eye would evaluate it. The "a" value measures redness when positive, gray when zero and greenness when negative, and "b" measures yellowness when positive, gray when zero and blueness when negative. The color meter was standardized to a white tile (standard no. c2-12418 with L = 92.37, a = -1.2 and b = 0.5) each time before measuring. One hundred grams of sample were placed in a clear dish placed under the port (10 cm diameter viewing port) and L, a, and b values were recorded. The sample was returned to the original container, gently mixed and another reading taken. Each sample was measured in triplicate.

3.2.7. Starch Gelatinization

During tempering treatment the native lentil starch was insoluble in water because the tempering temperature (10-15°C) was below its gelatinization temperature (65°C) (Schoch and Maywald, 1968). However, the starch granules swell slightly in cold water, owing to diffusion and absorption of water into the amorphous regions and this swelling is reversible upon drying (Biliaderis, 1989). When seeds were heated with infrared radiation, the processing temperature was much higher than the gelatinization temperature, and therefore the granule swelling became irreversible and some amylose leached out into the aqueous intergranular phase. Gelatinization of starch resulted in the disruption of granule structure, swelling, loss of birefringence and solubilizatin of starch molecules (Biliaderis, 1991). With lentils, the starch gelatinization could greatly improve the water-banding capacity and other functional properties of the seeds during cooking.

The percentage of starch that was gelatinized in the uncooked micronized lentils was determined using a modified procedure of Chiang and Johnson (1977) based on enzyme susceptibility. Total starch for each sample was determined by mixing 20 mg of ground sample with 3 mL distilled water and 1 mL 1.0 N NaOH in a 50 mL centrifuge tube. This hydrolysis was terminated after 5 min by adding 1 mL 1.0 N HCl. To determine the level of gelatinized starch, 20 mg of ground sample were mixed with 5 mL distilled water in a 50 mL centrifuge tube. The alkaline hydrolyzed starch and the gelatinized starch were converted to glucose by adding 25 mL of amyloglucosidase solution and incubating at 40°C for 30 min. After incubation, 2 mL of 25% trichloroacetic acid was added to stop the reaction and the mixture was votexed and

centrifuged at 17,300xg for 5 min. From the supernatant, 0.5 mL was transferred to a test tube and 4.5 mL of o-toluidine reagent added. The mixture was boiled for 10 min, cooled in cold water and 5.0 mL of glacial acetic acid added. The absorbance of the mixture was measured at 630 nm. The blank was prepared with 5 mL of distilled water, which was treated with the same incubation and boiling procedures. The results were compared to a standard curve prepared by treating known amounts of glucose with o-toluidine reagent, boiling, cooling and adding glacial acetic acid as above. The percentage of gelatinized starch was calculated from the glucose value obtained from the standard curves as follows (Arntfield et al., 1997):

% total starch in sample (3.2)

= [μgglucose (sample treated with alkali) x 0.9 x 32 x100%] / [sample weight (μg)]

% gelatinized starch in sample (3.3)

= [μgglucose (sample treated with water) x 0.9 x 32 x100%] / [sample weight (μg)]

Where μgglucose represents the amount of glucose (μg), whose concentration can be calculated from the absorbance value of the sample compared with the standard curve, 0.9 is the converting factor from glucose to starch, and 32 is the dilution factor for the sample during analysis. The percentage of the total starch in the sample that had been gelatinized was then calculated as:

% starch gelatinized of total starch (3.4)

= [% gelatinized starch / % total starch] x 100

3.2.8. Protein Solubility

The protein solubility is the one of the most critial factors of lentil proteins because it also affects many other functions of the proteins, such as foaming and emulsifying capacity and gelling ability (Kinsella, 1976). Those functional properties will affect the cooking quality and the texture of the food products. The protein solubility in the uncooked micronized lentils was determined following a spectrophotometric procedure using the Coomassie Protein Assay Reagent. One gram of ground sample was extracted in 10 mL 0.5 M NaCl for 30 minutes, centrifuged at 17,300xg for 10 min, 1 mL supernatant was diluted to 25 ml with 0.1 N NaOH. A 0.1 ml aliquot of the diluted sample was and 5 ml Coomassie Blue Protein Assay reagent was added to a test tube, votexed the test tube, and the absorbance of the solution was read immediately at 595 nm. The blank was prepared with 0.1 mL of 0.1 N NaOH and 5 ml Coomassie reagent. The standard curve was prepared using BSA solutions in the range of 200 – 600 μg/mL treated with the Coomassie reagent. The total protein contents of lentils were determined with Kjeldahl method (AOAC, 1990), and the factor used to convert N to protein was 5.7. Therefore, the percent protein solubility was calculated as below:

% protein solubility = [extracted protein (μg) / total protein (μg)] x 100% (3.5)

3.2.8. Texture Evaluation

Evaluation of the texture of micronized lentils was performed with a Lloyd Materials Testing Instrument (L1000R, Lloyd Instruments Ltd., Fareham, UK). The micronized lentils were cooked prior to texture measurement. Raw or micronized lentils were removed from the storage room (2-8°C) at least 12 hours before cooking. Fifty grams of sample were rinsed in distilled water at a ratio of 1:2.5 for 1 min and drained. The rinsed lentils were added to 500mL boiling deionized water, and boiled slowly for a set time. The boiling pot was covered with aluminum foil leaving a small vent for steam. Each sample was cooked in duplicate, drained thoroughly and placed in a 250mL covered plastic container to cool for 1 hour before texture measurements were taken. Cooking time was determined by cooking raw lentils for 12.5, 15, 17.5, 20 and 22.5 min. Twenty minutes was chosen as the cook time for both No.2 Laird and Eston lentils because this was the point where instrumental texture values began to level off with increased cooking time. Ten grams of cooked lentils were placed in the $10cm^2$ wire grid extrusion cell and a 1000N load cell was programmed to travel at 60 mm/min to compress those lentils, with the initial distance between the extrusion cell and the load cell being 77 cm. The instrument recorded the maximum force required to compress the lentils during the movement of the load cell. Six samples from the same cooking batch were measured. RControl software Ver.2.21 (Lloyd Instruments Ltd., UK) was used to program the test method and record the results of the texture testing.

3.2.9. Determination of Soluble, Insoluble and Total Dietary Fiber

The determination of dietary fiber in raw and micronized lentils followed the AACC (32-07) (1995) procedure. Each of the four 1g ground samples was subjected to sequential enzymatic digestion by heat-stable α-amylase, protease, and amyloglucosidase. Two of the digested solutions were filtered, and the residues were washed with 95% EtOH and acetone. Then they were dried at 103°C overnight and weighed, the average value of the residues was the gross weight of insoluble dietary fiber (IDF). One of these residues was analyzed for protein, and the second residue was analyzed for ash. The filtrates and water washings were combined separately and precipitated with 4 volumes 95% of EtOH; the precipitates were filtered, dried and weighed for soluble dietary fiber determination (SDF). Both SDF and IDF residues were corrected for protein, ash, and blank for the final calculation of SDF and IDF values. The other two digested solutions were precipitated right after digestion with 4 volumes of 95% EtOH. The precipitates were filtered and dried as well for the total dietary fiber determination (TDF). The TDF value was only corrected for protein and ash content.

3.2.10. The Determination of Antinutritional factors

3.2.10.1. Phytic Acid Determination

The content of phytic acid in lentils was determined following a modificated column chromatography procedure of Latta and Eskin (1980) (Appendix 2).

3.2.10.2. Phenolic Determination

The content of phenolic compounds of lentils was measured with a spectrophotometer procedure using Lowry reagent (Dorrel, 1976) (Appendix 3).

3.2.10.3. HPLC Determination of Oligosaccharies

In recent years, the high-performance liquid chromatography (HPLC) method has been developed for quantitative analysis of oligosaccharides in protein-rich food products (Knudsen and Li, 1991; Johansen et al., 1996). The sample was extracted with ethanol-water mixture and filtered to go through the column (Aminex® HPX-87N, 300 mm x 7.8 mm), and the results were recorded with a Shimadzu chromatopac recorder (Appendix 4). The contents of stachyose and raffinose were calculated from the sample curve and the standard curve and adjusted with the internal standard. In this study, the arabinose was used as an internal standard because the peak of arabinose couldn't be found from the sample curve and arabinose wouldn't affect the elution of the other sugars.

3.3. Data Analysis

Data analysis was carried out with the two-way ANOVA (StatEase Inc., USA) and the multiple comparision test (Tukey's test) (SigmaStat, 1997). The difference among least square means were studied at the $p \leq 0.05$ level.

CHAPTER 4

RESULTS & DISCUSSION

4.1. Chemical Compositions of Samples

Two batches of Laird and Eston lentils were used in this study. Table 4.1 lists the proximate contents of starch, protein, fat, ash, moisture content and color values (L, a, b) of the raw samples tested upon their arrival. The contents of those components were quite stable during one year's storage. Since the moisture content of the seeds was used to calculate the amount of water used in sample tempering, it was checked biweekly.

Table 4.1. General composition and color values[a] of raw Laird and Eston lentils

Content (%)	Sample			
	Laird		Eston	
	Batch 1	Batch 2	Batch 1	Batch 2
Starch	47.14	49.57	50.88	51.75
Protein	21.06	21.03	20.45	20.05
crude fat	1.39	1.37	1.20	1.22
Ash	2.72	2.68	2.49	2.43
Moisture	11.14	11.50	10.95	10.27
color L	49.1	47.9	44.9	47.4
color a	-2.9	-1.05	-1.8	-0.7
color b	15.2	14.8	13.5	15.1

[a] mean value

4.2. Tests with The Laboratory Scale Infrared Lamp

In this initial study, lentils were pretreated under different conditions. With water as the tempering solution, three tempering levels (20, 30, 40%) and two tempering times (12h, 24h) were tested. When tempering to a level of 40% moisture content, the final moisture contents of the seeds after micronization were too high for proper storage. Therefore, only two tempering levels (20, 30%) and one tempering time (12h) were evaluated when the three salt (150 ppm disodium EDTA, 2% sodium tripolyphosphate and mixture of 1% citric acid and 2% ascorbic acid) were included in the tempering solutions. The tempered seeds were heated with the infrared lamp for 1 min 45 sec to the final temperature was 120°C.

4.2.1. Final Moisture Content

The final moisture contents of the seeds after tempering and micronization treatment are recorded in Table 4.2. The final moisture contents were determined after the seeds had cooled to room temperature. The results showed that the moisture loss during micronization was about 10-20%, and the final moisture contents were dependent on the moisture level of the seeds following tempering. After infrared processing and cooling, the seeds were stored in the cold room where the temperature fluctuated between 2 and 8°C. It was found that the seeds with high final moisture contents (>20%) easily became moldy after storing for two or three days. The moisture contents of the seeds changed slowly during storage, reaching to 12-14% in a few weeks. With low final moisture content (<20%), the cooking quality of the seeds appeared to maintained after several months' storage according to their color changes and cooking characteristics.

Table 4.2. Final moisture contents[a] of micronized lentils of different pretreatment conditions using infrared lamp

Tempering		Final Moisture Content (%)	
Solution	level (%), time (h)	Laird	Eston
Distilled water	20, 12	11.81 ± 0.02	11.07 ± 0.08
	20, 24	11.76 ± 0.06	11.38 ± 0.01
	30, 12	15.81 ± 0.01	16.15 ± 0.03
	30, 24	16.75 ± 0.11	17.37 ± 0.04
	40, 12	21.33 ± 0.08	22.85 ± 0.02
	40, 24	23.08 ± 0.02	24.53 ± 0.10
150 ppm disodium EDTA	20, 12	12.45 ± 0.09	12.05 ± 0.14
	30, 12	16.98 ± 0.04	16.13 ± 0.08
2% sodium tripolyphosphate	20, 12	11.66 ± 0.07	12.14 ± 0.03
	30, 12	16.34 ± 0.10	17.75 ± 0.05
1% citric / 2% ascorbic acid	20, 12	12.83 ± 0.06	12.79 ± 0.06
	30, 12	17.14 ± 0.09	18.10 ± 0.12

[a] mean value ± std.

4.2.2. Starch Gelatinization

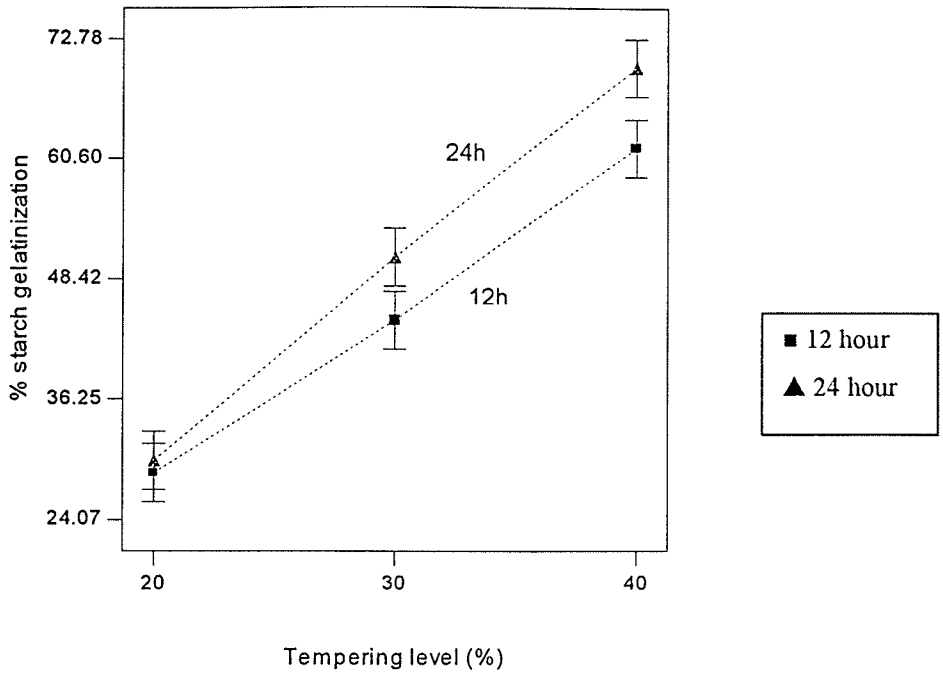
The starch contents of the untreated sample lentils were quite high, about 45-50% in Laird lentils and 50-55% in Eston lentils. As expected, the contents of total starch didn't change much after tempering and micronization treatments. The degree of starch gelatinization of micronized lentils, however, was increased between 20-60% depending on the pretreatment and infrared processing conditions (Appendix 5).

4.2.2.1. Tempered with Water

The effect of pretreatment conditions on the percentage of gelatinized starch in micronized lentils appears in Fig. 4.1(a) and Fig. 4.1 (b). For Laird lentils, the percentage of gelatinized starch was increased to about 70% after tempering to 40% moisture level and micronizing. The two-way ANOVA statistical analysis indicated that the higher tempering levels significantly increased n percentage starch of gelatinization ($p<0.0001$). Longer tempering times also helped to increase the amount of starch gelatinization ($p<0.05$). It seemed that the higher the moisture content, the more effective the tempering time was. That was consistent with the previous study by Arntfield et al. (1997).

Similar results were obtained from the experiments on Eston lentils, as both tempering level ($p<0.0001$) and tempering time ($p<0.01$) had significant effects on the level of starch gelatinization in Eston lentils. In Fig. 4.1(b), it was obvious that the changes in starch gelatinization in going from 20-30% were much greater than the changes going from 30-40%.

(a)



(b)

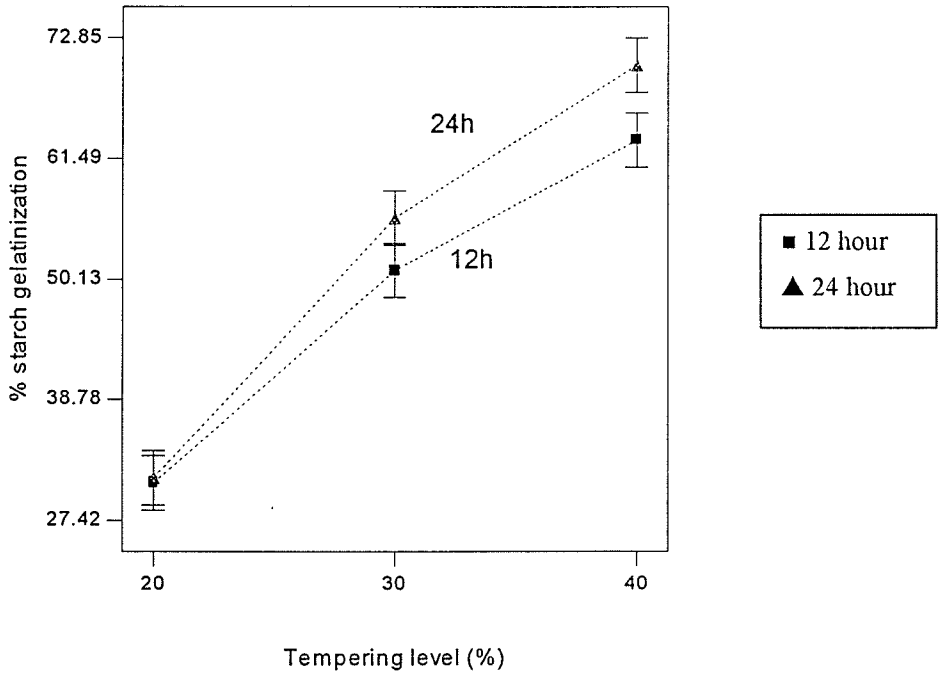


Fig. 4.1. Model graph of starch gelatinization of Laird (a) and Eston (b) lentils

4.2.2.2. Tempering with Salt Solutions

The degree of starch gelatinization of the lentils tempered with salt solutions prior to micronization was much higher than that of the untreated raw seeds. For both Laird and Eston lentils, salt solutions seemed to be more effective than water at the same tempering level. Tempering level still played an important role in the starch gelatinization ($p < 0.001$). Between 20% and 30% moisture, the starch gelatinization of Laird or Eston lentils was increased by about 20% for each tempering solutions.

Based on the two way ANOVA, different salt solutions appeared to have different effects on the percent starch gelatinized in micronized lentils (Laird: $p<0.01$, Eston: $p<0.001$). Therefore, pairwise multiple comparions (Tukey test) on the effects of the three tempering solutions were made using Sigma statistical software (version 2.0). The results are summarized in Table 4.3. With the exception of the 20% tempering level for the Laird lentils, tempering with the solution containing 1% citric acid/2% ascorbic acid resulted in a higher level of gelatinized starch than obtained with the 2% sodium tripolyphosphate. The addition of 150ppm sodium EDTA to the soaking solution gave starch gelatinization values between the other two salts but not significantly different from either regardless of the tempering level. The effects of the three tempering solutions on Laird lentils were about the same at the low tempering level (20%). All salts treatments resulted in higher levels of starch gelatinization than the distilled water treatment.

The high degree of starch gelatinization at high tempering levels was due to the swelling of starch granules, which made the gelatinization easier during micronization. Long tempering times helped the penetration and equilibration of water when the

solutions helped to increase the degree of starch gelatinization during micronization treatment.

Table 4.3. The effects of different salt solutions on the content of gelatinized starch in Laird and Eston lentils*

Salt solution	% starch gelatinization			
	Laird		**Eston**	
	Temp. level 20%	Temp. level 30%	Temp. Level 20%	Temp. level 30%
distilled water	28.8±2.4 [b]	44.2±3.5 [bc]	30.9±3.2 [b]	50.9±2.7 [c]
2% sodium tripolyphosphate	30.7±3.5 [a]	48.5±0.7 [b]	32.9±3.9 [ab]	53.3±0.6 [bc]
150 ppm disodium EDTA	33.7±2.8 [a]	51.6±1.0 [ab]	35.2±2.4 [ab]	56.8±1.5 [ab]
1% citric acid/2% ascorbic acid	35.4±3.5 [a]	55.2±2.3 [a]	39.0±0.0 [a]	60.0±0.5 [a]

* Column values followed by the same letter are not significantly different ($P \leq 0.05$)

4.2.3. Protein Solubility

The protein contents of the micronized seeds were determined by Kjeldahl nitrogen analysis. Micronization treatment had little effect on total nitrogen content in lentils. However, even with pretreatment at low tempering level (20%) and short tempering time (12h), the protein solubility of the micronized lentils was decreased by 20-25% compared with that of the raw seeds (Appendix 6). The two way ANOVA indicated that the protein solubility was significantly affected by tempering level and, at high tempering level, the type of tempering solution also played an important role in the decrease of protein solubility ($p<0.05$).

4.2.3.1. Tempering with Water

Fig.4.2(a) illustrates the effect of varying tempering time and moisture content during pretreatment on protein solubility of Laird lentils. Only tempering level significantly decreased the protein solubility ($p<0.0001$). In the graph of Eston lentils, the two lines, 12 h and 24 h, were close and, they even crossed at one point. Therefore, the extent of protein denaturation was mainly related with the initial moisture content and the heat treatment (Hansen et al., 1975; Neucere and Cherry, 1982; Nakai and Li-Chen, 1989). It would appear that the water was adequately distributed so as to promote protein denaturation after only 12 hour of tempering.

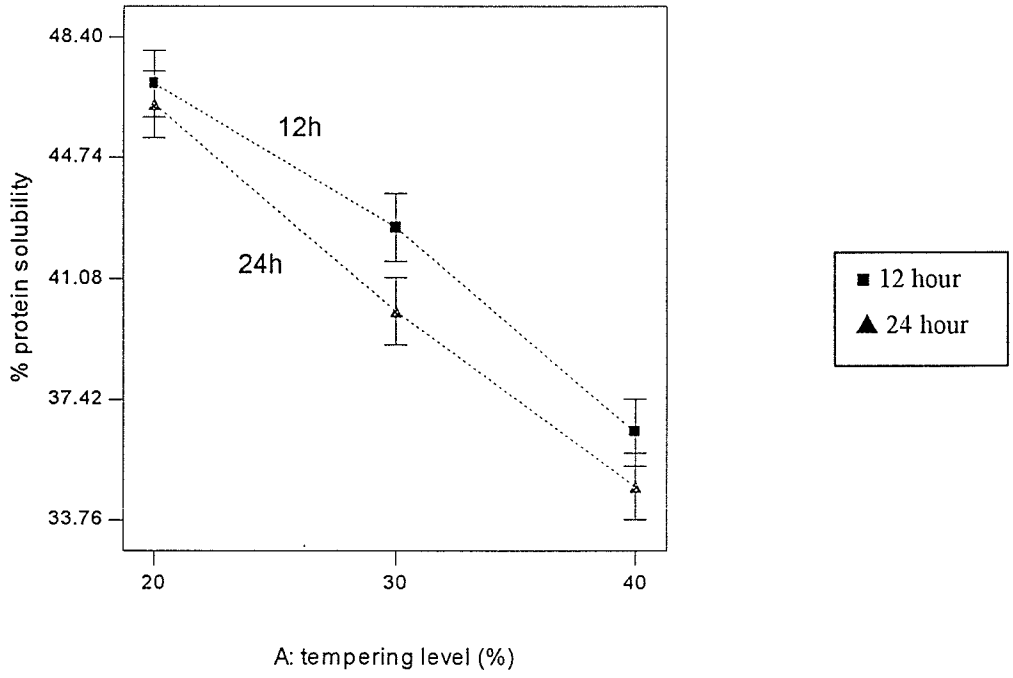


Fig. 4.2 (a). Model graph of protein solubility of Laird lentils

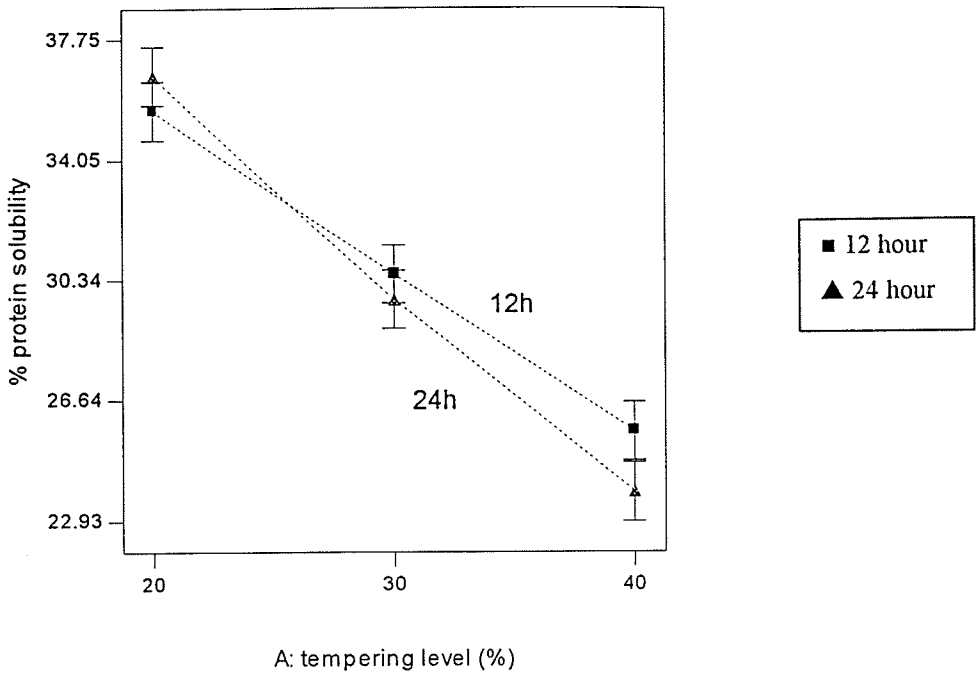


Fig 4.2 (b). Model graph of protein solubility of Eston lentils

4.2.3.2. Tempering with Salt Solutions

Both composition of the tempering solution as well as the tempering level had significant effects on the protein solubility of micronized lentils. The summary of pairwise multiple comparison for tempering solutions at different levels is shown in Table 4.4. As was the case with starch gelatinization, the greatest reduction in protein solubility came with 1% citric acid/2% ascorbic acid combinations where protein solubility values were significantly lower than those for 150 ppm disodium EDTA at all tempering levels. With the exception of the Eston lentils tempered to a 20 or 30% moisture level, protein solubility reduction was greater with the 1%citric acid/2%

ascorbic acid than with 2% sodium tripolyphosphate. Only for the Eston lentils tempered to 30% moisture were the data from EDTA and sodium tripolyphosphate different. The various solutions' ability to reduce protein solubility could therefore be ranked as follows: the mixture of citric acid and ascorbic acid, the sodium tripolyphosphate and then the disodium EDTA.

Table 4.4. The effects of different salt solutions on protein solubility of lentils *

Salt solution	% protein solubility			
	Laird		Eston	
	temp. level 20%	temp. level 30%	temp. level 20%	temp. level 30%
distilled water	46.9±1.4 [a]	42.6±1.3 [ab]	35.6±1.3 [a]	30.5±0.8 [ab]
1% citric acid/2% ascorbic acid	45.0±1.9 [b]	40.7±1.0 [b]	31.7±1.5 [b]	27.9±2.3 [b]
150ppm disodium EDTA	48.0±1.7 [a]	43.9±0.8 [a]	39.4±1.5 [a]	34.0±1.5 [a]
2% sodium tripolyphosphate	47.4±0.6 [ab]	43.6±1.3 [a]	36.3±1.5 [a]	29.0±0.9 [b]

* Column values followed by the same letter are not significantly different ($p<0.05$)

4.2.4. Color

The changes in lentil color after heat treatment were measured with the Hunterlab Color Meter (Appendix 7). The color of the final product was expected to be slightly changed (Arntfield et al., 1997), but the less the discoloration, the better the marketability of the micronized lentils.

4.2.4.1. Tempering with Water

The color values of both Laird and Eston lentils were significantly changed as a result of water-tempering and infrared treatment. The brightness (L value), redness (a value) and greenness (b value) of untreated Laird lentils were 49.1, -2.9 and 15.2 respectively. After processing, those values were reduced by 6-10, 0.2-1.0 and 5-8 (Fig. 4.3). Tempering level had significant effects on all the three values: L ($p<0.0001$), a ($p<0.0001$) and b ($p<0.0001$). Tempering time only had significant effect on L ($p<0.01$) and a ($p<0.01$). The changes in the seed color might due to the products from the Maillard reaction and decomposition during heat treatment (Yoshida et al., 1995).

The L, a and b values of untreated Eston lentils were 44.9, -1.8 and 13.5, respectively. The results of water-tempering Eston lentils were a little different from Laird lentils (Fig. 4.4). For Eston lentils, the tempering time didn't have significant effect on any of the color values, and the tempering level only had significant effects on L ($p<0.0001$) and a values ($p<0.0001$) only. Generally, the tempering level was the important factor in the discoloration of the final products. Higher tempering level decreased the degree of lightness and greenness of the seeds.

The results also indicated that the discoloration of the seeds was closely related to the heat treatment. The high heating temperature (>120°C) might accelerate the discoloration of the seeds (Yoshida et al., 1995). In addition, the heat treatment couldn't be very uniform with the infrared lamp because the sample tray was shaken by hand, which resulted in the burning of some seed coats.

(a)

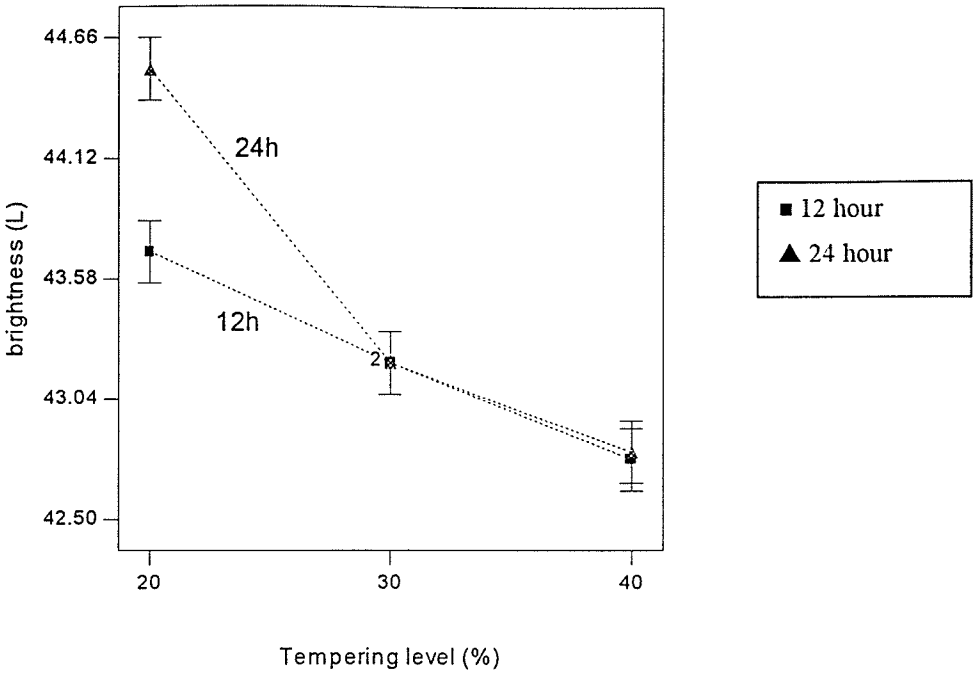


Fig. 4.3 (a). Model graph of Hunterlab L color values of Laird lentils.

(b)

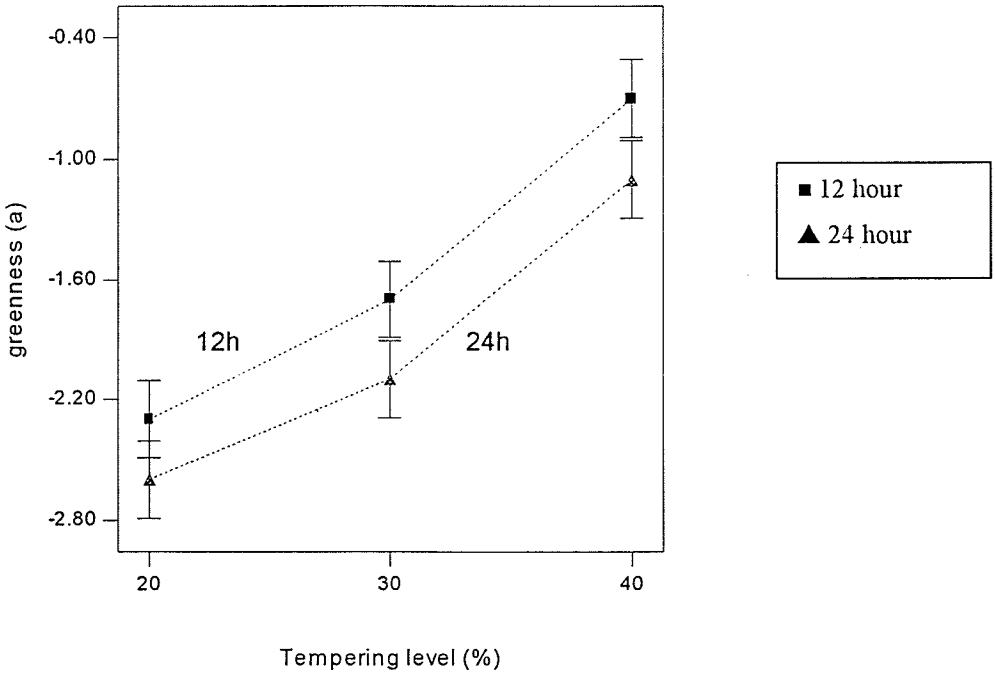
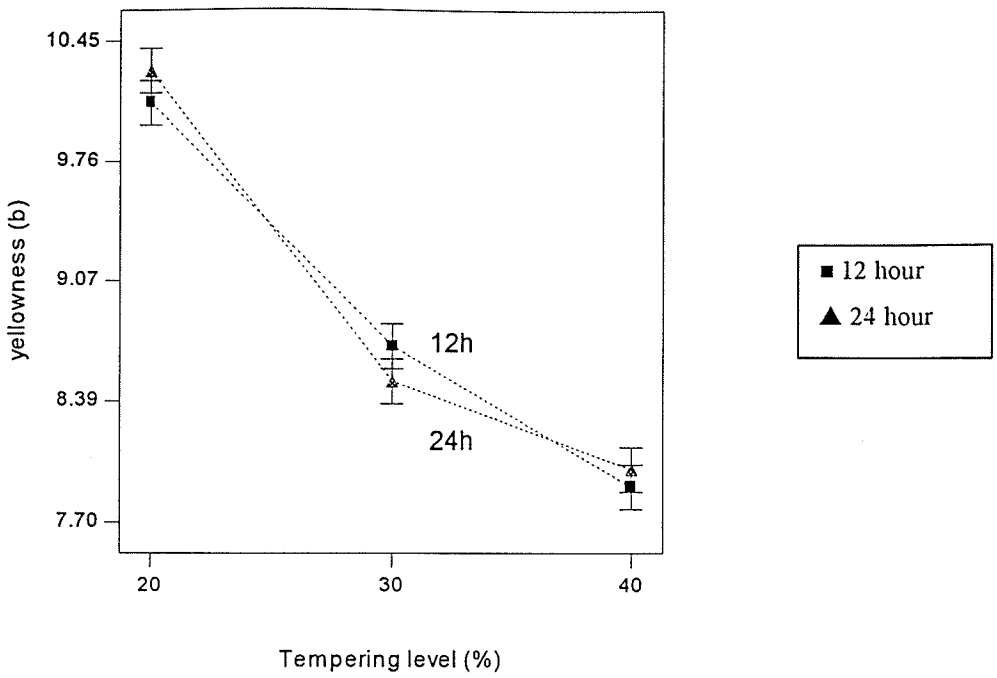


Fig. 4.3 (b). Model graph of Hunterlab a color values of Laird lentils.



ig. 4.3 (c). Model graph of Hunterlab b color values of Laird lentils.

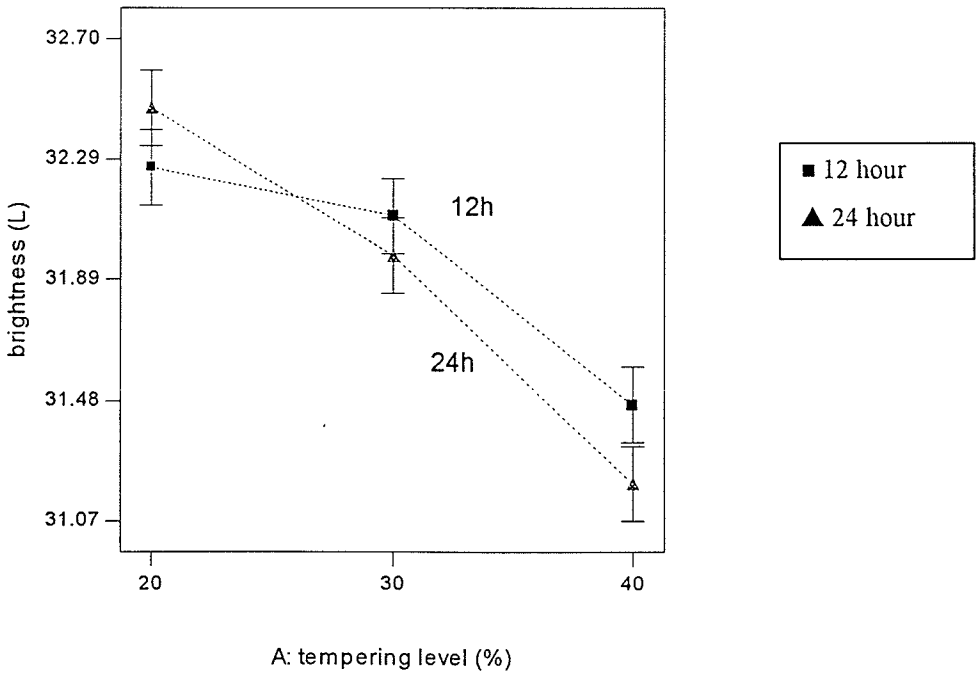


Fig. 4.4 (a). Model graph of Hunterlab L color values of Eston lentils.

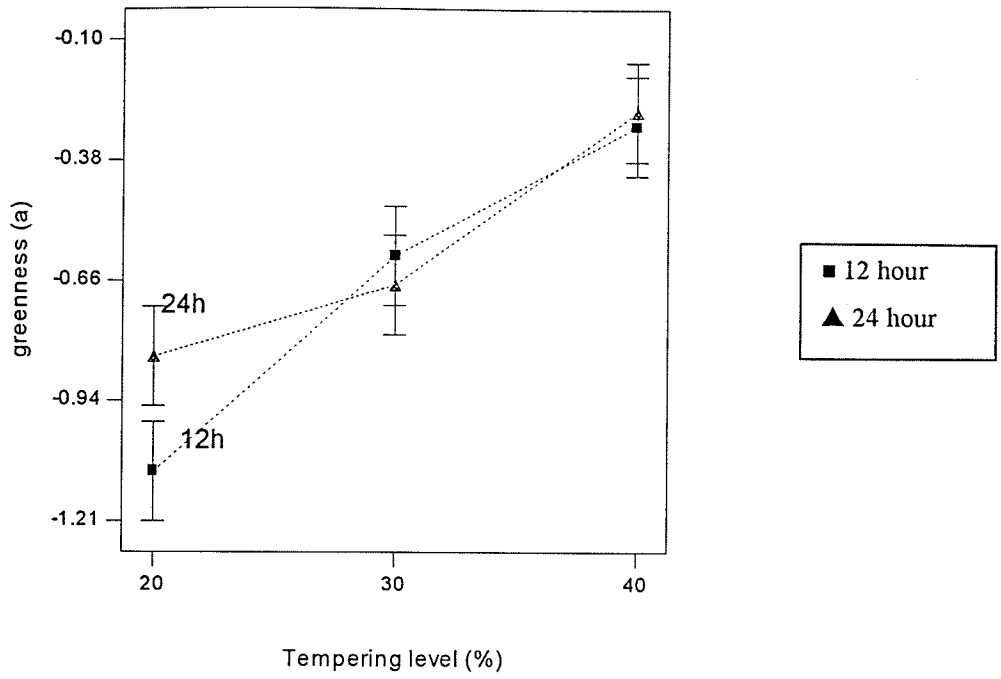


Fig. 4.4 (b). Model graph of Hunterlab a color values of Eston lentils.

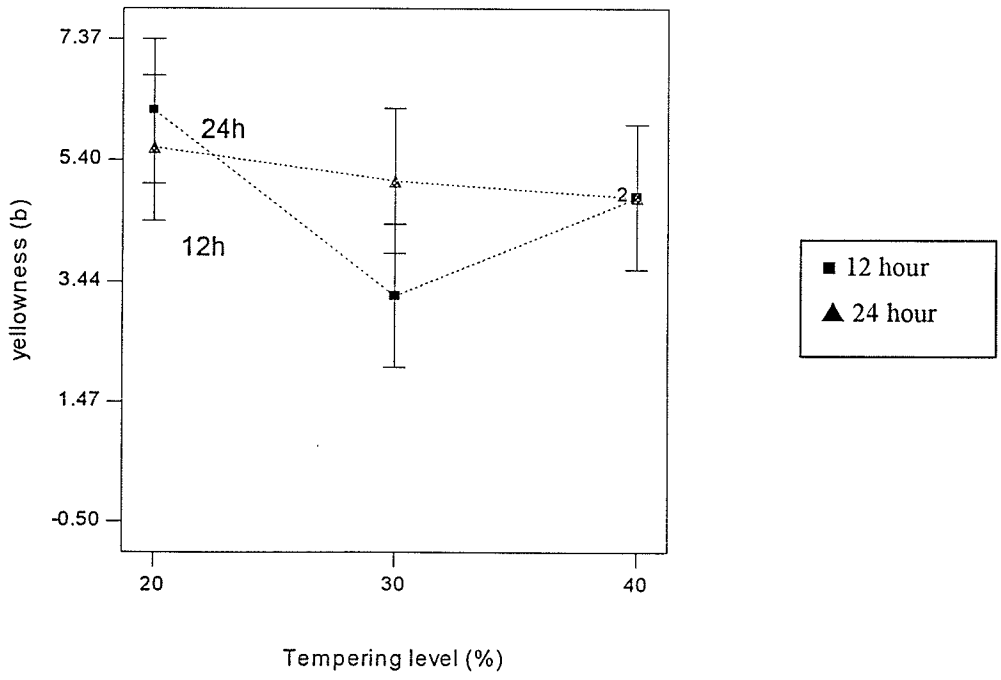


Fig. 4.4 (c). Model graph of Hunterlab b color value of Eston lentils.

4.2.4.2. Tempering with Salt Solutions

The three tempering solutions seemed to have significantly different effects on the discoloration of the lentil products (Table 4.5). The sample heated with 150 ppm disodium EDTA had the highest L values and lowest a values of the solutions tested. With the Eston lentils, however, the a values with disodium EDTA were not significantly different than those heated with 2% sodium tripolyphosphate. The sample heated with 1% citric acid/2% ascorbic acid, on the other hand had the lowest L values and higher a and b values for both lentil types. This is indicative of a less bright seed containing more red and more yellow, suggesting the seed had been browned more during the micronization treatment. In comparision to the samples treated with distilled water only (Table 4.5), the samples treated with 2% sodium tripolyphosphate appeared to have the most similar color; with the 150 ppm disodium EDTA samples appearing somewhat lighter; and the 2% sodium tripolyphosphate samples somewhat darker.

Table 4.5. The effects of different tempering solutions on lentil L, a and b Hunterlab color values*

Salt solution	Laird		Eston	
	temp. level 20%	temp. level 30%	temp. level 20%	temp. level 30%
L values				
Distilled water	43.7±0.2 [b]	43.2±0.1 [ab]	32.3±0.1 [a]	31.9±0.2 [a]
1% citric acid/2% ascorbic acid	40.4±0.3 [c]	40.2±0.1 [b]	27.8±0.2 [a]	26.7±0.2 [c]
150 ppm disodium EDTA	47.4±0.7 [a]	48.0±0.1 [a]	32.0±0.1 [c]	31.7±0.0 [a]
2% sodium tripolyphosphate	43.9±0.3 [b]	43.1±0.2 [ab]	30.3±0.2 [b]	29.1±0.2 [b]
a values				
distilled water	-2.27±0.21 [c]	-1.67±0.12 [b]	-1.1±0.1 [c]	-0.6±0.1 [b]
1% citric acid/2% ascorbic acid	0.03±0.03 [a]	0.13±0.03 [a]	0.08±0.03 [a]	0.1±0.05 [a]
150 ppm disodium EDTA	-2.57±0.06 [c]	-1.9±0.15 [b]	-0.9±0.1 [bc]	-0.5±0.1 [b]
2% sodium tripolyphosphate	-1.77±0.12 [b]	-1.63±0.15 [b]	-0.7±0.1 [b]	-0.43±0.15 [b]
b values				
distilled water	10.1±0.1 [c]	8.7±0.1 [d]	6.2±0.1 [c]	5.2±0.3 [c]
1% citric acid/2% ascorbic acid	12.1±0.2 [a]	13.0±0.1 [a]	8.4±0.1 [a]	9.7±0.1 [a]
150 ppm disodium EDTA	11.4±0.1 [b]	10.7±0.2 [b]	6.8±0.1 [b]	6.8±0.1 [b]
2% sodium tripolyphosphate	10.0±0.1 [c]	9.7±0.1 [c]	6.0±0.2 [c]	5.8±0.1 [b]

* For each of the L, a and b values, column values followed by the same letter are not significantly different ($p<0.05$)

4.2.5. Effects of Pretreatment and Infrared Heating

The results of this study indicated that the initial moisture contents had affected many of the properties of the lentils. Most effects were positive to the quality of the final products, except that higher moisture levels increased the discoloration of the seeds. At high tempering levels, the tempering time affected the degree of starch gelatinization, the protein solubility and the L value of lentil color. In the study of the next stage, the pilot scale gas-fired micronizer was used to process the lentils. More chemical properties of the products, such as the contents of dietary fiber and antinutritional factors after tempering and micronization treatment, and cooking quality were investigated to give some insight into the causes of the changes.

4.3. The Pilot Scale Micronizing System

Based on the results obtained with the small infrared lamp, the pilot scale micronizer was used to process Laird and Eston lentils. The final temperatures of the micronized seeds were taken at the end of the vibratory conveyor. Based on preliminary experiments, it was found that 85°C and 95°C were the optimal final temperatures to give an acceptable texture without burning for Laird and Eston lentils, respectively. During micronization, the moisture losses from the tempered lentils were in the range of 6-10%, therefore, the tempering level was chosen to be 20-30% and the tempering time was 6-18 hours. It was expected that micronized lentils which were tempered under optimum conditions would have low final moisture content (<20%) and protein solubility, high content of starch gelatinization, good cooking quality and little color change.

4.3.1. Results from Variations in Tempering Time and Level

4.3.1.1. Final Moisture Content

With the pilot scale micronization system, the tempering level had a significant effect ($p < 0.001$) on the final moisture of the lentils (Appendix 9). When the tempering level was high (above 25%), the final moisture contents of the micronized products were around or higher than 20% (Table 4.6). As discussed before, high final moisture content (above 20%) was not desired because of the mould problems.

4.3.1.2. Starch Gelatinization

With the gas-fired pilot scale micronizer, the content of gelatinized starch ranged from 15-35% (Appendix 8). This was lower than that of the lentils processed with the infrared lamp with the same pretreatment conditions, and might be due to the lower heating temperature and shorter processing time. The tempering level and tempering time still had significant effects ($p<0.05$) on the content of gelatinized starch (Appendix 9). The effects of tempering time and level on the starch gelatinization of lentils during micronization are shown in the following three-dimensional plots (Fig.4.5). With high intra- and intercellular water availability, the starch gelatinization occurs easily when using the infrared heat treatment (Garcia-Vela and Stanley, 1989). The percent of starch gelatinization was greatly increased at or above 25% tempering level. The influence of tempering time was more noticeable at lower tempering levels and seemed to be optimal between 10h and 14h for the Laird lentils and above 12h for the Eston lentils.

Table 4.6. The final moisture contents[a] of micronized Laird and Eston lentils

	Tempering		Final Moisture Content (%)	
No.	Level (%)	Time (h)	Laird	Eston
1	20	6	15.95 ± 0.28	15.35 ± 0.39
2	30	6	22.32 ± 0.04	17.90 ± 0.95
3	20	18	15.85 ± 0.19	16.00 ± 0.51
4	30	18	21.54 ± 0.09	18.87 ± 0.91
5	25	3.5	17.84 ± 0.17	18.24 ± 0.65
6	25	20.5	18.76 ± 0.28	17.01 ± 0.42
7	17.93	12	13.38 ± 0.18	13.40 ± 0.90
8	32.07	12	22.02 ± 0.06	21.18 ± 0.37
9	25	12	18.28 ± 0.01	17.76 ± 0.37
10	25	12	17.99 ± 0.06	17.41 ± 0.38
11	25	12	18.31 ± 0.10	17.33 ± 0.69
12	25	12	18.81 ± 0.35	16.64 ± 0.69
13	25	12	17.97 ± 0.07	17.53 ± 0.59

[a]average ± std.

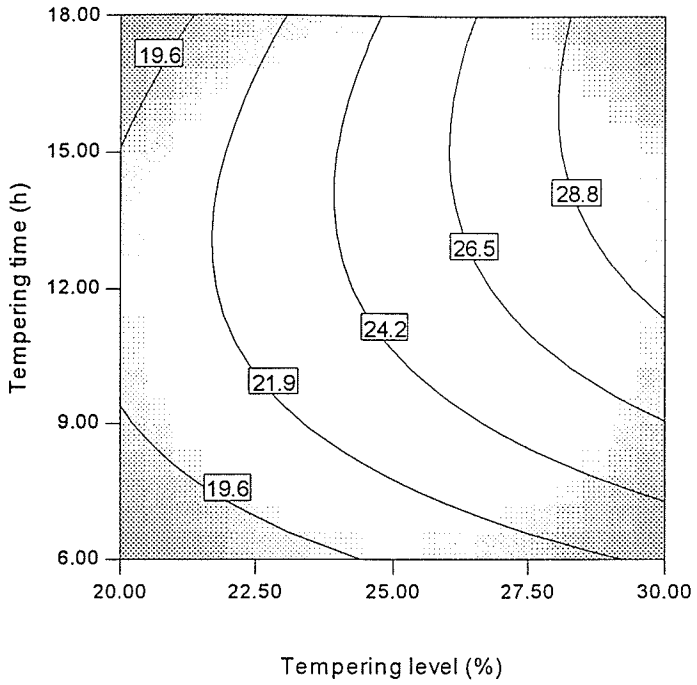


Fig. 4.5(a). Response surface plot for the percent gelatinized starch of micronized Laird lentils as a function of tempering level (%) and tempering time (h).

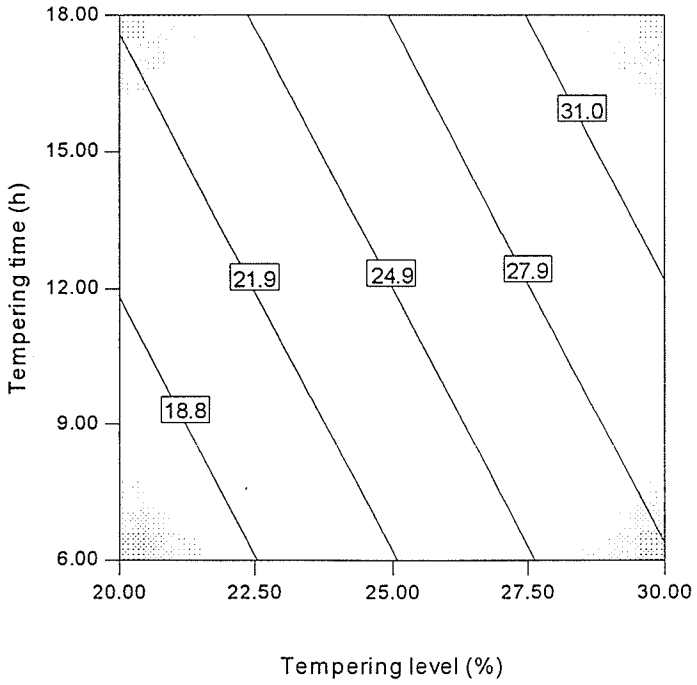


Fig. 4.5(b). Response surface plot for the percent gelatinized starch of micronized Eston lentils as a function of tempering level (%) and tempering time (h).

4.3.1.3. Protein Solubility

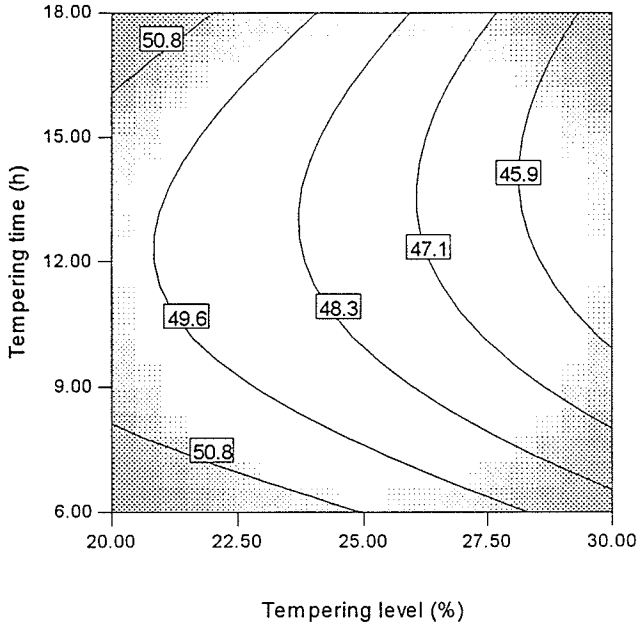
Zheng et al. (1998) found that micronization could reduce the hydrophilicity of legume proteins resulting in a reduction in nitrogen solubility between pH 6 and10. In our study, the protein solubility of Laird and Eston lentils decreased by 10-15% and 10-20% (Appendix 8), respectively, and the results showed high correlations ($p<0.01$) between the tempering level and the loss of protein solubility (Fig. 4.6). Higher initial moisture contents of the seeds might make the proteins more susceptible to hydrophobic aggregation of polypeptide chains following denaturation during the infrared heat treatment (Arntfield et al., 1997). While tempering time seemed to have some influence on the protein solubility of Laird lentils at low tempering levels (<25%), for Eston lentils protein denaturation was primarily dependant on the tempering levels and the influence of tempering time was minimal.

4.3.1.4. Color

It was found that the appearance of the lentils processed with the pilot-scale micronizer was closer to that of the untreated seeds than was seen with the infrared lamp. Most of the micronized seeds had intact seed coats, with only a very few seeds cracking at high tempering levels. The Hunterlab L, a, b values of untreated Laird seeds were 47.9, -1.05 and 14.8, respectively. After the micronization treatment, the L values were between 47 to 50, a values varied from –1.04 to 1.04, and b values from 15-16.5. Changes in color values of micronized Eston lentils were even less than those of Laird lentils (Table 4.7). The lack of color change resulting from the micronization of lentils

might due to the uniform heating with the pilot-scale micronizer, as well as the low processing temperatures and the short processing times used.

(a)



(b)

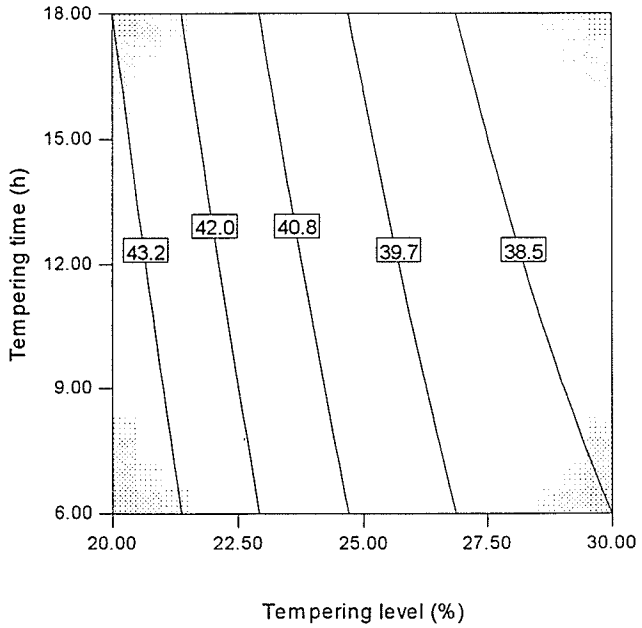


Fig. 4.6. Response surface plot for the percent protein solubility of micronized Laird (a) and Eston (b) lentils as a function of tempering level (%) and tempering time (h).

The ANOVA analysis of the data indicated that the changes in the color values due to different tempering conditions mainly depended on the tempering level ($p<0.01$) of the seeds (Appendix 9). At low tempering levels (17.93% and 20%), the brightness (L value) of the Laird and Eston lentils were 1.0-2.0 points higher than the samples tempered at higher moisture level (30 and 32.07%). Similarly, the greenness (a) was 0.01-0.4 points lower and the yellowness (b) of the seeds was increased by 0.5-1.0 points. It appears that treatment by tempering at a moisture level of approximately 25% could give a final product with an acceptable color. At this tempering level, the micronized lentils also had acceptable final moisture contents (17-18%), relatively high percent starch gelatinization (24-25%) and low protein solubility (45-47%).

4.3.1.5. Cooking Quality

The cooking quality of the micronized seeds was determined by the texture evaluation using the Lloyd Materials Testing Instrument. The lentils were cooked prior to texture measurement. The maximum load to compress 10g of cooked lentils was measured. The cooking curves of non-micronized Laird and Eston lentils are presented in Fig 4.7. At 15-20 min, the maximum load to compress both Laird and Eston lentils was greatly decreased, and at 22.5 min the maximum load of Laird and Eston lentils approached to 125N. Using similar test conditions, Scanlon et al. (1997) found that lentils were judged to be adequately cooked when they had a compressive force value of less than 125N. Since the micronization treatment should help to accelerate the softening of lentils during cooking and thereby reduce the force to compress at comparable cooking

times, 20 min was chosen as the cooking time so that micronized and non-micronized lentils could be compared.

Table 4.7. The Hunterlab color values[a] of the micronized Laird and Eston lentils using the pilot scale micronizer

Tempering		Color					
		Laird			Eston		
level (%)	time (h)	L	a	b	L	a	b
20	6	48.60±0.28	-1.04±0.02	15.35±0.21	48.53±0.04	-0.30±0.04	15.39±0.16
30	6	46.92±0.16	0.92±0.03	15.98±0.21	46.98±0.04	0.95±0.07	15.71±0.01
20	18	50.32±0.07	-1.02±0.02	15.24±0.33	48.45±0.07	-0.60±0.07	15.23±0.04
30	18	47.37±0.09	1.04±0.03	16.49±0.02	47.00±0.14	0.98±0.03	16.00±0.07
25	3.5	49.97±0.09	-0.83±0.11	15.62±0.21	48.37±0.05	-0.03±0.04	15.85±0.07
25	20.5	50.30±0.00	-0.62±0.01	15.39±0.02	48.40±0.04	-0.39±0.06	15.50±0.14
17.93	12	50.04±0.05	-0.61±0.66	14.82±0.12	48.70±0.07	-0.69±0.03	15.04±0.08
32.07	12	47.17±0.18	1.03±0.03	16.47±0.10	46.42±0.19	1.06±0.06	16.22±0.04
25	12	50.15±0.11	-0.73±0.00	15.43±0.00	48.28±0.18	-0.53±0.04	15.53±0.06
25	12	50.03±0.00	-0.63±0.14	15.45±0.03	48.09±0.05	-0.43±0.04	15.50±0.07
25	12	50.13±0.14	-0.77±0.03	15.07±0.10	48.31±0.04	-0.30±0.04	15.40±0.14
25	12	49.83±0.04	-0.53±0.04	15.43±0.07	48.25±0.04	-0.29±0.04	15.40±0.07
25	12	50.07±0.04	-0.70±0.10	15.20±0.21	48.55±0.07	-0.33±0.04	15.60±0.04

[a]average ± std.

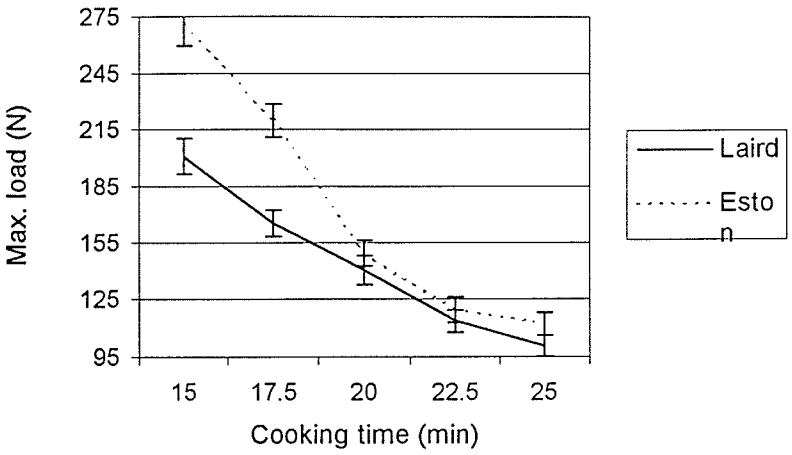
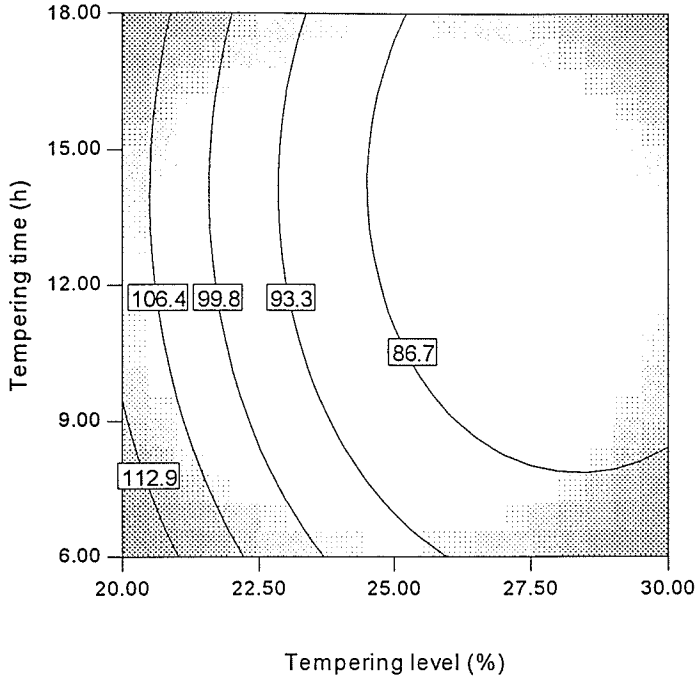


Fig. 4.7. Cooking curve of raw Laird and Eston lentils

The contour plots showing the effect of tempering time and level on cooked lentil texture are presented in Fig. 4.8. It was found that the cooked micronized seeds were much softer than the cooked non-micronized seeds. Therefore, micronization treatment significantly reduced the maximum force required to compress the cooked lentils. Cenkowski and Sosulski (1998) reported that micronized split peas absorbed about 7% more water then the untreated sample during the 15 min cooking period. Starch gelatinization and protein denaturation could be related to this water uptake cooking The softening of the texture of the cooked seeds at higher tempering levels ($p<0.0001$) could also be influenced by the water uptake. The maximum load to compress was also affected significantly by the tempering time ($p<0.05$). Long tempering time decreased the hardness of the cooked seeds. This was especially noticeable at higher tempering levels.

(a)



(b)

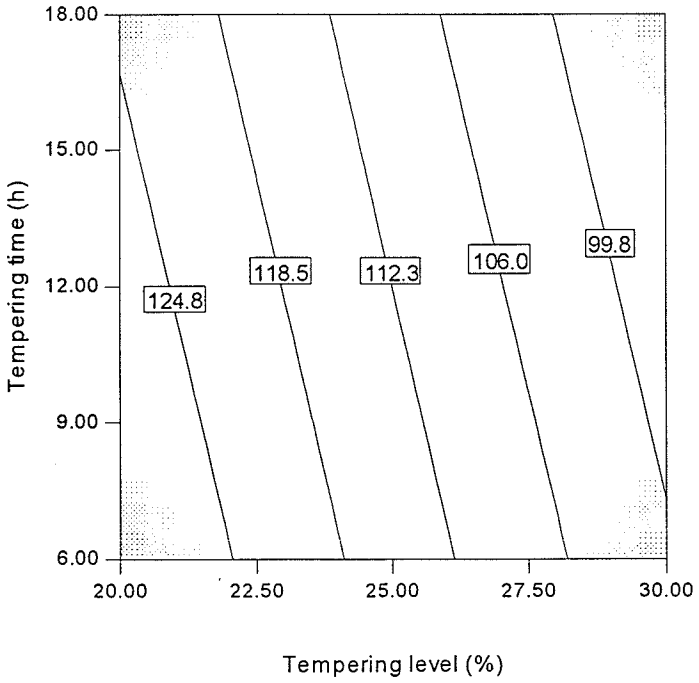


Fig. 4.8. Response surface plot for the maximum force (N) of micronized cooked Laird (a) and Eston (b) lentils as a function of tempering level (%) and tempering time (h).

Under the same treatment conditions, Eston lentils (Fig 4.8b) were harder than Laird lentils, the cooked Eston lentils were observed to be less likely to separate and lose their hulls during cooking. At 25% or higher tempering levels, the maximum loads to compress Laird and Eston lentils cooked for 20 min were quite low. A cooking curve of the tempered (25%, 12h) and micronized lentils was obtained with cooking of 12.5, 15, 17.5, 20 and 22.5 min (Fig. 4.9). Under that pretreatment condition, the cooking time of Laird lentils could be reduced to 16 min, and Eston lentils could be reduced to 18 min based on a maximum force to compress of 125N.

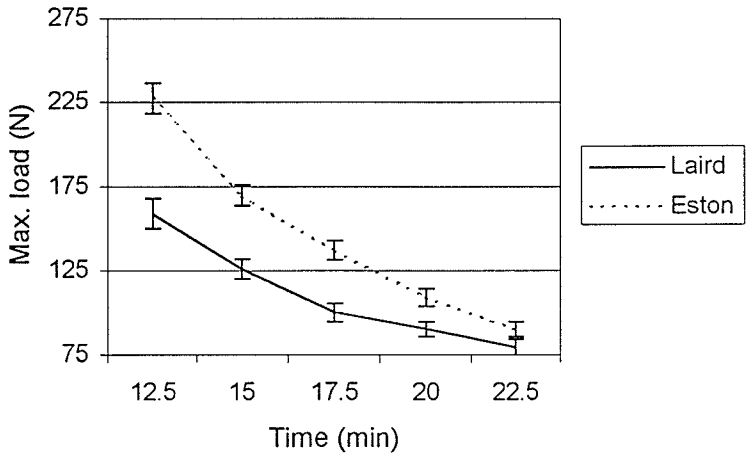


Fig. 4.9. Cooking curve of micronized (25%, 12h) Laird and Eston lentils.

4.3.2. Effects of Different Tempering Solutions

From our investigation of tempering solution composition, it was found the several salt solutions had effects on the physo-chemical properties of the tempered and micronized lentils. Furthermore, the study verified the previous results as seen with the infrared lamp, that all the three salt solutions tested had similar effects on the final moisture content and protein solubility (Table 4.8).

Table 4.8. Comparision of the effect[a] of different salt solutions on lentils[b] ($p \leq 0.05$)*

Tempering solution	Laird			Eston		
	final moisture (%)	starch gelatinization (%)	protein solubility (%)	final moisture (%)	starch gelatinization (%)	protein solubility (%)
distilled water	18.30±0.86 [a]	23.6±1.4 [b]	48.7±0.4 [bc]	17.24±1.14 [a]	24.6±1.3 [c]	39.9±0.2 [a]
150 ppm disodium EDTA	18.96±0.57 [a]	28.8±1.0 [a]	50.6±0.6 [a]	18.27±0.51 [a]	28.1±1.1 [b]	40.6±0.4 [a]
2% sodium tripolyphosphate	18.35±0.37 [a]	26.9±1.3 [ab]	49.8±0.4 [ab]	19.04±0.93 [a]	31.8±1.3 [a]	41.2±0.6 [a]
1%citric acid/2% ascorbic acid	18.95±0.11 [a]	29.0±1.4 [a]	48.3±0.2 [c]	18.53±0.72 [a]	27.1±0.9 [bc]	39.9±0.8 [a]
	Color			Color		
	L	A	B	L	A	B
Distilled water	50.0±0.08 [a]	-0.7±0.06 [d]	15.3±0.07 [b]	48.4±0.04 [a]	-0.2±0.06 [c]	15.5±0.07 [c]
150 ppm disodium EDTA	48.9±0.09 [b]	-0.4±0.06 [c]	15.6±0.05 [a]	48.2±0.09 [b]	-0.1±0.06 [c]	15.7±0.1 [b]
2% sodium tripolyphosphate	48.7±0.05 [c]	-0.06±0.05 [b]	15.4±0.10 [ab]	47.7±0.07 [c]	0.2±0.05 [b]	15.5±0.05 [c]
1%citric acid/2% ascorbic acid	48.3±0.07 [d]	0.4±0.05 [a]	15.1±0.06 [c]	45.7±0.06 [d]	1.0±0.07 [a]	16.1±0.05 [a]

[a] mean ± std.
[b] pretreatment treatment condition: 25% tempering level and 12 hour tempering time.
*column values followed by the same letter are not significantly different ($P \leq 0.05$)

The content of gelatinized starch in samples heated with the infrared lamp was higher than that of the samples heated with the micronizer, which may because of the different processing conditions applied on the two infrared systems (Table 4.8). With the same micronization equipment, the content of gelatinized starch in samples tempered with salt solutions was higher than that of the samples tempered with distilled water alone. The exceptions to this were the Laird lentils in 2% sodium tripolyphosphate and the Eston lentils in 1% citric acid / 2% ascorbic acid. With the infrared lamp, the only significant difference was for the Eston lentils where the 1% citric acid / 2% ascorbic acid was differ from the distilled water.

Color changes were noted with all treatments but the greater adverse change (the decrease of L value and increase of a value) were seen with the 1% citric acid / 2% ascorbic acid. This is similar to what was seen with the infrared lamp (Table 4.5). However, the L, a and b values were all higher for the micronizer compared to the infrared lamp.

Under these tempering conditions (25% tempering level and 12 h tempering time), the salt solutions were not significantly better than the distilled water at reducing the maximum compression force for the cooked Laird lentils (Fig. 4.10). However, for Eston lentils, the sodium tripolyphosphate significantly reduced the hardness of the seeds compared to water treatment. Fig 4.10 shows the maximum load of the cooked samples which were pretreated with tempering and heated using the micronizer.

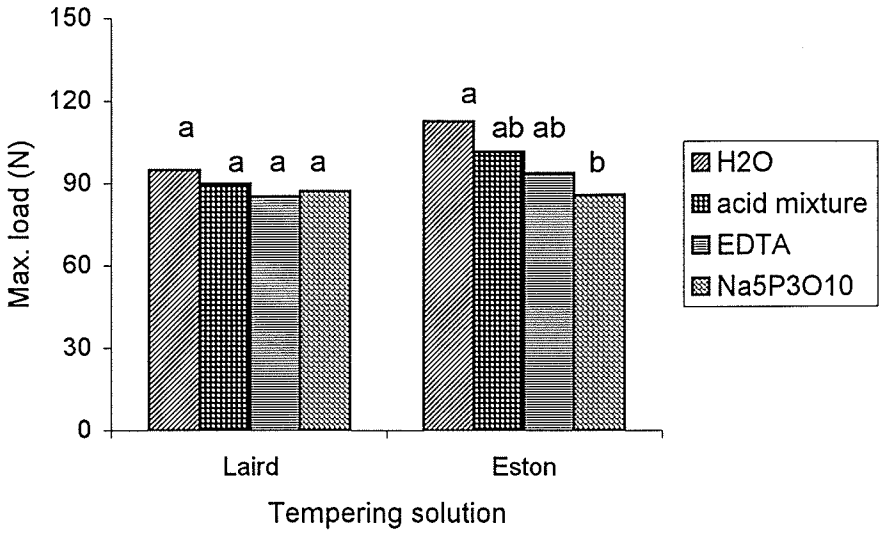


Fig. 4.10. Effect of tempering solutions on the texture of cooked lentils (20 min).

4.3.3. Effect of Micronization on the Other Properties

4.3.3.1. Dietary Fiber

Lentils contain a large amount of dietary fiber (DF). The Laird lentils contained approximately 15% dietary fiber, mostly as insoluble dietary fiber (IDF) and only about 1% as soluble dietary fiber (SDF). The total dietary fiber in Eston lentil was approximately 16%, the distribution between SDF and IDF was similar. Gooneratne et al. (1994) reported that the distribution of the nonstarch polysaccharides between soluble and insoluble fiber could be changed during processing due to the degradation and solubilization of pectic polysaccharides. Fig. 4.11 illustrates the changes of soluble and insoluble dietary fiber after tempering and heat treatment using the big micronizer.

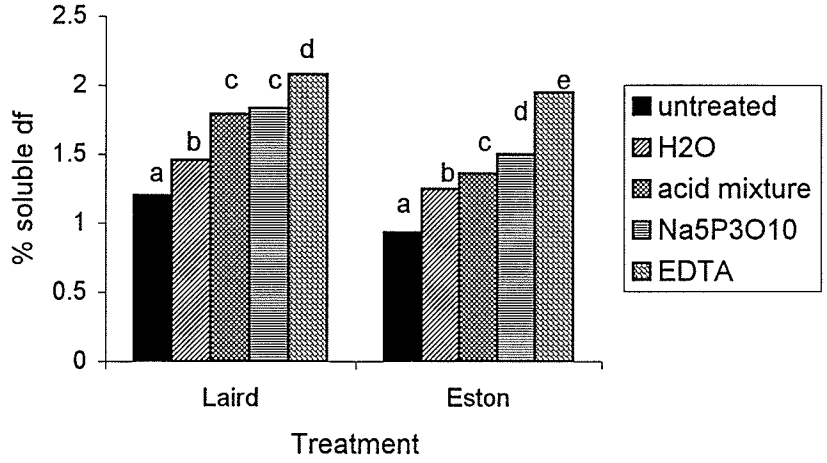


Fig. 4.11(a). Effect of tempering solutions on the soluble dietary fiber of lentils

The amount of SDF in Laird lentils and Eston lentils was increased by 30 and 20% respectively, after tempering with distilled water and micronization treatment. Salt tempering solutions, especially the 150ppm disodium EDTA and the 2% sodium tripolyphosphate greatly increased the SDF,. The increased solubility of DF caused a softening of the tissue and the constituent cell walls of the seeds thus reducing their cooking time. This might explain the remarkable effects of the disodium EDTA and the sodium tripolyphosphate on the cooking texture of the micronized lentils. In addition, an increase in soluble dietary fiber could improve the nutritional quality of lentils. The IDF contents of the two type lentils were decreased correspondingly after micronization treatments. However the TDF contents were remained almost unchanged in the micronized lentils.

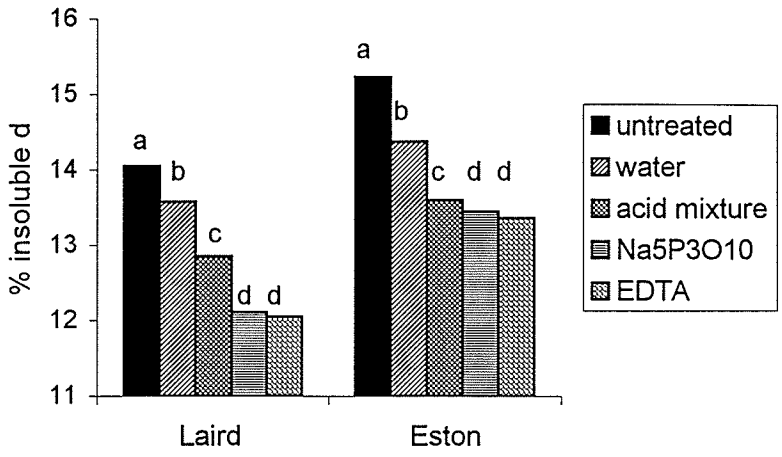


Fig. 4.11(b). Effect of tempering solutions on the insoluble dietary fiber of lentils

4.3.3.2. Antinutritional Factors

The reductions in the levels of the phytic acid and phenolic compounds in micronized lentils are shown in Figs.4.12 and 4.13 respectively. The samples were heated with the big micronizer. Results shown are the mean of determinations made in triplicate for the tempered and micronized product. With the treatment using the salt solutions, the contents of phytic acid and phenolic compounds were reduced by 10-40%. The reduction in phytic acid and phenolic compounds should increase the digestibility and utilization of the lentil proteins. The tempering solutions containing disodium EDTA showed remarkable effect on both antinutritional factors. Both phytic acid and the phenolic compounds are heat-stable components, therefore, a single heat treatment couldn't destroy them. Barampama and Simard (1994) reported that soaking or fermentation treatment could significantly reduce the amounts of phytic acid and phenolic compounds in dry beans, the reduction was probably due to the diffusion of those antinutritional

factors in water or the effects of some enzymes, such as the phytases. It was assumed that those enzymes were activated at high moisture content during tempering treatment.

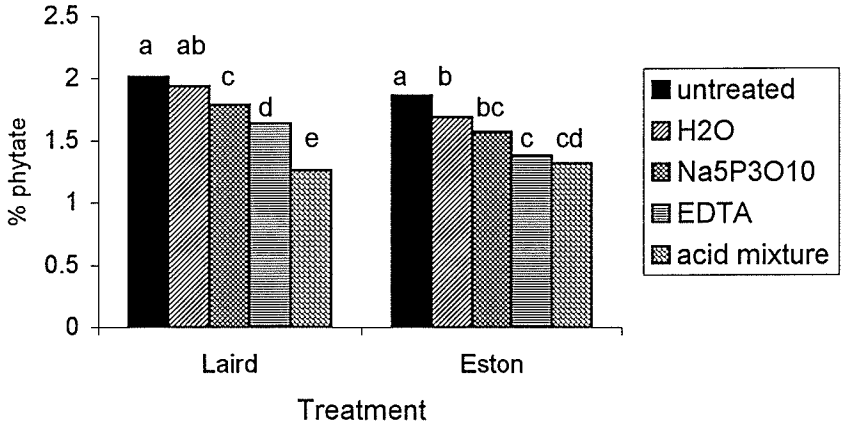


Fig. 4. 12. Phytate acid content in lentils subjected to various treatments

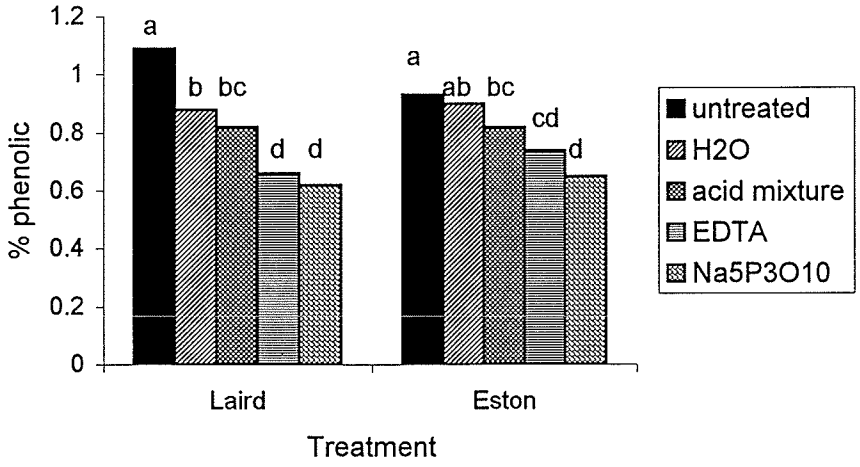


Fig. 4. 13. Polyphenolics content in lentils subjected to various treatments

The notable reduction of raffinose sugars has also been reported by Kosson and Bakowski (1986) for soaked and cooked beans, by Ibrahim and Antai (1986) for fermented beans, and by Nnanna and Phillips (1990) for germinated cowpeas. The decreases were caused by a loss of sugar to water or by diffusion or by enzymatic degradation in germinated or fermented beans. The raffinose sugars are water soluble and heat stable components, however, Nnanna and Phillips (1990) found that a single soaking treatment had little effect on the reduction of the raffinose sugars. In our study, a HPLC analysis procedure was applied to determine the changes of raffnose and stachyose in the micronized products. The results only showed a slight reduction of the raffinose sugars in lentils tempered with salt solutions (Fig. 4. 14). That might because the tempering condition used (25% tempering level, 12 hour tempering time and room temperature) did not provide high tempering and high water availability for those reactions to take place.

Lentils also contain very low amounts of other antinutritional factors, such as the hemagglutinins, the trypsin inhibitor, the cyanogenic glycosides and the α-amylase inhibitors. The infrared heat treatment might have some effects on those antinutrients, especially those heat sensitive factors.

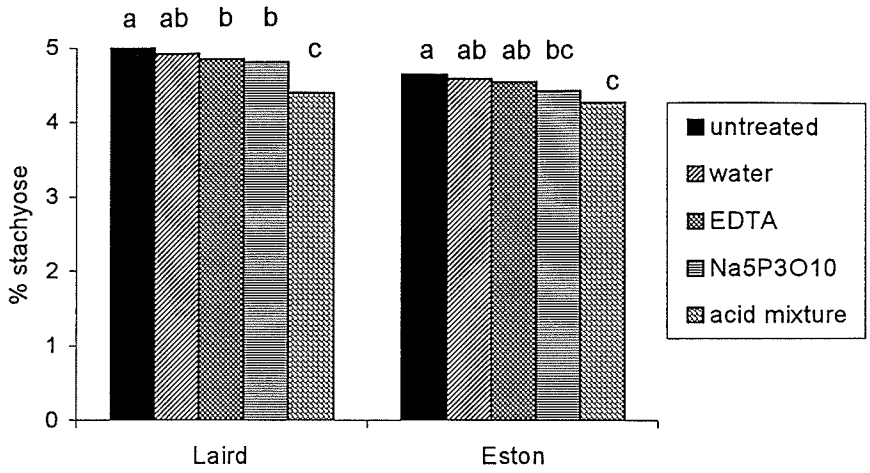


Fig. 4.14. Stachyose content in lentils subjected to various treatments

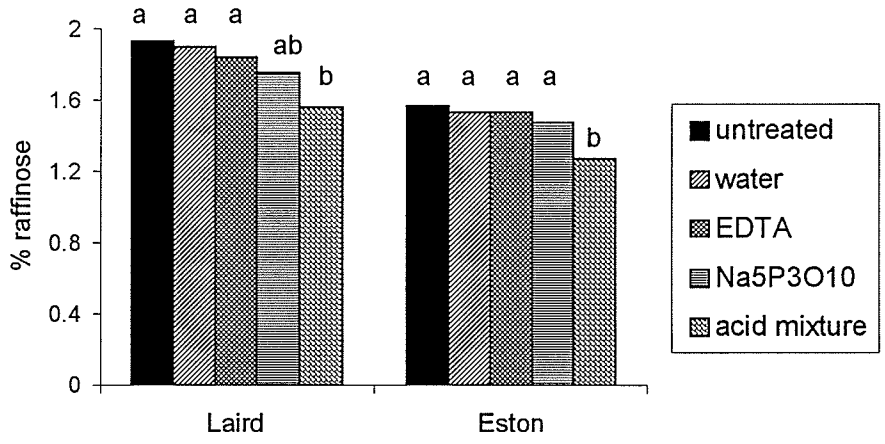


Fig. 4.15. Raffinose content in lentils subjected to various treatments

CHAPTER 5

CONCLUSIONS & RECOMMENDATIONS

With both infrared heat systems, the water tempering pretreatment could significantly affected the quality of the end products. High tempering levels increased the content of the gelatinized starch, decreased the protein solubility, and reduced the cooking time required to reach a compression force of 125N. However, high tempering level also caused high final moisture content and significant color changes in the products. Within test conditions, the optimum tempering level for the two types of lentil was around 25%. Tempering time affected the degree of starch gelatinization and cooking quality at high tempering levels but did not affect the final moisture content of the products. Therefore, the optimum range of tempering time is dependent on the tempering levels. The higher the tempering level, the longer the tempering time needed. At 25% tempering, 12h tempering time would be enough for the lentils. With tempering and micronization treatments, the cooking time of Canada No.2 Laird lentils could be reduced to about 16 min and the cooking time of Canada No.2 Eston lentils could be reduced to about 18 min.

Among the four tempering solutions tested, 150 ppm disodium EDTA and 2% sodium tripolyphosphate could be used to improve the cooking quality of the lentils without affecting the appearance of the products any more than the distilled water treatment. Two percent sodium tripolyphosphate had the greatest effect on the cooking quality of Eston lentils, producing the lowest force to compress at a set cooking time. The micronized seeds tempered with 150 ppm disodium EDTA and 2% tripolyphosphate had

a high content of gelatinized starch and low protein solubility. The 1% citric acid/2% ascorbic acid mixture also had significant effects on protein, starch and cooking time of the micronized lentils, however, the color of the seeds was noticeably darker and more brown when this tempering treatment was used.

The pretreatment and infrared processing caused a change in the relative amounts of the soluble and insoluble DF of the lentils. The SDF content was increased and this may have helped improve the texture of the cooked seeds. The 150 ppm disodium EDTA caused the greatest increase in the SDF content. It was also found that tempering and micronization could reduce the contents of phytate and phenolic compounds of lentils to a certain extent. The tempering solutions containing disodium EDTA or sodium tripolyphosphate were able to significantly reduce the contents of those two antinutritional factors compared to water and the mixture of 1%citric acid and 2% ascorbic acid. It seemed that the micronization treatment didn't affect the contents of the raffinose sugars.

With lower heating temperature and shorter processing time, the samples processed with the pilot scale micronizer had more stable quality and much less color change compared with the infrared lamp. The micronizer has high efficiency and it is very easy to operate. Results from the infrared lamp, however, gave similar responses in terms of micronization effects and thus provide a good indicator of the parameters required for the larger pilot-scale unit. The results of this study indicate that the infrared treatment has potential as an economical heat treatment for lentils. It will increase the marketability of end products and decrease the energy cost involved in subsequent cooking.

Recommendations for future research based on this study would be:

1. Improve methodology for measuring the temperature of the seeds during the process of micronization to get the end products with more consistent quality.
2. Determine the impact of micronization on the other nutritional properties of the lentils, such as the changes of the amino acids in lentils before and after micronization treatment.
3. Evaluate the sensory properties of the micronized lentils (e.g. to compare and characterize the micronized-cooked and unmicronized-cooked lentils) using a trained sensory panel, and relate the results of the sensory measurements to the results from instrumental and chemical tests.
4. Further investigate the relationship between the solubility of dietary fiber and the cooking quality of the micronized lentils.
5. To reduce the cost of tempering treatment, the minimum concentrations of the salts used in the tempering solutions could be established based on their regulated concentrations. Continuous study should be carried out to find the optimum combination of the salt concentration and their tempering levels, such as the disodium EDTA.
6. Examine the effects of micronization on some other food legumes, such as soybeans and dry beans.

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Appendix 1. Oven Drying Procedure for Moisture Determination

1. Pre-dry alumni dishes for 2 hours at 100°C.
2. Cool dishes in a desiccator for 10 min and weigh them on the analytical balance. Handle dishes with tongs.
3. Weigh approximately 2 g of ground sample into each dish.
4. Dry the samples at 100°C for 18 hours.
5. Cool samples in a desiccator for 30 min and weigh them.
6. Calculate %moisture = (g lost) x (100) / (g original sample weight)
7. Perform 3 replicates for each sample.

Appendix 2. Phytic Acid Determination

1. Sample extraction

   Weight 0.5 g of sample (in duplicate) into a 25 ml flask and place on a magnetic stirrer. While stirring, add 10 ml of 2.4% HCl and mix them for 1 hour. Centrifuge the sample at 10,000 rpm in 50 ml tubes for 10 min, dilute 3 ml of the supernatant to 25 ml.

2. Column chromatography

   Place a glass column (0.7cm x 27 cm) in a ring stand and pack with some glass wool and 0.5 g of 200-400 mesh AG1-X8 chloride anion exchange resin (Biorad). Prepare column with 15 ml of 5% HCl and rinse with 20 ml distilled water. Pipet 10 ml of sample onto column, then add 15 ml of 0.1 M NaCl. Once the 0.1 M NaCl passes through, place a 25 ml volumetric flask under the column and add 15 ml of 0.7 M NaCl to the column. The eluant is collected and diluted to 25 ml with distilled water.

3. Preparation of standards and Wade reagent

   Wade reagent – combine 0.09 g anhydrous $FeCl_3$ (0.15g $FeCl{\cdot}6H_2O$) and 1.5 g sulpyosalicylic acid in water and dilute to 500 ml.

   Phytic acid standards – Contain 5, 10, 20, 30 and 40 μg/ml. Store in fridge and bring to room temperature prior to use.

4. Color test

Pipet 3 ml of blank (water), standards and samples into 15 ml conical centrifuge tubes. Add exactly 1 ml of Wade reagent then cover with parafilm and mix on vortex. Centrifuge for 10 min and pour supernatant into a cuvet and read absorbance at 500nm using water to zero the colorimeter. The readings of samples and standards are subtracted from the blank reading to obtain the final reading. The content of phytic acid is calculated from the standard curve and adjusted with the moisture content of the sample.

Appendix 3. Phenolic Determination

1. Sample extraction

   Accurately weigh 100 mg ground sample and transfer to a 250 ml round bottom flask. Reflux with 12.5 ml of 80% ethanol at pH 4.0 for 30 min, the boiling point of ethanol is about 78°C. Cool down, and centrifuge the sample at 2,000 rpm for 5 min. Adjust the volume of supernatant to 25 ml with distilled water. Refulx 12.5 ml of 80% ethanol as the blank.

2. Standard preparation

   Accuratly weigh 0.1 g of tannic acid and disolve to 500 ml with distilled water, then prepare 5, 10, 15, 20 and 25 μg/ml solutions with the stock solution.

3. Phenolic compound extraction

   Take 0.5 ml of the samples, standards and blank into 10 ml graduated test tubes and diluted them to 7 ml with distilled water, respectively. Vortex the test tube for approximately 2-3 second, then add 0.5 ml Lowry reagent and vortex again. Let the tubes stand for 3 minutes, and add 1 ml of saturated sod. Carbonate. Dilute each of them to 10 ml with distilled water and stand for 1 hour at room temperature. Determine the max. wavelength of the sample solutions between 700-800 nm with Diode Array, then read absorbance at max. wavelength. Calculate the concentrate of phenolics from the standard curve and convert to the content of phenolics in the sample.

Appendix 4. Oligosaccharides Determination

1. Sample extraction

   The seeds are finely ground and the flour can pass a 0.5 mm screen. Weigh 0.5 g of sample into a roound bottom flask and add 10 ml 50% ethanol-water mixture (v/v) containing 1 mg/ml aradinose as internal standard. Reflux the suspension at 50°C for 1 hour and stir occasionally, cool down. Centrifuge the suspension at 2,000 rpm for 15 min and adjust the volume of the aliquots to 10 ml with distilled water.

2. Sample cleanup

   Filter the supernatant through a Sep-Pak C_{18} cartridge, the Sep-Pak C_{18} cartridge is prewetted with 1 ml of methanol, 2 ml of distilled water and 1 ml of the extraction solution. Centrifuge the filtrate, 2 ml of the filtrate is dried at 50°C with N_2, and finally redissolve the residue in 2 ml of distilled water. Filter the solution through a 0.45 μm filter to remove particulates.

3. Standard preparation

   The standard solution contains 1 mg sugar/ml water. Accurately weigh 50 mg of glucose, fructose, raffinose, sucrose stachyose, raffinose and arabinose, and dissolve them to 50 ml with distilled water. The 1 mg/ml arabinose is internal standard. The standard solutions go through the cleanup procedure if necessary.

4. Separation and quantification by HPLC

The column is a Aminex HPX-87N (300 mm x 7.8 mm) column. Apply 20 μl of the sample solution to the chromatography. The elution solvent is distilled water (air-removed), flow rate is 0.6 ml/min. The column temperature is 85°C. The content of stachyose and raffinose in the sample is calculated from the standard curve and adjusted with the internal standard.

Appendix 5. Data showing the degree of starch gelatinization of tempered and micronized lentils using infrared lamp

Tempering		Starch gelatinization(%)[a]	
Solution	level (%), time (h)	Laird	Eston
Untreated seed		*9.0 ± 2.0*	*11.4 ± 1.5*
Distilled water	20, 12	28.8 ± 2.4	30.9 ± 3.2
	20, 24	30.0 ± 5.7	31.4 ± 2.9
	30, 12	44.2 ± 3.5	50.9 ± 2.8
	30, 24	50.6 ± 2.1	55.9 ± 3.2
	40, 12	61.7 ± 0.9	63.3 ± 3.2
	40, 24	69.8 ± 3.0	70.3 ± 1.5
150 ppm disodium	20, 12	33.7 ± 2.8	35.2 ± 2.4
EDTA	30, 12	51.6 ± 0.9	56.8 ± 1.5
2% sodium	20, 12	30.7 ± 3.1	32.9 ± 3.9
tripolyphosphate	30, 12	48.5 ± 0.6	53.9 ± 0.6
1% citric /	20, 12	35.4 ± 3.5	38.9 ± 0.0
2% ascorbic acid	30, 12	55.2 ± 2.3	59.9 ± 0.5

[a]mean value ± std.

Appendix 6. Data for the degree of protein solubility of tempered and micronized lentils with the infrared lamp

Tempering		Protein solubility (%)[a]	
Solution	level (%), time (h)	Laird	Eston
Untreated seed		*60.9 ± 0.9*	*54.6 ± 1.2*
Distilled water	20, 12	46.9 ± 1.4	35.6 ± 1.3
	20, 24	46.3 ± 1.4	36.6 ± 1.0
	30, 12	42.6 ± 1.3	30.5 ±0.8
	30, 24	40.1 ± 1.3	29.7 ±1.0
	40, 12	36.4 ± 0.6	25.6 ± 0.9
	40, 24	34.8 ± 0.7	23.8 ±0.8
150 ppm disodium	20, 12	48.0 ± 1.7	39.4 ± 1.5
EDTA	30, 12	43.9 ± 0.8	34.0 ± 1.5
2% sodium	20, 12	47.4 ± 0.6	36.3 ± 1.5
tripolyphosphate	30, 12	43.6 ± 1.3	28.9 ± 0.9
1% citric /	20, 12	44.9 ± 1.9	31.7 ± 1.5
2% ascorbic acid	30, 12	40.7 ± 1.0	27.9 ± 2.3

[a]mean value ± std.

Appendix 7. Hunterlab color values[a] for tempered and micronized lentils with infrared lamp

Tempering		Laird			Eston		
Solution	Level (%), time (h)	L	a	b	L	a	b
distilled water	20, 12	43.7±0.2	-2.3±0.2	10.1±0.1	32.3±0.1	-1.1±0.1	6.2±0.1
	20, 24	44.5±0.1	-2.6±0.2	10.3±0.2	32.5±0.2	-0.8±0.1	5.6±0.4
	30, 12	43.2±0.1	-1.7±0.1	8.7±0.1	32.1±0.1	-0.6±0.1	5.2±0.3
	30, 24	43.2±0.1	-2.1±0.1	8.5±0.1	31.9±0.2	-0.7±0.1	5.1±0.1
	40, 12	42.8±0.3	-0.7±0.4	7.9±0.2	31.5±0.2	-0.3±0.2	4.9±0.1
	40, 24	42.8±0.1	-1.1±0.4	8.0±0.2	31.2±0.1	-0.3±0.2	4.8±0.1
150 ppm	20, 12	47.4±0.7	-2.6±0.1	11.4±0.1	32±0.1	-0.9±0.1	6.8±0.1
EDTA	30, 12	47.9±0.1	-1.9±0.1	10.7±0.2	31.7±0.2	-0.5±0.1	6.1±0.1
2% sodium	20, 12	43.9±0.3	-1.8±0.1	10±0.1	30.3±0.2	-0.7±0.1	6.0±0.2
tripoly-	30, 12	43.1±0.2	-1.6±0.2	9.7±0.1	29.1±0.2	-0.4±0.2	5.8±0.1
phosphate							
1%citric/	20, 12	40.4±0.3	0.03±0.03	12.1±0.2	27.8±0.2	0.08±0.03	8.4±0.06
2%ascorbic	30, 12	40.2±0.1	0.1±0.0	13±0.1	26.7±0.2	0.1±0.05	9.7±0.06
acid							

[a]mean value ± std.

Appendix 8. Data[a] of the 13 treatment combinations for Laird and Eston lentils using pilot scale micronizer

No.	Tempering (level, time)	Starch gelatization (%)		Protein solubility (%)		Max. load (N)	
		Laird	Eston	Laird	Eston	Laird	Eston
1	20, 6	16.7±3.5	16.1±1.4	51.1±2.1	44.5±0.2	123.4±7.1	128.75±14.62
2	30, 6	21.4±1.3	22.8±2.1	48.4±0.9	39.3±0.3	96.4±13.3	101.7±3.8
3	20, 18	17.1±2.5	22.2±0.6	51.9±0.1	43.2±0.1	113.8±7.5	126.9±12.3
4	30, 18	30.1±2.1	35.9±1.3	45.8±2.3	38.0±0.4	84.9±10.2	89.7±8.7
5	25, 3.5	17.0±2.9	20.6±2.2	54.2±0.3	40.6±0.6	98.1±6.3	120.6±2.3
6	25, 20.5	23.5±0.5	24.8±0.1	50.3±0.5	39.1±0.1	89.9±6.3	110.6±7.9
7	25, 12	27.5±2.6	24.8±1.1	48.3±0.9	39.8±0.0	80.5±13.2	115.8±3.1
8	25, 12	22.9±4.8	24.8±2.5	48.7±1.5	40.4±0.6	84.7±7.5	113.4±9.9
9	17.93, 12	18.8±1.7	16.5±0.8	51.2±2.7	45.9±0.0	123.9±9.2	131.0±18.3
10	32.07, 12	31.9±0.5	35.9±4.4	43.5±2.1	36.5±0.2	83.2±5.5	90.2±13.7
11	25, 12	27.3±2.5	28.8±0.9	49.5±2.3	39.7±0.3	86.3±13.4	108.3±3.2
12	25, 12	22.8±0.9	25.5±1.2	46.7±3.3	40.1±0.0	89.8±6.0	111.8±5.7
13	25, 12	23.8±0.5	25.1±0.9	45.7±1.4	39.9±0.1	88.5±8.9	110.7±3.7

[a] mean ± std.

Appendix 9. The ANOVA table for the response surface tests with the pilot scale micronizer*

Response 1: Final moisture content

	Temp. Factor	DF	Mean Square	F Value	Prob > F
Laird	level	1	73.68	188.66	<0.0001
	time	1	0.022	0.057	0.8185
Eston	level	1	33.71	36.99	0.0005
	time	1	0.001785	0.001958	0.9659

Response 2: Degree of starch gelatinization

	Temp. Factor	DF	Mean Square	F Value	Prob > F
Laird	level	1	164.04	43.22	0.0003
	time	1	41.83	11.02	0.0128
Eston	level	1	286.03	40.40	<0.0001
	time	1	79.00	11.16	0.0075

Response 3: Protein solubility

	Temp. Factor	DF	Mean Square	F Value	Prob > F
Laird	level	1	48.46	27.49	0.0012
	time	1	6.69	3.79	0.0924
Eston	level	1	70.17	249.77	<0.0001
	time	1	2.79	9.92	0.0118

Response 4: Color-L

	Temp. Factor	DF	Mean Square	F Value	Prob > F
Laird	level	1	9.44	24.04	0.0017
	time	1	0.87	2.21	0.1804
Eston	level	1	4.84	211.42	<0.0001
	time	1	0.000039	0.000257	0.9875

Response 5: Color-a

	Temp. Factor	DF	Mean Square	F Value	Prob > F
Laird	level	1	5.02	69.16	<0.0001
	time	1	0.024	0.33	0.5844
Eston	level	1	3.52	74.64	<0.0001
	time	1	0.076	0.65	0.4372

Response 6: Color-b

	Temp. Factor	DF	Mean Square	F Value	Prob > F
Laird	level	1	2.22	55.17	0.0001
	time	1	0.000698	0.017	0.8989
Eston	level	1	0.95	41.38	<0.0001
	time	1	0.017	0.70	0.4208

Response 7: Cooking ability-Max. load

	Temp. Factor	DF	Mean Square	F Value	Prob > F
Laird	level	1	1609.10	84.82	<0.0001
	time	1	133.63	7.04	0.0327
Eston	level	1	1858.97	201.84	<0.0001
	time	1	97.94	10.63	0.0086

*Values of "Prob>F" less than 0.05 indicate factors are signifcant.