School of Public Health

Cooking Quality: Physical and Biochemical properties of Lentils (*Lens culinaris*)

By

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Abstract

Lentils, one of the cool-season pulses, are consumed as a staple food in most developing countries. The demand for pulses in western societies is increasing because of its valuable nutritional quality and an increased awareness of health issues. Australia has a good reputation for producing clean low moisture legume products and could increase its market share of lentil production by identifying, developing and promoting good quality varieties.

Lentils which are graded as good quality varieties must have a short and uniform cooking time, without 'hard to cook' seed, have the hull stay attached to the seed during cooking, and have a final acceptable taste, texture, flavour and appearance after cooking (Bhatty 1990). Cooking quality in this study is defined as the maximum force (N) that is required to compress the whole seed cooked product after cooking for a standard period of time. This study aims to develop an objective measurement to determine the cooking quality of lentils and thereby evaluates the relationships between lentil cooking quality and some of its physical and biochemical properties. Four cultivars used (Cassab, Digger, ILL 7180 and Matilda) were grown during 1999 at Mullewa and Pingaring, Western Australia. The relationship between the cooking quality of lentil and water absorption, seed size, seed coat thickness, phytic acid, mineral composition and initial moisture content was investigated.

Texture measurement was carried out using the TA.XT2i meter as an alternative to the subjective method *"Cooking time test"*. By comparing the cooking time determined by *'Cooking time test'*, 220 N was established and suggested as an optimal peak compression force to determine the adequate cooking time for lentils. Both methods showed that 35 minutes cooking time was adequate for red lentils (Cassab, Digger, and ILL 7180), and 45 minutes for green lentils (Matilda).

Cooking significantly reduced the hardness of the seeds ($R = -0.752$ to -0.89) and promoted mineral leaching ($P < 0.05$). The interaction between environment and genotype had a significant effect on seed size, seed coat thickness, mineral composition (Phytic acid, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Fe^{2+} and Cu^{2+}) and hardness ($P < 0.05$). Seed coat thickness did not correlated with the rate of water uptake and cooking quality. Growing environments had a greater influence on the cooking quality than genotypes. Lentils grown at Pingaring are generally had a higher in Phytic acid content, better mineral retention and were harder in texture than those grown at Mullewa.

The results of this study implicated that the peak compression force (220 N) was identified as an indicator to determine the cooking time of lentil cultivars. Texture Profile Analysis (TPA) is a useful method to evaluate various texture characteristics (hardness, cohesiveness, chewiness, springiness, gumminess and adhesiveness) of lentil cultivars. Cooking quality of lentil is significantly affected by the effect of varieties and growing locations. However, not the various biochemical compositions (phytic acid and minerals) and the thickness of seed coat have no significant effect on the cooking quality of lentil.

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Chapter I
Introduction

1.1 *Introduction*

Lentils (*Lens culinaris)* are a species of cool-season pulses/grain legumes which originated from southwest Asia in about 7,000 BC. Lentils are best adapted to either the cooler temperate zones of the world or the winter season in Mediterranean climates (Dowsley et al. 1996). They require moderate rainfall but a sustained dry period for seed ripening. The rate of seed deterioration depends on the moisture content and temperature after reaching peak maturity on the mother plant (Roberts 1972).

Lentils are a good source of nutrients, fibre, complex carbohydrates, iron and folic acid and are an excellent protein food for developing countries. Lentils are also low in calories, fat and free of cholesterol. Comparing FAO reference standard, lentil proteins are deficient in sulfur amino acids, but are rich in lysine contents, which is relatively low in cereal grain proteins. A diet blending cereal with legumes provides a higher nutritional value than cereal or legumes alone (Adsule, Kadam and Leung 1989). Lentils contain insignificant levels of antinutritional factors, are low in flatulence-causing sucrose α–glactosides and produce low postprandial (after meal) glycaemic response in normal and diabetic volunteers (Bhatty, 1988). A diet containing lentils may thus help in dietary management and control of diabetes (Bhatty, 1988).

Owing to increased awareness of the factors of a healthy diet in western countries and the population growth in developing countries such as India, Pakistan and Bangladesh, there is an increased demand for lentils. The adoption of low fat diets and a move towards vegetarianism in Western Countries has increased the consumption of processed pulses, especially in the United States, European Union, Canada and Australia. These trends create an enormous demand for lentils and the long term outlook for this market in the world is promising.

Presently, India is the largest lentil producer and consumer, followed by Turkey. America and Canada are the major exporter of green lentils. In Australia, the area of lentil production has increased from < 1000 ha in 1993 to about 117 000 ha in 2000. Victoria ranks first in lentil production (80 000 ha), followed by South Australia (30 000 ha) and Western Australia (5 000 ha). The dramatic growth of lentil production is attributed by the commitment of the industries to develop good quality of pulses to meet the market demands and to improve varieties to suit Australia growing conditions. Currently, there is a new red lentil variety (Cassab) produces good yields and superior seed quality and shows good adaptation to the growing environment in Western Australia.

In 2000, the indicator price given to lentils was as high as $560/t for a relatively low cost of production. The average yields of 1.37t/ha have been profitable. However, lentils were a minor crop in Australia until recently. It received little attention from breeders and agronomists because it requires special cultivation conditions and little was known about its agronomy. Appropriate management can ensure a profitable lentil production yield.

Currently, there is an increased demand for good quality pulses in Southeast Asia, Indian subcontinent and the North Asia due to the high economic growth rates. In the short term production is unlikely to meet their increasing demand (Siddique 1993). Australia has an excellent reputation for producing clean and low moisture product (Community Farmer Federation 1994)[a] and is well placed to capture this opportunity to increase market share in the world. There is an immediate need to identify, develop and promote good quality lentils varieties, which are able to cater for the needs of export markets.

Several studies have been carried out which examined aspects of lentils and human consumption. These have provided information concerning physical and chemical characteristic of lentils and the effect of growing location and food processing such as milling, cooking, germination, canning, on the nutritional bioavailability of lentils. Numerous studies have examined storage conditions on the effect of seed hardening and poor cooking problems, which led to the identification of a "Hard-to-Cook" phenomenon.

Poor cooking quality has been related to the thickness of seed coat which acts as a protective barrier for the embryo and other seed components against adverse environmental conditions such as fluctuation in humidity and temperature: the harder the seed coat, the better the protection; the longer the cooking times of seeds and poorer the cooking quality (Mohamed-Yasseen et al. 1994).

Seed hardness has been correlated with the content of the chelating agent phytic acid. Phytate chelates calcium and magnesium ions and prevents formation of calcium and magnesium cross-linkages between the pectate molecules of the middle lamella tissue. The degree of solubility of the intercellular material of seeds is positively correlated with phytic acid content in seeds (Bhatty 1995). The present challenge to researchers is to identify and evaluate the cooking quality of various lentil cultivar with a reliable test and it will be the focus for this research.

1.2 Statement of the Problem

The production of lentils in Australia is mainly for export purposes. The developments of objective screening and quality evaluation methods are extremely important to evaluate the quality of lentil varieties developed in Australia compared to the commercialised world products. As lentils are mainly used for domestic consumption, the main concern for the lentil market is to evaluate the processing quality and nutritional value.

At present the most important lentil quality parameters as foods are seed hardness, lysine and methionine, protein content, and protein digestibility (Williams 1988). A short and uniform cooking time, no 'hard to cook' seed, the ability of the hull to stay attached to the seed during cooking, and a final acceptable taste, texture, flavour and appearance after cooking are also important parameters to rank the quality of lentil varieties (Bhatty 1990).

Good cooking quality has allowed Australian lentil products to be widely accepted into the world market. An objective screening and quality evaluation method is an important stepping stone to allow the quality of newly developed Australian lentil varieties to be evaluated. However, there are limited studies, which have evaluated the quality of

Australian-cultivated lentil varieties, especially the cooking quality. Also, no objective measurements have been developed to determine the cooking quality of lentils.

The main focus of this study is to evaluate the possibility of using texture analyser (TA-XT2i) to determine the cooking quality of lentils, thereby investigate the possible reasons contributed to the variability. Therefore, how various lentil varieties are consumed commercially is not relevant to this research. In this study, both green and red lentil varieties available to the researchers are treated under the same experimental conditions to determine the variability of cooking quality and the possible reasons contributed to the differences.

1.3 Definition of Terms

1. "Cooking time" is defined as the time taken between starting to cook the seeds until the time the seeds are ready for consumption.

2. "Seeds" are defined as "cooked" after the process of cooking, the process of starch gelatinisation, the breakdown of protein and the reduction of cell wall tissue are completed, and when seeds are soft enough to masticate without having to chew.

3. "Cooking quality of lentils" in this study is referred to as peak compression force (N), which involves the gelatinisation of starch and the break down of protein after a fixed time of cooking.

4. "Water absorption" is defined as the mean percentage increase in the weight of seeds, which considers the loss of weight due to solid loss during soaking, as a result of water imbibition.

1.4 Research Hypotheses

Derived from the earlier discussion, the following hypotheses are investigated in this study.

1. It is possible to use the TA-XT2i texture analyser to evaluate the cooking quality of lentils and to replace the old subjective method "The cooking time test".
2. There is no variation between cultivars of lentils in terms of cooking quality.
3. The chelating reaction of phytic acid with divalent ions affects the cooking quality of lentils.
4. Mineral retention in the lentil seeds after cooking has an effect on the cooking quality of lentils.
5. Cooking quality is related to the initial moisture content and water absorption of the lentil seeds.
6. Water absorption of lentil seeds is related to thickness of the seed coat.

1.5 Objectives

This research is designed to investigate the relationship between lentil cooking quality with some physical and biochemical properties of lentils. The procedures to test the investigations are as follows:

- To develop an objective measurement to determine the cooking quality of lentils;
- To develop an objective method to determine the cooking time of lentils;
- To investigate the interaction effect of environment and genotype on the variations of lentil cooking quality among cultivars
- To investigate the relationship between cooking quality and water absorption of seed
- To evaluate the effect of seed coat thickness on water absorption of seed
- To evaluate the relationship between cooking quality and the content of phytic acid and minerals.

1.6 Samples

The samples of lentil varieties Cassab, Digger, ILL 7180 and Matilda were from research trials, which aimed to identify the superior lentil genotypes in Western Australia. Cassab and Digger are commercial varieties of red lentil. Matilda is a commercial variety of green lentil. ILL 7180 is a newly developed red lentil variety which is still undergoing trial testing and will be released in Victoria by 2001. Samples came from Pingaring and Mullewa in Western Australia.

The trial sites from which the samples were collected had identical soil treatment, trial size, seeding rate and fertilizers, but different growing environments. In 1999, Pingaring was reported as comparatively drier than Mullewa (Regan *et al.* 2000). This difference provided important information concerning the influences of genotype, environment and their interaction on the cooking quality characteristics of lentil cultivars.

Commercially, lentil is either utilised as whole seed, splits (de-hulled seeds) or ground into flour. Whole seeds are normally consumed along with cereals. Dehusked seeds/splits are consumed as a vegetable. Lentil flours blends with wheat flour and is used in baked products. Unlike other pulses such as faba beans, peas and chickpeas, lentil consumption after a soaking process is not a common practice. Lentils are cooked directly. Therefore, there is no pre-treatment required before conducting a cooking quality test.

In this study, samples of each cultivar were used as a whole and unbroken seed free of foreign matter. Non-soaked samples were tested with different physical and biochemical properties analysis after a standard set period of cooking time. The interrelationship and the significance of testing between physical and biochemical properties on the cooking quality of lentils were determined with the assistance of statistical analysis.

1.7 *Benefits of the Study*

The results of this study will provide immediate benefits to breeders, farmers, food technologists and especially benefits to the Australian export industry. The beneficial details are described as follows:

Cooking quality is one of the most important quality parameters of lentils as food. Consumers are seeking to purchase good quality lentil varieties not only with superior physical characteristics (seed size, uniformity of size, colour and loss on decortication) but also with good cooking qualities (short and uniform cooking time, acceptable taste, texture, flavour and appearance after cooking). Production of good cooking quality varieties allowed farmers to sell their product for "premium" prices. Farmers can be assured of a better profit with similar or even lower costs of production. In addition, release of a variety of lentils with good cooking quality allows for the expansion of the Australian export industry and an upgrading of the reputation of Australian cultivated lentil varieties to compete with the world market standard.

In order to capture the world market, breeders are required to release new lentil varieties with good cooking quality to farmers for cultivation. Owing to the inadequate studies on the evaluation of Australian cultivated lentil varieties, breeders have limited knowledge on the quality of their newly cultivated varieties. In the past, subjective sensory testing was widely used for cooking quality evaluation. However, this involved enormous financial expenditure on the recruitment and training of expert panelists. The development of an objective standard of measurement for the evaluation of cooking quality reduces the cost of production of lentils. This will provide immediate benefits not only to the breeders and farmers but also to the traders and consumers.

In addition, as this research tests the cooking quality of four cultivars (Cassab, Digger, ILL 7180 and Matilda) at two locations, it will provide important information concerning the effect of genotype, environment and their interactions on the cooking quality of lentils. A developed objective "cooking quality" measurement provides a quick and effective tool to allow breeders to evaluate the quality of their newly developed varieties during the early

stages of their breeding programmes. Consequently, good cooking quality lentil varieties with high yield can be released to farmers which in turn will benefit the whole pulse agribusiness.

The evaluation of the potential effect of biochemical compositions and permeability of seed coat on the variation of cooking quality among genotypes and between environments benefits both breeders and food technologists. This allows breeders to identify the impact of potential differences in genotype and cultivated environment on the nutrient uptake among cultivars. This allows food technologists to identify the potential physical and biochemical factors that may alter the functional properties of lentil cultivars as added value food products.

1.8 Limitations of the study

1.8.1 Samples

- Sample handling after harvest might significantly affect the physical and biochemical properties of samples. For example, hot and humid storage conditions leads to the hardening of seeds or even to the "Hard-to-cook" phenomenon (HTP) defect and to the poorer cookability of samples (Liu 1997 &Stanley and Anguilera 1985). However, this is not under the control of the researcher and divergent research outcomes may result.

- Lentils cultivated in Australia are of all good cooking varieties. The unavailability of comparatively good, medium and poor cooking lentil varieties limited the variations of cooking quality that could be demonstrated and amplified the significance of the research outcome.

- The only samples available to the researcher were four lentil varieties each grown at cultivated locations. This limitation restricted the interpretation of the interaction effect of genotype and environment on the cooking quality of

lentils. A minimum of five varieties grown at five sites is generally regarded as the minimum requirement.

1.8.2 Time constraints:

- There are many potential factors that may vary the cooking quality of lentils. For example the variation of chemical composition after prolonged storage, different storage conditions and cooking conditions. In this research, only some of the physical and biochemical factors (thickness of seed coat, seed size, water absorption, phytic acid content, initial mineral content and moisture content) that are identified as critical factors that may affect the cooking quality of lentils, were evaluated.

- The evaluation of the force-time curve allowed the extraction of seven texture parameters (hardness, cohesiveness, adhesiveness, springiness, gumminess, fracturability and chewiness). Sensory evaluation was not conducted and the researcher did not evaluate potential use of the predictive value of sensory attributes.

The process of recruiting, screening and training for a group of expert panels to evaluate the various sensory attributes requires a prolonged period of time and great financial support from the industry. Due to the limitations of time and financial support to the researcher, this study did not examine or provide information on relationships between personal preferences and the degree of hardness, cohesiveness, adhesiveness, springiness, gumminess, fracturability and chewiness. However, this could be very important to test and evaluate the effectiveness and potential uses of objective instrumental measurements by correlating the results with various sensory attributes.

- Due to the limitations of expert knowledge and time constraints, researchers have not yet explored the uses of complex principle component analysis to examine the important relationships that may exist between the seven texture parameters (hardness, cohesiveness, adhesiveness, springiness, gumminess, fracturability and chewiness) and cooking time and between the various minerals retention after cooking, and cooking quality.

1.8.3 Equipment constraint

- It was not possible to determine the hardness of the uncooked samples or even partially cooked samples due to constraints of the Texture analyser (TA-XT2i). The change of cooking hardness could only be evaluated for 25 minutes and longer time cooked samples. It might be possible to determine the hardness with a stronger load cell that is able to compress at a higher force, for example, 50 kg load cell. This equipment was not available to the researcher.

- It is not possible to evaluate the seed size density due to the constraints of equipment availability. Seed size density can be evaluated using an IBAS Image Analysis System or equivalent system. It can also be evaluated with a set of screen, for example 3, 4, 5, 6 mm. However, this equipment was not available to the researcher. So, seed size in this study could only be determined by the mean weight (g) of 100 seeds.

Chapter II
Literature Review

2.1 *Pulse: Origin, Production and Distribution*

'Legume' is derived from a Latin word *Legumen*, meaning seeds harvested in pods, and includes peas, bean and lentils (Coyle 1982 & Kadam and Salunkhe 1989). Legumes are cultivated in all the agricultural regions of the world and used mainly for both human and animal feeding, but they also have other uses, such as timber and in the preparation of pharmaceuticals (Borget 1992).

According to the Food and Agriculture Organisation (FAO), the word 'legume' is used for all leguminous plants. Many leguminous crops are referred to as 'pulse', an alternative term for edible seeds of leguminous plants. 'Pulse' is derived from the Latin *puls*, meaning a vegetable boiled to make a thick soup or porridge (Salunkhe & Kadam 1989). The word 'pulse' is used for seeds containing a small amount of fat, such as lentils, field peas, faba beans, chickpeas and lupins (Salunkhe & Kadam 1989). The term "leguminous oilseed" is used for seeds containing a high proportion of fat, such as soybean and peanuts (Salunkhe & Kadam 1989). Pulses are primarily used for human consumption (Siddique, Loss and Pritchard 1995). The botanical classification of food legumes places lentils in the family of leguminosae and subfamily of papillionoideae (Figure 2.1).

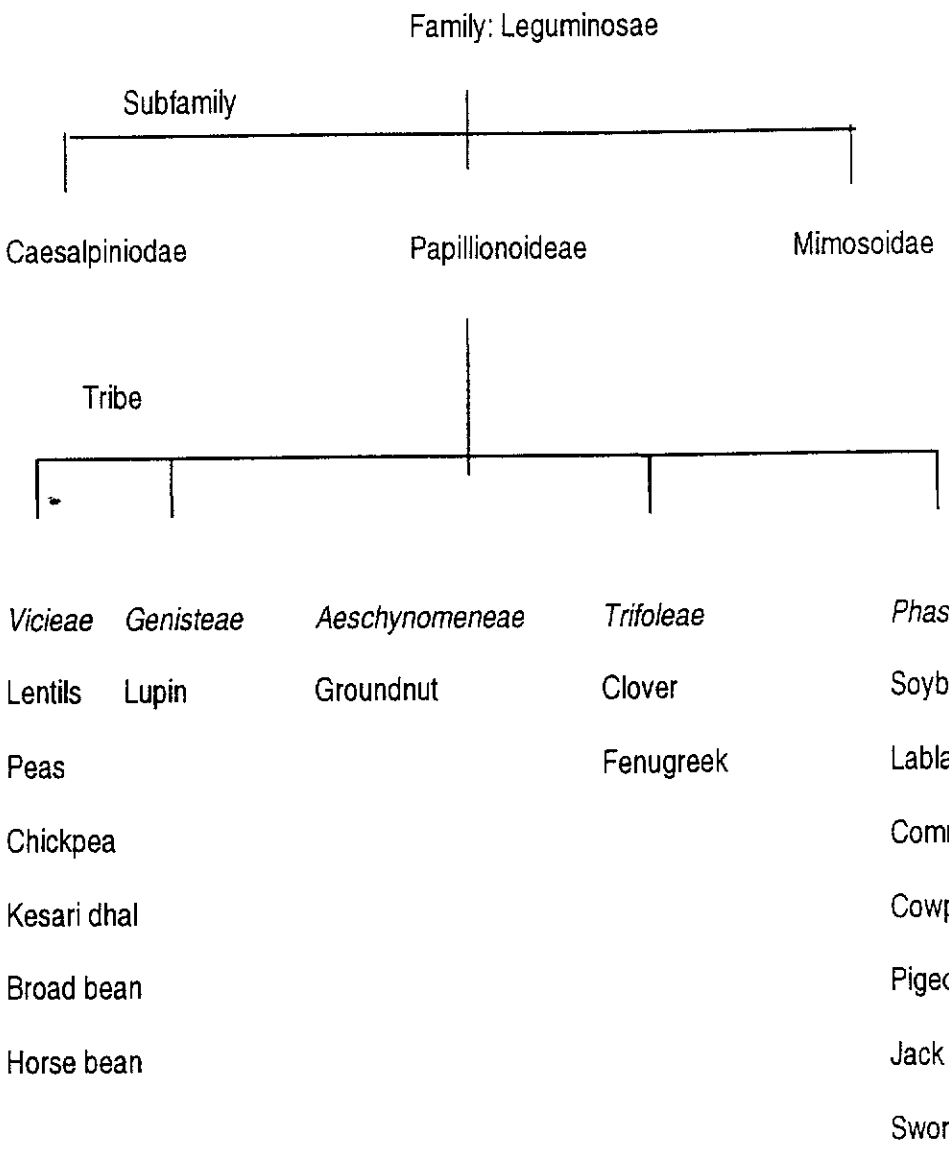


Figure 2.1 Botanical Classification of Food Legumes
(Source: Sathe 1996, p. 15)

Oilseeds (soybean and groundnuts) and pulses (dry beans, peas, broad beans, chickpeas and lentils) are the major food legumes grown in all continents of the world. As food legumes play an important role in agricultural systems and human nutrition. India is the country with the highest production of pulses, however, it is also the greatest importer of many pulses such as chickpeas, lentils and field peas (Siddique, Loss and Pritchard 1995). Additional demand for pulses is more likely to be increased as the population of India continues to rise towards being the largest by the year 2000. Based on the present population growth in the Middle East continent, Siddique, Loss and Pritchard (1995) estimated that the demand for additional pulses would increase from three to six million tonnes per year by year 2000.

2.1.1 Pulses in Australia

The major pulse crops grown in Australia can be divided into cool- and warm-season species. Cool-season species dominate pulse areas and production in Australia (Siddique and Sykes 1997). Lentils are one of the winter-growing legumes (Table 2.1).

Table 2.1: Current cool- and warm-season pulse crop species of commercial significance in Australia

Cool Season	Warm Season
Narrow-leafed lupin	Mung bean
Field Pea	Navy - culinary bean
Chickpea - desi and kabuli	Adzuki bean
Faba bean	Cowpea
Lentil - red and green	Pigeon pea
Albus lupin	Lablab
Vetch	Lima bean

(Source: Siddique and Sykes 1997, p. 104)

In Australia, the adoption of pulses in farming systems appears to be the most significant recent aspect of field crop production (Siddique and Sykes 1997). Traditionally, Australian farming practices are based mainly on a "ley" farming system. It consists of rotating production of several years of cereal crops with a

pulse crop, which is utilised by grazing livestock. However, during the 1970s and 1980s, there was an emphasis on changing the traditional system to a more intensive and yet sustainable system by including pulses in cropping rotations. The pulse-production in Australia increased rapidly and it has grown from about 2.5 X 10^2 t/year in 1980 to around 2 X 10^6 t/year today. Currently, Australian pulse crops are approximately worth A$400 million at the farm gate (Siddique and Sykes 1997). Seventy percent of the crop is exported. The trends in Australian pulse production in recent years are shown in Figure 2.2.

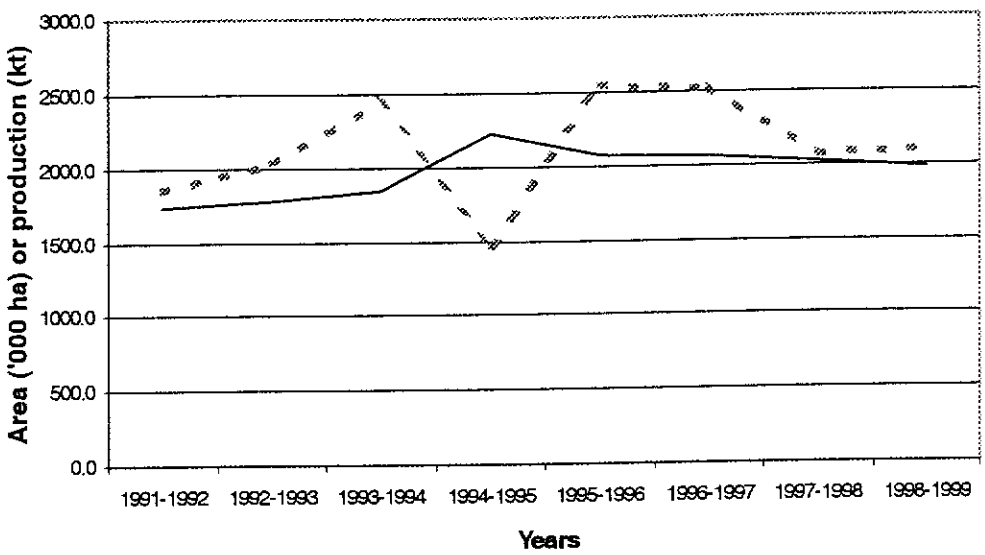


Figure 2.2: Trends in the total area (——) and production (" " " " ") of pulses in Australia.
(Source: Australian Bureau of Agricultural & Resource Economics 1999)

The financial and rotational benefit includes increased soil nitrogen, which is an important nutrient for plant growth; nitrogen is a limited nutrient in Australian soil and nitrogenous fertilisers are expensive. In addition, reduced disease incidences are noted when pulse crops are included in the production systems (Siddique and Sykes 1997). It increases the yield and protein content of the subsequent cereal crop and provides a useful summer fodder which can be obtained from the residual crop stubble and seed (Petterson, Sipsas and Mackintosh 1997).

The production of pulses in Australia has increased about 30-fold in the past 20 years (Petterson, Sipsas and Mackintosh 1997). There are several factors contributing to the increased production;

1. the need to improve the rotation systems by adding inexpensive nitrogen to the soil;
2. the need to allow crops suitable for acid, sandy and gravel soils which is met by development of varieties of lupins;
3. the need to increase the variety of legume crops to suit intensive rotation practices in Australia's wide range of environments
4. response to an increased import of pulses for the animal feeding industry in the European Union and also because they encourage pulses for domestic consumption;
5. an increased wheat and oilseed production which has pushed the farming of pulse crops in India down, allowing increasing imports of Australian field peas and chickpeas, and
6. the need to support long-term research and development and extension programs in crop breeding and agronomy.

(Source: Petterson, Sipsas and Mackintosh 1997)

2.2 Lentils Production

2.2.1 Classification of Lentils

The cultivated lentil is classified botanically as *Lens culinaris.* It is one of approximately four species of *Lens*, members of the pea family Leguminosae (Everelt 1981). The genus *Lens* is composed of four species, namely: *L. culinaris L. odemensis, L. nigricans* and *L. ervoides.*

Lens culinaris is further divided into two subspecies, the cultivated lentil ssp. *culinaris* and its wild relative ssp. *orientalis.* Lentils are part of the Tribe Vicieacae which also comprise *Pisum L.* (peas), *Lathyrus L.* (grasspea) and *Vicia L.* (beans) (Figure 2.1) (Dowsley, Carter, and Materne 1996).

2.2.2 Origin and Distribution of Lentil

Cultivated lentil originates from the Near East and Mediterranean region and was known to ancient Egypt and Greece, where it is still cultivated (Dowsley, Carter, and Materne 1996 & Duke 1981). Soon afterward, lentils spread northward into Europe as far as the British Isles, east to India and much of China and south to Ethiopia. Carbonised remains of cultivated lentils date back to 7000-6000 B.C. in early Neolithic settlements (Dowsley, Carter, and Materne 1996). It is now introduced and cultivated in most subtropical and warm temperate regions of the world, and high altitudes of the tropics, as well as in Chile and Argentina (Duke 1981).

L. *Culinaris* ssp. *orientalis*, which closely resembles the cultivated species, is believed to be the progenitor species (Dowsley, Carter, and Materne 1996). In the wild, ssp. *orientalis* is found throughout the Near East as far as Afghanistan in rocky and stony habitats with very little soils (Dowsley, Carter, and Materne 1996).

2.2.3 World Lentil Production

Lentil is an important food legume in the developing countries and it ranks about fifth in the world production of pulses (Williams, Erskine and Singh 1993). It is an important crop in Turkey, India, Canada, Bangladesh, Syria, Nepal, USA, Iran, Spain, Ethiopia, and Pakistan, in order of production levels (Table 2.2).

Asian countries produce up to 75% of the total lentil production in the world. India is the largest producer and consumer of lentils, with most produced lentils being used domestically. Turkey is the second largest producer of lentils and the largest exporter of red lentils. America is the third largest producer of lentils followed by Canada which is the major exporter of green lentils. In countries such as Bangladesh, Iran and Ethiopia, all lentils produced are consumed domestically (Dowsley, Carter, and Materne 1996).

Table 2.2: Major Nations Producing Lentils

Nation	Production ('000 tonnes)					
	1989-91	1993	1994	1995	1996	1997
Australia	3	5F	6	8	18	57
Pakistan	69	63	52	61	65	69
Africa	110	102	103	106	115	114
Syria	134	105	118	126	141	120
Nepal	121	164	171	148	157	160
Iran	128	239	174	175	175	175
Bangladesh	211	207	208	208	206	206
Canada	158	328	386	327	332	329
N America	215	400	473	404	398	407
Turkey	858	712	646	640	620	553
India	1127	1204	1224	1200	1180	1200
World	3242	3465	3440	3310	3307	3287

(Source: FAO Production Year Book 1995 & 1997)

2.2.4 Australian Lentil Production

In the past, lentils have received little attention from breeders and agronomists in Australia due to lack of adapted cultivators and suitable production packages (Siddique and Sykes 1997). In the late 1980s, a small lentil industry was established in Victoria and recently, a more viable Australian lentil industry has

been expanded in Victoria and established in South Australia. In 1993-4, The Lentil Company released some early flowering red and green lentil varieties that are better adapted to Australian conditions (Siddique and Sykes 1997). The mission of The Lentil Company is "*to supply premium lentils and other specialty crops to niche local and international markets through a multi level marketing system with the ultimate objective of maximising returns to our growers*" (The Lentil Company, 1999).

In 1998, CLIMA and Agriculture Western Australia released two new red lentil varieties (Cassab and Cumra) into the market. They were tested in more than 30 comparative sites and agronomic trails between 1996-1998 at various regional locations in Western Australia, South Australia, Victoria, New South Wales and Queensland. Cassab and Cumra are red lentil cultivars that produce good yield and excellent seed quality. They showed good adaptation to low and medium rainfall areas of Southern Australia (Siddique 2000a and Sidique 2000b).

Presently in Australia, lentils are a minor crop, as they need specific soil requirements. However, the area of lentil production has increased from < 1000 ha in 1993 to about 117 000 ha in 2000. Victoria ranks first in lentil production (80 000 ha), followed by South Australia (30 000 ha) and Western Australia (5 000 ha) (Table 2.3 and Table 2.4).

Table 2.3: The total Area sown to Lentils in Australia by States (1996-2000)

	Area Sown ('000 ha)			
Year	Victoria	South Australia	Western Australia	Total
1996-1997	15	2	0	18
1997-1998	50	5	1	57
1998-1999	70	9	2	82
1999-2000	55	17	2	75

(ABARE 2000b & Pulse Australia)

Table 2.4: The total Production to Lentils in Australia by States (1996-2000)

	Production ('000 t)			
Year	Victoria	South Australia	Western Australia	Total
1996-1997	35	2	0	38
1997-1998	28	6	1	36
1998-1999	30	13	2	46
1999-2000	77	23	2	103

(ABARE 2000b & Pulse Australia)

Loss and Siddique (1997) noted that 1996 was the third year of commercial lentil production in Western Australia. The area of lentil production was increased from 200 ha in 1994 to 2 000 ha in 2000. As evaluated by Loss and Siddique (1997), the soil types and likely rotations throughout the WA wheat belt demonstrate the potential for about 0.5 million ha of pulse production within the next decade and includes more than 5 000 ha of lentils.

Therefore, although Australia is a relatively small producer of lentils at present, the production can be increased. Through the releasing of better adapted varieties, improving knowledge of agronomic practices, increasing of research and advisory services in the public sector, farmer experience and the promotion and marketing of lentils through The Lentil Company, the increase in production of lentils is promising (Dowsley, Carter, and Materne 1996).

2.2.5 Trade of Lentil

Lentils traded for human consumption offer a great opportunity for Australian growers both domestically and overseas. Australia is an importer of lentils (Table 2.5), however there are opportunities for interested growers to supply lentils under contract (Dowsley, Carter, and Materne 1996). Recently, The Lentil Company began selling seed of the following varieties: Cobber, Digger and Matilda. This is done on a buy back contractual agreement. Seed is bought at a price based on a pool return from lentil sales both domestically and overseas (Dowsley, Carter, and Materne 1996).

Australia has the ability to produce an excellent product, which is noted in world markets as being clean and of low moisture (National Farmer Federation 1994). Production over and above domestic needs should find a ready export market although it will be competing with lentils from Turkey (red lentils) and Canada (green lentils), the major exporters of lentils (Dowsley, Carter, and Materne 1996). Researchers from the Department of Agriculture show that Australia may be in a position to capitalise on any shortfall, at least in red lentils, caused by climatic fluctuations in Turkey (National Farmer Federation 1994).

Static production along with increasing population is creating an enormous demand for pulses in countries such as India, Pakistan and Bangladesh. In the year 2000, there will be an additional demand for pulses of about 3-6 million tonnes per annum, as predicted by looking at the present population growth rate and improvements in the economy (Loss and Siddique 1997). For many centuries, populations of the Middle East have regarded pulses as one of their cultural food ingredients, so the improvement of their economies may only have limited changes in social economic food consumption behaviours.

A report on the market potential for Australian pulses identifies an export potential of about 120 000 tonnes of lentils with significant markets in India, Germany, Spain, Venezuela, Algeria, South Africa and Mauritus (Dowsley, Carter, and Materne 1996). Therefore, the long-term outlook for pulse markets is very promising.

In Australia the consumption of pulses is increasing per capita of population, which may be related to the migration of people from around the Mediterranean region to this country. It is also attributed to increasing knowledge of cooking with pulses and their health benefits. Lentils are part of this trend (Dowsley, Carter, and Materne 1996).

Table 2.5: Australian Lentil exports and imports in quantity (tonnes)

Year	Quantity (Tonnes)	
	Exports	Imports
1990-91	600	1 876
1991-92	5 916*	1 994
1992-93	435	n.a.
1993-94	422	1 703

*Includes an unknown (large) amount split vetch improperly described as lentils
(Source: Australian Bureau of Statistics)

In order to increase the supply of lentils, for the future, the Australian pulse industry will need to firstly establish facilities for product development and processing opportunities. Secondly, they need to place greater emphasis on niche marketing and crop diversification. Finally, an arrangement for the international testing of Australian cultivars in colour, taste, cooking time, growth inhibitors and other impediments to acceptance of the Australian product is necessary to obtain more substantial market information. These are important for expanding the market needs as well (Rees *et al.* 1994).

2.2.6 Health Benefits of Lentils

Lentils sometimes are described as 'poor man's meat' (Bhatty 1995). They are packed with nutrients, fiber, complex carbohydrates, and folic acid. In addition, they are a low calorie, low fat, and cholesterol free food and they are relatively cheap compared to e.g. poultry. So, they have an important health benefit for the general population, especially for women, the elderly and vegetarians.

a. Health Benefits for Women

Folic acid is one of the important nutrients found in lentils. The Australian Recommended Dietary Intake (RDI) recommend all women of childbearing age consume 200mg of folic acid per day. During 1998 the Australia New Zealand Food Standards Council (ANZFA) approved a number of foods and food products which included lentils to make a

health claim about the benefits of folate in reducing neural tube defects such as spina bifida in babies.

In addition, lentils are an important source of iron (Ensminger *et al.* 1994), especially for women whose iron needs are greater. Eating lentils with foods rich in Vitamin C, such as tomatoes, green peppers and broccoli helps the body absorb iron more efficiently (USA Dry pea and Lentil Council 1988).

b. Health Benefits for Elderly

With advancing age, the risk of developing malnutrition increases, particularly among institutionalized patients. This may be due to age-associated reductions in food intake combined with the presence of debilitating diseases, social isolation, altered health status, economic limitations and multiple hospital admissions (Bruyere and Rapin 1998). In Australia, the number of elderly, which includes migrants, those aged 65 or above, will increase from 2.2 million in 1995 to 4.1 million in 2021 and to 6.4 million in 2051 (Australian Bureau of Statistics 1995-2051). Moreover, the leading cause of death in Australia is Ischaemic heart disease and the health risk is increased with the increase in age.

Lentils are rich in protein but low in fat and have no cholesterol. It is a perfect food product for the elderly to meet their protein needs without increasing their fat consumption. Lentils are one of the recommended food items suggested by the Australian Heart Foundation, to reduce the amount of fat consumption. One cup of cooked lentils provides 90% RDI of folate. Lentils provide more folic acid than any other unfortified food, so it is rich source of folic acid. Folate rich food products have an important role in the prevention of arteriosclerosis.

The soluble fibre found in lentils act as a "scrubbing brush", for cleaning the digestive system. This type of fiber also decreases serum glucose and cholesterol and decreases insulin requirements for the elderly with diabetes (USA Dry pea and Lentil Council 1988).

c. Health Benefits for Vegetarians

Arthur (1992) conducted a survey in the USA and found that there is a rising preference for vegetarian dishes in restaurants. In Australia, the apparent consumption of meat and meat products for the long term pattern of consumption is decreasing, even though there is a slightly increased consumption by 2.4% to 79.8 kg per capita in 1993-1994 (ABS 1993-1994). This may suggest that there is an increased population of vegetarians in Australia.

Food proteins are divided into two categories, which include essential and non-essential amino acids. Essential amino acids, which cannot be synthesised by the body, are required to be supplied by the diet while the body can manufacture non-essential amino acid. So, in order to meet dietary requirements of protein intake, vegetarians need to improve their protein intake by consumption of high protein legume products. Lentils are high in protein content (Ballentine 1985).

Although lentils are rich in protein, they are deficient in the amino acid methionine, which is an essential amino acid and is supplied amply by the protein in cereal grains (Ensminger *et al.* 1994). Cereals are short of lysine, an amino acid that is present more abundantly in lentils (Ensminger *et al.* 1994). Adding cereals with eggs, nuts, seeds, meat, dairy products will provide a more complete protein diet (Ensminger *et al.*

1994). Lentils consumed as a meal is as digestible as casein protein and it is essential to have a mixed combination of grain products and lentils to form a complete protein vegetarian diet (Bhatty and Christison 1984).

2.3 Types of Lentils and Their Uses

2.3.1 Types of Lentil

Lentils are a high value crop and are one of the most drought tolerant pulse crops (Siddique, Loss and Pritchard 1995). Lentils are mainly divided into two main groups with the distinction based on the seed size and cotyledon colour. Red lentils also known as Persian lentils have small seeds with a reddish-purple coat and orange-red cotyledons (Dowsley, Carter, and Materne 1996 & Siddique, Loss and Pritchard 1995). The seed size of red lentils ranges from 2 to 6 mm in diameter and in 100-seed weight, from 2 to 6 grams (Dowsley, Carter, and Materne 1996). The seed coat is usually removed from red lentils and the seed is split. The currently grown red lentil varieties in Australia are Aldinga, Callisto, Cobber, Cassab, Cumra, Digger and Kye (Dowsley, Carter, and Materne 1996).

Green lentils are also known as brown, yellow, Chilean, macrosperma or Continental lentils (Dowsley, Carter, and Materne 1996). They have larger seeds than red lentils, with a pale green coat and light brown cotyledons (Dowsley, Carter, and Materne 1996 & Siddique, Loss and Pritchard 1995). Seed size ranges from 6 to 9 mm in diameter and 4 to 7 grams per 100 seeds. Matilda, Laird and Invincible are green lentil varieties currently grown in Australia (Dowsley, Carter, and Materne 1996).

Spanish Brown or Lentils de Puy is the other lentil type, which has yellow cotyledons and speckled seed coats. French Green lentils have yellow cotyledons and a green and black mottled seed coat (Dowsley, Carter, and Materne 1996).

2.3.2 Uses of Lentils

Lentils are usually consumed whole, decorticated, decorticated and split or ground to flour (Nygaard and Hawtin 1981). The seeds are soaked and dried, and then passed between rollers, which are 'decorticating'. They are then cleaned to separate the seed coats (Nygaard and Hawtin 1981). The split cotyledons may then be polished with magnesia powder to improve their appearance (Nygaard and Hawtin 1981).

Green lentils are generally used whole as a basic ingredient in many traditional dishes, such as 'myaddarah' (lentil and rice) in Middle Eastern countries (Dowsley, Carter, and Materne 1996). On the other hand, split red lentils are consumed in curries, soup and Sa'amya and as stuffing. They are boiled to make Indian 'dhal' and lentil soup. Lentil flour is used to make pappadams, or added to cereal flour for making bread, cakes and baby foods. Lentils may be deep-fried and consumed as a snack, and young immature pods and sprouted seed may be eaten as vegetables (Dowsley, Carter, and Materne 1996).

The seed coat, by-product of the decortification process contains approximately 13 percent protein and is useful for feeding stock (Dowsley, Carter, and Materne 1996). In some countries, lentil stubble is highly valued and used as stock feed. There are some medicinal uses of lentils. Lentils are used as a remedy for constipation and other intestinal afflictions. In India, lentils are poulticed onto the ulcers that follow smallpox and other slow healing sores (Duke 1981).

2.4 Seed Structure

Legumes are classified into two categories; one with energy stored as fat or oil and the other one with energy as starches or gums. However, the basic seed structures of legumes are similar (Kadam *et al.*1989). Mature legume seeds have three major components: the seed coat, the cotyledons, and the embryo axis (Figure 2.3). The average diameter, thickness, unit mass and volume of mature legume seeds are 6.64mm, 2.65, 0.07g and 49.08mm respectively and 6.5% moisture content bulk (Carman 1996).

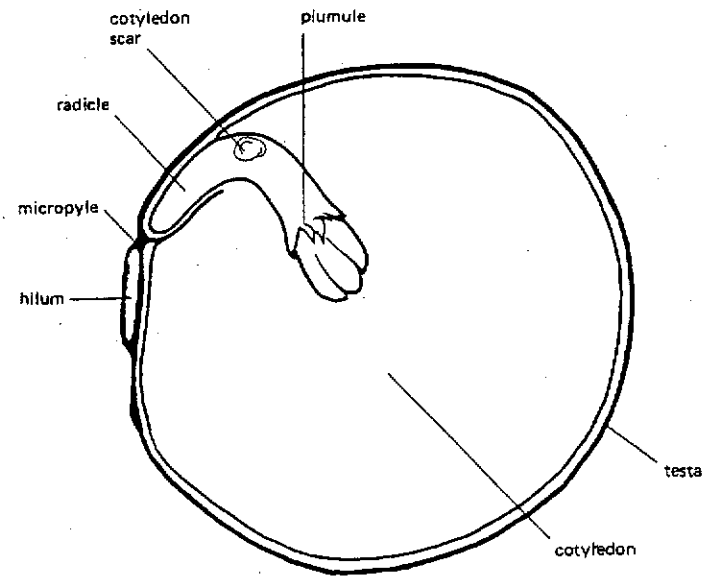


Figure 2.3: Transverse section of a mature lentil seed with one cotyledon removed.
(Source: Webb, C. and Hawtin, G.H. 1981, pg. 49).

In general, seeds have a short living endosperm at the early development stage and it is reduced to a thin layer surrounding the cotyledons or embryo during maturation. The outermost layer of the seed is the seed coat or testa (Figure 2.3) which is the only protective barrier to seeds. Without it, seeds will become unusually vulnerable to breakage, especially after dry and rough treatment (Kadam, Deshpande and Jambhale 1989). Legumes generally have a moderately thick layer of seed coat. The radicle, plumule, and cotyledons can be damaged rather easily as well (Kadam, Deshpande and Jambhale 1989). The plumule, or embryonic stem, is reasonably well developed in the seed and is located between two cotyledons or seed leaves (Kadam, Deshpande and Jambhale 1989).

Unlike other legumes, the surface of the lentil seed coat is uneven and covered with distinctive projecting conical papillae (Hughes and Swanson 1986). Near the middle edge of the seed, there is a large oval scar known as "hilum" which is where seeds are broken away from the stalk. There is a small opening in the seed coat beside the helium known as "micropyle". Micropyle is the original site where the pollen tube enters the valve. Together with the seed coat, hilum and micropyle (Figure 2.3) are suggested to be related to the permeability of the seed coat and water absorption in various legumes (Kadam, Deshpande and Jambhale 1989).

Palisade cells, which are derived from the outer epidermis of the outer integument (Figure 2.4), are located next to the cuticle or epidermal layer on the outer part of the seed coat. It is suggested that as the seeds mature, the wall of the palisade cell contract and cause the hardness and impermeability of the dried testa. The tightly packed palisade cells are suggested to have an important effect on the hydration properties of mature legume seeds. The hourglass cells of legumes are situated next to the palisade cell layer. The rest of the testa is composed of mesophyll cells. Mesophyll cells located near the hilum, are smaller

and more compressed in size and often contain deposits which fill the entire cell cavity (Kadam, Deshpande and Jambhale 1989).

Cotyledons are composed of numerous parenchymatous cells, which contain mostly starch granules (Figure 2.4). The outer cells of the cotyledons, is where protein bodies are situated. There is a distinct cell wall and the middle lamella attached to each individual cotyledon cell and between the two adjacent cells, there is a visible intercellular space. In the seed cotyledons there are a few scattered vascular bundles, which contain a large number of closely packed cells, which supply nutrients for the development of parenchyma cells during seed ripening (Kadam, Deshpande and Jambhale 1989).

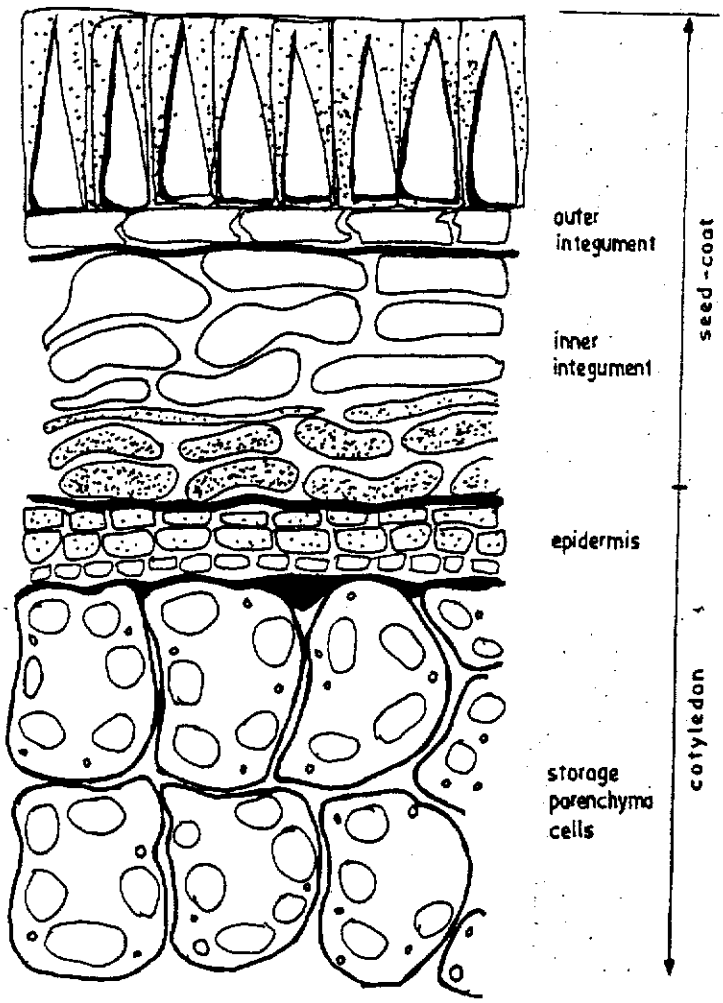


Figure 2.4 Lentil seed coat and cotyledon

2.5 Chemical Composition and Nutritive Value

The embryo is rich in nutrients, but only accounts for a small portion of the total food value of the whole seed. Seed coat is low in nutrients but has a larger proportion than the embryo (Kadam, Deshpande and Jambhale 1989). The distribution of nutrients in seed or fractions of the whole seed show that the major portion of protein, ether extract, phosphorus, and iron is located in cotyledons, whereas crude fiber and calcium are mainly found in the seed coat (Table 2.6) (Kadam, Deshpande and Jambhale 1989). Except for crude fiber and calcium the removal of the seed coat and embryo during milling, does not affect the nutritional value of all pulses (Kadam, Deshpande and Jambhale 1989) (Table 2.7).

Table 2.6: Percent distribution of the Chemical constituents in Lentils.

Seed part/Sample	Seed coat	Cotyledons	Embryo
Ash	6	92	3.1
Protein (Nx6.25)	4	91	5
Ether extract	2	90	6
Crude fiber	75	28	1.5
Phosphorus	3.3	92.7	5.1
Calcium	38	60	4.5
Iron	13	79.1	5.2

(Kadam, Deshpande and Jambhate 1989)

Table 2.7: Major Nutrients in Lentil per 100 grams edible portion

Food Composition	Lentil dried	Lentil dried, boiled
Water (g)	10.30	73.80
Energy (KJ)	1090.00	294.00
Protein (g)	24.20	6.80
Fat (g)	2.00	0.40
Carbohydrate (g)	35.00	9.50
Sugars (g)	1.50	0.50
Starch (g)	33.50	9.00
Dietary Fibre (g)	13.70	3.70
Sodium (mg)	5.00	8.00
Potassium (mg)	840.00	220.00
Calcium (mg)	73.00	17.00
Magnesium (mg)	82.00	25.00
Iron (mg)	7.50	2.00
Zinc (mg)	3.00	0.90
Retinol Equiv. (ug)	3.00	1.00
B-Car. Equiv. (ug)	19.00	5.00
Thiamin (mg)	0.40	0.08
Riboflavin (mg)	0.16	0.06
Niacin Equiv. (mg)	6.30	1.80
Niacin (mg)	2.30	0.70
Vitamin C (mg)	3.00	0.00

(English and Lewis 1991)

2.5.1 Protein

The protein content of lentil ranges from 20.4 to 30.5% and about 91% is found in the cotyledons (Table 2.8) (Kadam, Deshpande and Jambhale 1989). It is a relatively higher protein source than other pulses. Table 2.8 (Amino acid composition of lentils) shows that lentil proteins are rich in glutamic acid and aspartic acid but deficient in sulfuric amino acids such as methioine.

Lentil proteins are primarily divided into two types: metabolic proteins (both enzymatic and structural) and storage proteins. Metabolic proteins are responsible for normal cellular activities including the synthesis of structural proteins, and the storage proteins are relatively smaller in number and account for about 70% of seed nitrogen (Adsule and Kadam 1989). There are several enzymes and enzyme inhibitors, structural and recognition proteins (Adsule and Kadam 1989). The enzyme inhibitors of lentils will be discussed later.

In most pulses, globulin is the major storage protein, followed by glutelin, albumin and prolamin (Adsule and Kadam 1989). However, the different fractions of legume seed proteins do not have similar amino acid composition. In most cases, it is observed that albumin fraction of proteins has better amino acid balance than the globulin fraction (Adsule, Kadam and Leung 1989). The albumin fraction of lentil protein contains more tryptophan, lysine, threonine, valine, and methionine and less arginine, leucine, and phenylalanine (Adsule, Kadam and Leung 1989). Lentil contain some uncommon basic amino acids such as α-hydroxyornithine, α-hyroxyarginine and homarginine (Adsule, Kadam and Leung 1989). A portion of common amino acids also occurs in the free form (Adsule, Kadam and Leung 1989)

Lentil is a good protein source but has poor protein quality. The amino acid composition of lentil is low in both sulphur amino acids and tryptophan (Petterson, Sipsas and Mackintosh 1997). Adsule Kadam and Leung (1989) reviewed studies of nutritional values on some other pulses such as soybean, lima bean and cowpeas, and concluded that lentils exhibited the maximum protein digestibility. The amino acid composition, the presence of protease inhibitors and the interference in protein utilisation by other antinutritional factors are the attributers which depress protein value and digestibility. However, cooking or some other form of heat treatment improves the nutritive value and protein digestibility of pulses. Lentil proteins are deficient in methioine so supplementing lentil diets with methionine is able to improve the protein quality. A diet containing 40% raw lentil and 0.5% methionine exhibited the best protein efficiency ratio (Adsule, Kadam and Leung 1989).

Table 2.8: Amino acid composition of Lentil.

Amino acid	% in seed	Range (g/16gN)	Mean	FAO reference protein	
				Infant	Adult
				Mean (Range)[a]	
Alanine	0.85	2.61-4.07	3.38	--	--
Agrinine	1.70	4.42-9.01	6.68	--	--
Aspartic acid	2.31	6.28-11.13	9.18	--	--
Cystine	0.35	1.37-1.49	1.41	--	--
Methionine	0.11	0.14-0.75	0.42	--	--
Glutamic acid	3.46	9.49-16.04	13.75	--	--
Glycine	0.85	3.05-3.75	3.34	--	--
Histidine	0.46	1.29-2.38	1.83	1.63 (1.13-2.25)	1.00
Isoleucine	0.88	3.17-3.99	3.51	2.88 (2.56-3.31)	0.81
Leucine	1.42	4.72-6.83	5.60	5.81 (5.19-6.69)	1.19
Lysine	1.37	4.13-6.49	5.43	4.13 (3.31 - 4.75)	1.00
Phenylalanine	0.91	3.19-4.58	3.60	--	--
Tyrosine	0.57	1.92-2.63	2.28	1.06 (1.00 - 1.06)	0.31
Proline	1.04	3.91-4.48	4.13	--	--
Serine	0.73	1.72-4.70	2.89	--	--
Threonine	0.82	1.96-4.83	3.23	2.69 (2.50 - 2.81)	0.56
Valine	1.00	2.86-4.63	3.99	3.44 (2.75 - 4.81)	0.81
Cys + Met	0.53	2.01-2.25	2.13	2.63 (1.81 - 3.75)	1.06
Tyr + Phe	1.50	5.12-6.91	5.87	4.50 (4.25 - 7.38)	1.19

[a] Amino acid compositin of human milk (16-19)

(Source: Petterson, Sipsas and Mackintosh 1997, p. 33, Adsule, Kadam and Leung 1989, p134 and FAO 1991, 23)

2.5.2 *Carbohydrates*

a. Starch

Lentils contain about 60% carbohydrates most of which are in the form of starch and the content may vary from 35 to 53% (Adsule, Kadam and Leung 1989 and Bhatty 1988). The amylose content of lentil starch was in the range of 20.7 to 45.5% and the majority of the remaining content is amylopectin (Adsule, Kadam and Leung 1989). Lentils contain resistant starch which is not digestible or absorbed by the human digestive system. The resistant starch content in lentil flour is 0.71± 0.08% fresh matter (Saura-Calizto *et al.* 1993). Lentil starch granules show a dark cross and divide into four segments. Starch granules dispersed fully and are elipsoid, kidney shaped. The granule size diverges from 15 to 30 μM in length and 10 to 25 μM in width (Bhatty 1988) and it is classified as type A starch granule.

Functional Properties of Starch

i. Swelling and Solubility

Legume starches have a limited swelling ability. The swelling of starch occurs in various stages and depends on the severance of different attractive forces (strong or weak). These swelling stages arise from crystalline vs. the amorphous regions of starch granules. The swelling and solubility depend on the starch source, temperature, and pH.

ii. Water Absorption

Water absorption of legume starches is directly related to swelling and inversely related to solubility (Reddy *et al.* 1989c). Water-binding capacity of lentils (92.4 to 98.0%) is higher than faba bean, pea and *Phaseolus* bean starches, but not much different from wheat starch (83.0 to 91%) (Bhatty 1988).

iii. Gelatinisation and Pasting

Gelatinisation occurs when starch granules lose crystallinity after being heated in the presence of water. The viscosity and translucency of starch increases as a result of starch gelatinisation (Reddy *et al.* 1989c). The gelatinization temperature for legumes is varied; for lentil starch it ranges from 64 to 74°C (Adsule, Kadam and Leung 1989).

b. Dietary Fibre

The dietary fibre existing in lentils ranges from 3.8 to 4.6%. It consists of hemicellulose (6%), cellulose (4.1%), and lignin (2.6%). As most of the crude fibers are located in the seed coat, dehulled products such as *dhal* contain very low amounts of fiber (Adsule, Kadam and Leung 1989).

c. Sugars

Sugars present in the lentil seed are divided into two categories: reducing and non-reducing sugar. Most of the sugars present in lentils are non-reducing sugar. There is approximately 4.2 to 6.1% soluble sugars (oligosaccharides), which include sucrose, raffinose, stachyose, verbascose and ajugose (Adsule, Kadam and Leung 1989). Stachyose is the major sugar forming 35 to 64% of the total free sugars, verbascose varies from 17 to 23 % and raffinose varies from 6 to 12 % (Bhatty 1988).

These sugars are of concern because of their implication in causing flatulence. The cause of flatulence in humans who ingest a large quantity of lentils, results in discomfort, abdominal rumblings, cramps, pain or diarrhaea (Reddy, Sathe and Salunkhe 1989). It is suggested the enzyme (α–1-6 galactosidase) that is responsible to cleave the galactose linkages from oligosacharides is not present in human intestinal mucosa. These unhydrolysed oligosaccharides pass into the

large intestine and produce carbon dioxide, hydrogen and traces of methane after anaerobic fermentation. However, the removal of oligosaccharides from beans did not completely eliminate flatulence. It is suggested soaking beans and discarding soaked water before cooking will remove most of these sugars from beans and reduce flatus formation (Reddy *et al.* 1989c).

Lentils contain about 1% unknown sugars, not known to be present in other legumes. The newly identified disaccharide in lentils is galactopinitol (α-D-glactopyranosyl-pinitol). It acts as a transfer-intermediary for galactose to biosyntheses raffinose, stachyose and verbascose (Schweizer *et al.* 1978). It is being investigated as a factor that may contribute to the flatus formation. However, results do not support this suggested role and its chemical and physiological properties are still unknown (Adsule, Kadam and Leung 1989).

2.5.3 Lipids

Lentils contain of < 1% ether extract: their gross energy of lentils (4.2 kcal/g) is similar to that of wheat and barley (Bhatty 1988). Nearly all the ether extract (90%) is located in the cotyledons, 6% in the embryo and only 2% in the seed coat (Singh *et al.* 1968). Lipids are situated in spherosomes or in lipid-containing vesicles of cotyledons.

Total lipids in lentil seeds range from 0.6 to 3.8% (Adsule and Kadam 1989). The total unsaturated fatty acids in lentils are 62.39%. Linoleic acid and oleic acid are the main fatty acid components in this group. The total saturated fatty acid in lentils is 14.52% and palmitic acid is the main fatty acid belonging to this group (Salunkhe, Sathe and Reddy 1989). As lentils have a lower unsaturated fatty acid composition, the lentil lipid may be less susceptible to rancidity than pulses, such as black gram bean (76.3%) and peanuts (76.16%), which have a higher level of

unsaturated fatty acid proportion (Salunkhe, Sathe and Reddy 1989 & Adsule, Kadam and Leung 1989).

2.5.4 Vitamins

Lentils contain B vitamins, which include thiamin, riboflavin and niacin, as most other legumes do and they contain only a small amount of vitamin A. The vitamin content in lentils especially vitamin C is reduced dramatically after cooking (Table 2.9). The bioavailability of vitamins may also be changed after modification and processing of food lentils.

Table 2.9: Main Vitamin Content in Lentils per 100 grams edible portion

Food Composition	Lentil dried	Lentil dried, boiled
Retinol Equiv. (Vit. B_{12} Equiv.) (ug)	3.00	1.00
B-Car. Equiv. (Vit. A) (ug)	19.00	5.00
Thiamin (Vit. B_1) (mg)	0.40	0.08
Riboflavin (Vit. B_2) (mg)	0.16	0.06
Niacin Equiv. (Vit. B_3 Equiv.)(mg)	6.30	1.80
Niacin (Vit. B_3)(mg)	2.30	0.70
Ascorbic acid (Vit. C) (mg)	3.00	0.00

(English and Lewis 1991)

2.5.5 Minerals

Overall, lentil seeds contain 2.4 to 4.2% minerals (Adsule, Kadam and Leung 1989). Almost all the phosphorus and iron are found in the cotyledon of the whole seed (Table 2.10). There is about 67% of the calcium located in the cotyledon. However, the seed coat, which accounts for only 8% of the whole seed, contributes nearly 40% of the calcium.

Table 2.10: Distribution of Phosphorus, Calcium and Iron in different anatomical parts of lentils (mg/100g dry seed)

Seed Component	Phosphorus	Calcium	Iron
Seed Coat	12.3	58.0	1.0
Cotyledon	336.0	93.0	5.7
Embryo	18.0	7.0	0.3
Whole seed	363.0	140.0	7.4

(Source: Adsule, Kadam and Leung 1989 p. 132)

According to English and Lewis (1991), potassium is the most abundant element in lentils (Table 2.11) and iron, zinc and magnesium are relatively good sources in lentils. However, part of these minerals may be bound to phytic acid and are therefore not available for absorption. Lentils have a low content of phytate, about 1 to 8 g/kg, and condensed tannins of about 5g/kg. This allows increase in the bioavailability of the minerals, especially iron (Petterson, Sipsas and Mackintosh 1997). Therefore, lentils are considered a good source of iron.

Table 2.11: Main Mineral Content in Lentils per 100 grams edible portion

Food Composition	Lentil dried	Lentil dried, boiled
Sodium (mg)	5.00	8.00
Potassium (mg)	840.00	220.00
Calcium (mg)	73.00	17.00
Magnesium (mg)	82.00	25.00
Iron (mg)	7.50	2.00
Zinc (mg)	3.00	0.90

(English and Lewis 1991)

2.5.6 *Anti-nutritional Factors*

'Anti-nutritional factors' are chemical substances which, although non-toxic, generate adverse physiological responses in animals consuming these legumes and, in many cases, interfere with the utilization of nutrients in these products (Nwokolo 1996). Most raw legumes, which include lentils, contain some anti-nutritional factors. However, lentil seeds contain relatively lower levels of anti-nutritional factors than the rest, such as soybeans and lupins. These factors include trypsin inhibitors, haemagglutinins, polyphenols, phytic acid, flatulence factors, α-amylase inhibitor, cyanogens and saponins (Adsule 1996).

a. Trypsin inhibitors

The enzyme trypsin is the chief proteolytic enzyme in pancreatic fluid. Inhibition of trypsin may change the digestibility of proteins by preventing complete hydrolysis, resulting in certain amino acids being available (Quesda *et al.*. 1995). Lower protein digestibility may indirectly affect starch digestibility, as enzymes are attacking less starch (Quesda *et al.* 1996).

Trypsin inhibitors are well-known growth-depressing factor in legumes (Adsule, Kadam and Leung 1989). They not only reduce the digestibility of protein, but may also "lock in" a significant fraction of total cysteine of bean protein which is already in short supply (Adsule and Kadam 1989). However, among all legumes, lentil seeds have the lowest trypsin inhibitor activity and highest protein digestibility (Table 2.13).

Table 2.12: Presence of Trypsin inhibitors in Lenil and some Legume Seeds [a].

Legume Seeds	Trypsin Inhibition (IU/g)	% of inhibitor [b]
Soybean	94	100
Bean	76	78
Lentil	15	16
Pea	13	14
Horse bean	3	3
Maize	3	3

[a] Values are given in inhibitory units (IU) per gram of sample. An IU represents the amount of inhibitor inhibiting 1 unit of the corresponding enzyme.
[b] Inhibition of the corresponding enzyme by soybean inhibitors was taken or 100%. The other values have been calculated to the number.
(Source: Blahovec 1991, p.277)

The activity of the trypsin inhibitor is significantly reduced after germination and soaking and is almost completely destroyed after cooking (Adsule 1996). The study of Frias *et al.* (1995) shows that germination of lentils in the dark for 10 days with daily rinsing decreased substantially 45% of trypsin inhibitors. It indicates that trypsin inhibitors may be utilised as a source of energy and nitrogen during germination.

b. Lectins

Lectins are proteinaceous toxic compounds, which have hemagglutinating activity and inhibit growth (Adsule, Kadam and Reddy 1989). The word "lectin" is used interchangeably with phytohemagglutinin (Adsule, Kadam and Reddy 1989). However, the mechanism of how phytohemagglutinins inhibit growth is not fully understood.

Hamagglutinin combines with the intestinal cell walls, causing nonspecific interference with absorption of nutrients and also disruption of the brush borders of duodenal and jejunal enterocytes (Adsule and Kadam 1989).

The feeding of lectins significantly reduced the growth of animals (Jindal, Soni and Singh 1983). There was a decrease in the activities of intestinal disaccharidases and proteases and an increase in the activities of intestinal alkaline and acid phosphatases and Ca^{++}ATPase after feeding with lectins. The activities of some hepatic dehydrogenases were decreased (Adsule, Kadam and Leung 1989). This shows that the activity of lectin on raw lentils does have its effect on growth. However, mono- and oligosaccharides (< biblio >) inhibit the level of lectin activity of lentils. The haemagglutinating activity of lentils is completely destroyed by autoclaving for 20 minutes (Adsule 1996)

c. Polyphenols

Polyphenols are also known as tannins, which are a water soluble compound, having a molecular weight between 500 and 3000 Dalton. Polyphenols are located mainly in the seed coat (testa), and low or negligible amounts are located in the cotyledons (Jadhav, Reddy and Desphande 1989). The content of polyphenol in lentils is 490 mg/100g (catechin equivalent) (Adsule, Kadam and Leung 1989).

There are two major polyphenol structural groups– proanthocyanidins (PA; also called condensed tannins) and hydrolysable tannins (Figure 2.5). Proanthocyanidins are nearly insoluble above approximately 7000 Dalton (i.e. above 20 flavan-3-ol units). Insoluble polymeric PA can range from 12% to 80% of total PA depending on the type of tissue, plant species and extraction method. Hydrolysable tannins are conventionally divided into gallotannins and ellagitannins. Both types are esters of a carbohydrate core (mostly glucose) with gallic acid or hexahydroxydiphenic acids, or their derivatives (Hättenschwiler and Vitousek 2000).

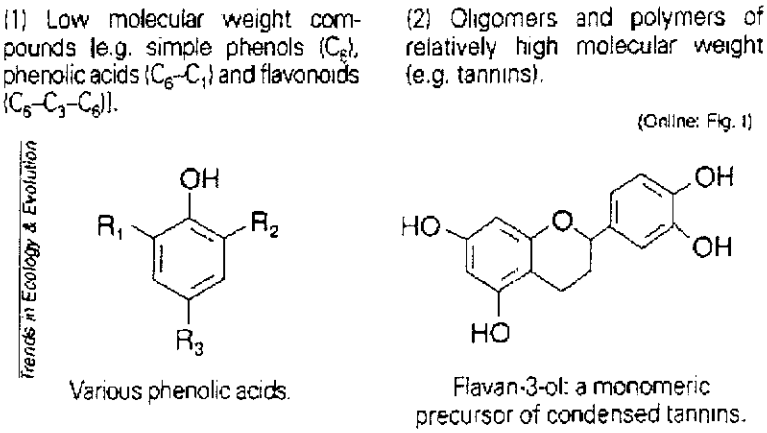


Figure 2.5: Two Main Groups of Polyphenols (A simplified overview)
(Source: Hättenschwiler and Vitousek, 2000)

One polyphenol molecule is able to bind two or more carboxyl oxygen molecules of the peptide group with possible formation of cross-links between protein chains. The degree of cross-linking depends on the number and accessibility of peptide carboxyl groups per protein molecule as well as the relative concentration of the reactant (Adsule and Kadam 1989).

The formation of tannin-protein complexity is reported as responsible for low protein digestibility, decreased amino acid availability and increased fecal nitrogen (Adsule and Kadam 1989). However, de-hulling significantly reduced the activities of polyphenols of lentils, as they are mainly present in the seed coat (Adsule 1996).

d. Phytic acid

Phytic acid (myoinositol hexaphosphate) (Figure 2.6) is composed of a significant amount of phosphorus in cereals and legumes. Phytic acid, phytate and phytin is referred to as free acid, salt and calcium/magnesium salt, respectively. The terms phytic acid and phytate are used interchangeably as phytic acid primarily exists as a salt of mono- and divalent cations (Reddy *et al.* 1986).

A wide range of phytic acid content (100-810 mg/100g) has been found in lentil seeds. The phytate content of dry whole and split lentils is 0.495 and 0.526 g/100g respectively. The phytate phosphorus amounted to about 50% of the total phosphorus of lentil seeds and splits (Adsule, Kadam and Leung 1989).

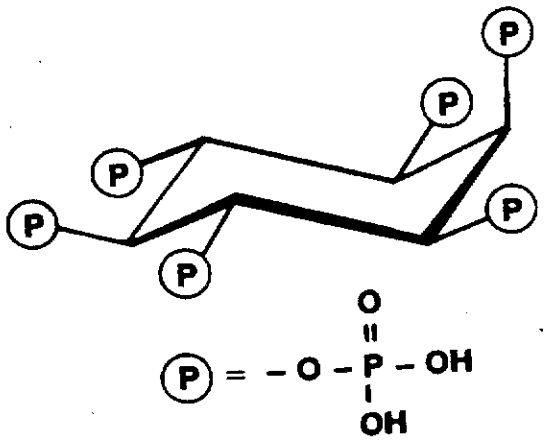


Figure 2.6: The chemical structure of phytic acid
(Source: Luo and Andrade 2000)

i. **Phytic acid Distribution and Content**

Large amounts of phytate present in cotyledons of dicotyledonous seeds in the electron-dense globoid crystals are located within the proteinaceous matrix of protein bodies, and not in the seed coats. Phytic acid can comprise up to 80% of the dry weight of globoids. Phytate accumulates rapidly in globoids during seed maturation. (Reddy *et al.* 1989a).

Phytate accounts for a major portion of the stored reserves of phosphorus in legumes. It is utilised as a phosphorussource during germination of seeds, supports seedling growth and supplies certain biosynthetic needs in the growing tissues. It is suggested that phytate has five physiological roles in seeds: (1) as a phosphorus store, (2) as an energy store, (3) as a source of cations, (4) initiation of dormancy, and (5) as a source of myoinositol which is utilised by the young seedling as a substrate for the myoinositol oxidation pathway and for the formation of the cell wall (Reddy *et al.* 1989a).

ii. **Environmental Influences on the phytic acid content**

The phytate content in seeds is influenced by the environmental and growing conditions, including environmental fluctuations such as change of climate and rainfall, locations, irrigation conditions, soil type, use of fertilisers and the growing year. The content of phytate in seeds increased if there is additional nitrogen and phosphorus fertilisers used during cultivation (Reddy *et al.* 1989a).

iii. **Interaction of Phytic acid with Protein and mineral**

Phytic acid is unstable and decomposes in the free acid form, but it is stable when it is present in the dry form as salt. This suggests that phytic acid has a potential for binding positively charged proteins and/or multivalent cations or minerals in foods (Reddy *et al.* 1989a).

1. Phytic acid - Protein Interaction

Phytic acid interacts with protein to form phytate-protein complexes at both acidic and alkaline pH conditions. Formation of these complexities leads to decrease in the solubility of proteins (Adsule and Kadam 1989). At low pH, phytic acid has a strong negative charge, while many plant proteins would be positively charged as the isoelectric pH value is near 4.5 to 5. This allows compounds to interact with each other; $-NH_3^+$ groups on protein are bound to the phosphate groups of phytic acid. Divalent metals ions may also interact with phytic acid under these acidic pH conditions (Reddy *et al.* 1989a).

Both phytic acid and proteins have a net negative charge in the intermediate pH range that provide the isoelectric pH of the protein. The complexation forms by either direct binding of phytic acid to α–NH_2 terminal group and ε–NH_2 groups of lysine, as they are still protonated or a multivalent cation-mediated interaction. In high pH level (pH>9), the mechanism of phytic acid and protein interaction is not clearly

understood. The interaction between phytic acid and protein is decreased when the pH is very high (Figure 2.7) (Reddy *et al.* 1989a).

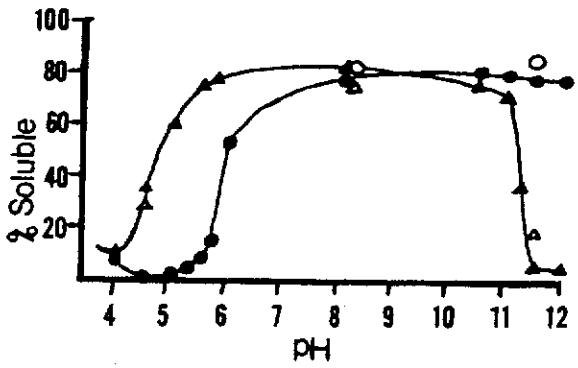


Figure 2.7 Influence of pH on the solubility of nitrogen and phytate of soy flour, nitrogen (○- - -○) and phytate (▲- - -▲).
(Source: Reddy *et al.* 1989a)

2. Phytic acid - Mineral Interaction

Phytic acid, which is a strong acid, forms a variety of insoluble complexes with minerals at physiological pH values. Copper and zinc appear to have a high affinity for phytate to form complexes. The values of pH affect the interaction of phytic acid and calcium complex formation (Reddy *et al.* 1989b).

Calcium and phytic acid are soluble below pH 4 at all molar ratios. The solubility decreases as the pH value is above 4 and the extent depends upon the calcium: phytic acid ratios. The highest calcium precipitation occurs when pH is above 6.0, at calcium to phytic acid ratios of 4.0 to 6.5. Phytic acid solubility decreases with increasing calcium: phytic acid ratio and complete precipitation occurs at ratio of 5. When calcium is limited, phytic acid remains in solution as the pH is increased to 7.0.

Magnesium-phytic acid complexes are soluble at pH below 5; the solubility decreases when the pH is more than 5.0 and when there is

increase in magnesium-phytic acid ratio. Magnesium is more soluble than phosphorus when the magnesium: phytic acid ratio is 4.0 or less and pH is more than 5.0. The magnesium is more soluble than phosphorus when the ratio is more than 6.0 (Reddy *et al.* 1989b).

iv. Digestibility and Bioavailability of Phytic acid

Insoluble complexes are formed when phytic acid combines with cations in the digestive tract of humans. Once the complexes are formed, humans cannot utilize the phosphorus constituent unless phytate is hydrolysed by phytase. The presence of phytate, reduces the availability of phosphorus from plant sources. The formation of minerals and phytic acid complexes, make minerals unavailable for absorption. The reduced bioavailability of minerals from legumes to humans due to phytate or phytate-protein complexes, is related to several factors. Nutritional status of humans and the concentration of minerals and phytate in foodstuffs are directly related to the formation of complexes. The ability of endogenous carriers in the intestinal mucosa to absorb essential minerals bound to phytate and other dietary substances, the digestion or hydrolysis of phytate by phytase and or phosphatase enzyme in the intestine are related to the ability to absorb the minerals and protein (Reddy *et al.* 1989a).

The processing of products or methods of processing which include unit food processing operations and the digestibility of the foodstuff are the contributing factors relating to the bioavailability of minerals and proteins (Reddy *et al.* 1989a & Reddy *et al.* 1989b). Cooking significantly reduces the phyate phosphorus and oligosaccharides in lentil seeds (Adsule 1996).

e. Saponins

Saponins are composed of a steroidal or triterpene aglycone linked to one, two or three saccharide chains of variable sizes and complexities via ester and ether linkages (Ruiz *et al.* 1997). Saponins have both deleterious and beneficial effects. They are toxic to fish, and inhibit the growth and sporulation of a wide range of fungi, and may cause to the bitter and astringent sensory characteristics in processed grain legumes (Ruiz *et al.* 1997). Saponins also have the ability to cause lysis of erythrocytes and to make the intestinal mucosa permeable.

Beneficial effects of saponin consumption are the ability to lower the level of plasma cholesterol in humans (Ruiz *et al.* 1997). They may exhibit anticancer activity and have an inhibitory effect on the infectivity of human immuno-deficiency virus *in vitro* (Ruiz *et al.* 1997). However, all the above functional properties are related to specific saponin structures rather than to the class as a whole.

Lentils have a relatively low saponin content when compared to soybean, haricot or kidney beans. It has a level comparable to that of pea, but a higher level when compared to lupin (Ruiz *et al.* 1997). The seed size and testa colour changes the saponin content (Ruiz *et al.* 1997). The total saponin content of seeds with a brown testa is significantly lower than those with a green testa (Ruiz *et al.* 1997).

f. Other Anti-nutritional factors

Apart from trypsin inhibitors, lectins, polyphenols and phytates, there are some other anti-nutritional factors in lentil, which include oligosaccharides, cyanogens, saponins and α-amylase inhibitor. Adsule, Kadam and Leung (1989) concluded that flatulence is not a particular problem with lentils and the content of cyanogens in lentils is only 0.46

mg HCN/100g, far below the permitted toxic range. However, the activities of other factors are not well studied, so information on the presence and nutritional significance is not available. The studies and reviews indicate that lentil seeds contain relatively low levels of anti-nutritional factors as compared with other pulses.

2.6 *Interaction of Environmental and Genetic Factors on Chemical Composition and Cooking Quality*

Location, growing season and genetics play a significant role in determining the seed yield, chemical composition and cooking quality of lentils and other legumes (Bhatty *et al.* 1984 and Chernick and Chernick 1962). Many researchers have done extensive study on the effects of environmental and genetic factors on crop yield and the protein and amino acid content in seeds. There is a negative correlation between seed yield and seed protein content (Erskine *et al.* 1985 & Stoddard *et al.* 1993). The protein content of seeds will increase after nitrogen and phosphorus fertiliser is applied to the soil (Kadam, Deshpande and Jambhale 1989 & Erskine *et al.* 1985).

The mineral content in seeds is influenced by the availability of nutrients from the soil during plant growth (Adsule, Kadam and Leung 1989). Additional phosphorus and potassium fertiliser improved the cooking quality of field peas; the ash, total (both organic and inorganic matters) and phytin phosphorus content of field peas increased after the application of phosphorus fertilizer (Halstead and Gfeller 1964). A faster cooking lentil is produced after plants are watered with adequate N, P, Ca, Mg, S, B, Cu, Fe, Mn, Mo, Zn and a high level of K (210ppm) fertiliser. The cooking quality of lentils improved with a combination of high level potassium and sodium in seeds (Wassim *et al.* 1977). However, the degree uptake of phosphorus and other mineral from soil depends on the growing environment. For example, lentils grown in Saskatchewan on stubble under low moisture conditions take up minimal amounts of phosphorus even if there is enough phosphate in the soil (Bhatty 1995).

2.7 Processing

Lentils are generally purchased raw and prepared at home, so there is a limited requirement for commercial processing. In addition, the preparation methods for lentils vary widely among different ethnic groups. The main purpose of processing is to reduce the activity of anti-nutritional factors, which inhibit the digestibility of lentils. Processing methods include milling, cooking, soaking, germination, canning and fermentation. Details of the main processing will be discussed as follows:

2.7.1 Milling and De-hulling

Lentils are prepared by removing the seed coat (hull), dehulling and splitting the kernel into two cotyledons (de-hull) and the detail milling process is shown in Figure 2.8 (Adsule, Kadam and Leung 1989). The different varieties of legume influence the milling characteristics. The differences include; thickness, texture of husk, waxiness of husk, thickness of the grain layer binding the seed coat to kernel, shape, size, uniformity and hardness of the grain, and storage conditions (Kadam and Salunkhe 1989).

A study on the effect of genotype and location on splitting and de-hulling of lentils showed that de-hulling efficiency was of minor importance in the difference among sites (Erskine, Williams and Nakkoul 1991). However, there was more variation among genotypes for de-hulling efficiency (80.8 – 87.7%) and for % split de-hulled seed (62.1 – 80.2%) than between locations, and seed processing variables showed medium to high broadsense heritabilities. As correlation between seed processing characteristics and agronomic variables were generally low, selection of one group of variables will be unlikely to affect the other.

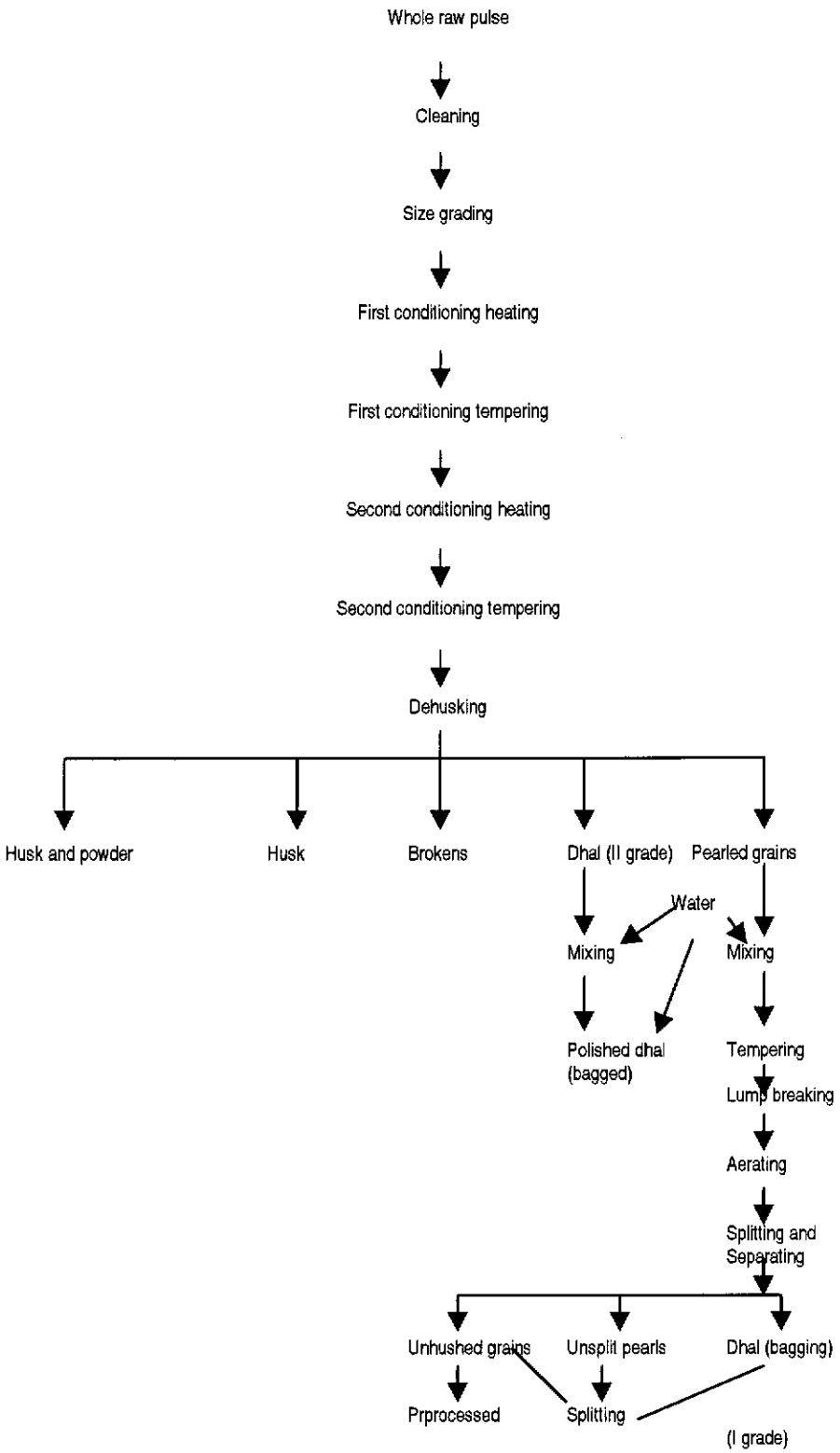


Figure 2.8 Improved dhal milling process developed by Central Food Technological Research Institute, Mysore, India
(Source: Kadam and Salunkhe 1989)

A study of the effect of seed size and different pre-treatments on splitting and de-hulling of lentils showed that there is a decreased of 0.31% de-hulling efficiency with large seeds and a decrease of 0.16% with small seeds for every minute of immersion in water. There is no difference between the air drying temperatures of 19 °C and 36 °C. The omission of drying after immersion gave the highest efficiency. Seed tempering, which is defined as defrosting frozen foods until they are firm but not frozen (Freeland-Graves & Peckham 1996), for 24 hours increased the yield of split and de-hulled seed by 5%, compared with immediate de-hulling (Erskine, Williams and Nakkoul 1991).

Milling influences the nutritional and cooking quality of legumes. As most of the dietary fiber and calcium is present in the seed coat, de-hulling significantly decreases these nutritional contents (Kadam and Salunkhe 1989). The dhal yields for lentil is between 62.1% and 80.2% (Erskine, Williams and Nakkoul 1991). The removal of the seed coat decreases the tannin content of lentils from 314-334 mg/100g to 180 mg/100g (Bressani and Elias 1988) and increases the digestibility of lentils. De-hulling improves water and oil absorption, which may be due to the interactions between seed coat materials and the endosperm protein components, which lead to decrease in protein content (Kadam and Salunkhe 1989). Dehulling decreases the cooking time and facilitates improved palatability (Kadam and Salunkhe 1989).

2.7.2 Soaking

Soaking forms an integral part of pulse processing, particularly in cooking, germination, fermentation and puffing (Deshpande, Sathe and Salunke 1989). Soaking is used to reduce the cooking time of pulses (Kadam and Salunkhe 1989 & Scanlon *et al.* 1998). However, different varieties of legume seeds have different water absorption rates (Deshpande, Sathe and Salunke 1989). The seed coat, hilum and micropyle are related to the permeability of legume seeds

(Deshpande, Sathe and Salunke 1989). If the seed coat is too thick, there will be a slow initial rate of water absorption individually (Deshpande, Sathe and Salunke 1989). Soaking removes soluble antinutritional factors and nutrients, especially when done in warm water. Consequently, it improves lentil digestibility and nutritional quality (Vidal-Valverde *et al.* 1994).

2.7.3 Cooking

Cooking is the oldest method for legume processing. It is a process to produce a tender, edible product, to develop aroma and to inactivate anti-nutritional factors (Chavan, Kadam and Salunkhe 1989). Cooking can be done at atmospheric pressure and temperature such as boiling. It can also be done at high pressure and temperature such as autoclaving. The time required for lentils reach to the cooked state ranged from 29.9 to 45 minutes (Erskine *et al.*. 1985).

Cooking influences the chemical composition and nutritional quality of lentils (Matthews 1989). There is a significant decrease in mineral content through leaching, washing and cooking. Twelve percent of available lysine is lost after one hour of cooking under atmospheric pressure (Adsule, Kadam and Leung 1989).

After one hour of boiling, the trypsin inhibitor activity of lentil will be effectively destroyed. There is a complete removal of trypsin inhibitor activity in lentil seeds soaked before cooking (Vidal-Valverde *et al.* 1994). In addition, autoclaving of lentil seeds will completely destroy the activity of the trypsin inhibitor (Adsule, Kadam and Leung 1989). A 72% removal of raffinose oligosaccharides from lentils occurs after cooking in boiling water for 30 minutes and discarding the cooking water (Adsule, Kadam and Leung 1989).

However, cooking may cause an apparent increase in the content of oligosaccharides and flatus-producing capacity of some legumes (Adsule, Kadam

and Leung 1989 & Rao and Belavady 1978). Oligasaccharides may bound either to proteins or other macromolecules or be present as constituents of high molecular weight polysaccharides in the raw pulses. Cooking may release the bound oligosaccharides and increase the content. Finally, cooking whole and split lentils caused a 9 and 55% loss in phytate content respectively (Adsule, Kadam and Leung 1989). As concluded, cooking is an important process procedure to reduce the activity of antinutritional factors and improve the nutritional quality of lentils.

The term *"cookability" is* defined, as the cooking time required to reach the cooked texture that is considered acceptable for eating. The cookability of lentils depends on genetic factors, physical properties, chemical composition, seed structure, processing and storage and details discussed below.

I. Heat Treatment of Lentils

a. Genetic Factor

Different legumes differ in their cooking behaviour, (Lyer, Kadam and Salunkhe 1989), which may be mainly due to their seed size differences (Tang *et al.*1995). Seed size is suggested to be used as a cooking quality predictor of lentils (Erskine *et al.* 1985).

b. Physical Properties

The optimal cooking time is linearly related to the thickness of seeds (Lyer, Kadam and Salunkhe 1989). Varieties that have a faster water absorption rate during the early stages of cooking required a shorter cooking time (Lyer, Kadam and Salunkhe 1989). There is a linear relationship between the hardness of legume seeds and the optimal cooking time (Figure 2.8) (Lyer, Kadam and Salunkhe 1989).

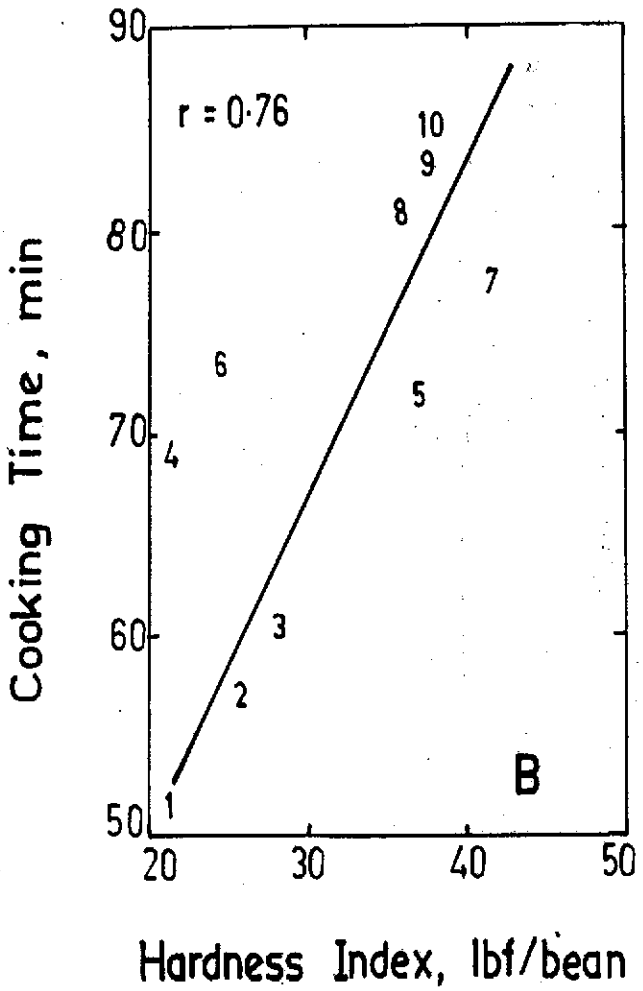


Figure 2.9: Dependence of optimal cooking time of dry beans on true water uptake at 20 min (A) and hardness of beans (B).
(Source: Lyer, Kadam and Salunkhe 1989, p. 143).

c. Chemical Composition

While the following research relates to beans, it also reflects useful information for this present study of lentils. During the cooking process, the middle lamella of the bean softens which allows the separation of adjacent whole cells (Jones and Boulter 1983). This phenomenon is suggested to be related to either the transport or removal or both of divalent cations from the middle of the lamella during cooking (Rockland and Jones 1974; Varriano-Marston and Omana 1979; Jones and Boulter 1983 & Moscoso *et al.* 1984). In regard to lentils, most researchers agree there is a similar process during cooking.

The degree of solubility of the intercellular material depends mainly on the amount of calcium and magnesium interacting with the pectic substances (Lethan 1962 and Shah 1966). This is related to the process of chelation of divalent cations from the middle lamella by phytate (Jones and Boulter 1983 and Moscoso *et al.* 1984). Mattson did the first observation on peas in 1946. He suggested that the cooking quality of peas deteriorate when the phyate level in peas is low. The pectin in the middle lamella formed insoluble calcium and magnesium pectates, caused poor cooking quality (Mattson 1946). Several researchers who studied the loss of cooking quality of stored dry beans found that the level of phytic acid decreases as the seed hardens (Jones and Boulter 1983; Moscoso *et al.* 1984; Hincks and Stanley 1986; Sievwright and Shipe 1986). As lentils are under the same tribe as peas similar chemical reactions can be assumed as found in peas.

Moscoso *et al.* (1984) suggested that phytate content could be an index of measuring cookability in beans. Several studies support the existence of the relationship between phytate content and cookability in lentil also. Phytate chelated calcium and magnesium ions prevent formation of calcium and magnesium cross-linkages between the pectate molecules

of the middle lamellar tissue. Higher phytate content in beans favours a rapid rate of softening and dissolution of pectic substances that would result in shorter cooking times (Reddy *et al.* 1989). Conversely, the lower phytate content in beans permits more interpectate cross-link formation that would result in a slower dissolution of pectic substances and in longer cooking times (Reddy *et al.*. 1989).

There is evidence that the development of lignification in black beans, also under the same tribe as lentils during storage reduces the bean's cookability (Varraino-Marston and Jackson 1981 and Hincks and Stanley 1987). Small polypeptides and free aromatic amino acids are hydrolysed from large proteins and may lead to polyphenol synthesis. At a period of relatively high temperatures and humidity in storage these compounds presumably migrate to the middle lamella. The actions of mobilised enzymes are thought to promote the lignification. These reactions are known to be catalysed by peroxidases, which were detected in extracts from the seeds. The production of lignin or other insoluble polymers in the middle lamella could certainly lead to a failure of the bean cells to separate upon cooking. This suggested polymers are built up within the cell walls and in the middle lamella as the bean seeds age. Because of the deposited polymer, water movement may be restricted within the cotyledons (Hohlberg and Stanley 1987). This present study confirms the above, by demonstrating a linear relationship between thickness of seed coat of lentils and cooking time.

d. Processing

Soaking and dehulling reduces the cooking time of lentils.

e. Storage

Prolonged storage leads to the development of hard-to-cook phenomenon (HTP), defined as seeds absorbing enough water but failing to soften upon cooking (Liu 1997 & Stanley and Anguilera 1985). Seeds with HTP defect have poor cookability. They require longer cooking time to achieve proper tenderness and they may even be resistant to softening during cooking (Liu 1997). Storage of legumes in a relatively humid and high temperature increases lentil cooking time (Lyer, Kadam and Salunkhe 1989). Storage increases the firmness of seeds mainly due to loss of phytic acid which enhanced the formation of a calcium or magnesium pectate complex (Chavan, Kadam and Salunkhe 1989). Some of the phytic acid is bounds with protein via a calcium or magnesium bridge, and firmer cotyledons are a result (Chavan, Kadam and Salunkhe 1989).

Structural changes reduce the cookability of seeds. During storage, cytoplasmic organelle disintegrates and the cell wall loosens the attachment with plasmalemma, affecting seed cookability (Chavan, Kadam and Salunkhe 1989). The insoluble salts of pectates, pectinates and protein components, located in the middle lamella often promote lignification that affects the cooking quality of seeds, with prolonged storage (Chavan, Kadam and Salunkhe 1989).

As storage reduces the phytic acid content in lentils (Bhatty 1990), so, the loss of phytic acid enhances the formation of a calcium or magnesium pectate complex (Chavan, Kadam and Salunkhe 1989). Some of the phytic acid interacts with protein via a calcium or magnesium bridge to produce firmer cotyledons (Chavan, Kadam and Salunkhe 1989). Consequently, the seed structure of lentil seeds change. This phenomenona changes the cell wall into a multilayer structure during storage and reduces the cookability of legumes (Bhatty 1990).

The storage of legumes reduces the protein digestibility (Chavan, Kadam and Salunkhe 1989).

However, Bernal-Lugo *et al.* (1990) suggested that cell wall, rather than the contents of phytate and its rate of hydrolysis, is the major contributor to the seed susceptibility to storage-induced hardening of different bean varieties. During storage, the cell walls are biochemically-changing leading to higher lignification. The degree of methylation of pectin decreases and the amount of insoluble pectin increases. These alternations of cell wall components reduce the "thermal degradation" and increase the cooking time of stored beans (Bernal-Lugo *et al.* 1990).

II. Measuring of Cooking Quality

Tang *et al* (1990) measured the texture of lentils with a Kramer shear press fitted with a thin multi-blade shear compression cell. In the test, the maximum compression-shear force expressed as Kg/g was used as the index of cooking quality. The shear force of a fully cooked condition in lentils is less than 4 Kg/g (Tang *et al.* 1990).

Bhatty (1995) compared the good-cooking or poor-cooking lentils by measuring their differences in texture. A good cooking lentil has a short cooking time, cooks uniformly, is firm and not mushy (Bhatty 1995). The texture of lentils are measured with a Texture Measuring System (Model TMS 90) under several conditions, which include force transducer, 0-300 lb (1 lb $\approx$ 0.45 Kg); multi-blade compression test cell, CS-2, RAM stroke, 0.7 cm/s. The measured force was divided 2.2 and the dry lentil weight to obtain shear force is measured in kg/g dry seed. The good- or poor-cooking lentils have a shear force smaller or greater than 4.0 kg/g respectively.

A rapid screening method was developed for the cooking time of chickpeas. It mentioned that seed density and hydration and swelling indices are of no practical value for predicting cooking time. However, seed size gave a rapid indication of potential cooking time (Williams, Nakoul and Singh 1983).

The texture of over-, under- and optimally cooked lentils was defined by using sensory and instrumental methods, and to investigate the effectiveness of various pretreatments for reducing lentil cooking time (Scanlon *et al.* 1998). As cooking time increased, sensory scores for hardness, chewiness and particle size decreased, as did peak-force values. Soaking and tempering of lentils reduced the cooking time. Moreover, the reagents that could be used in tempering solutions without affecting the appearance or taste, include 2% sodium tripolyphosphate, 1% citric or 2% ascorbic acid mixtures and 150 ppm disodium EDTA.

2.7.4 Others

The nutritional quality of lentils is greatly influenced by process practices. Germination improves amino acid availability, increases the availability of vitamins and decreases concentrations of phytic acid, trypsin inhibitors (Vidal-Valverde *et al.* 1994) and oligosaccharides (Rao and Belavady 1978). Fermentation improves nutritional quality by solublising proteins, inactivating antinutritional compounds, and increasing water-soluble vitamins (Kozlowsak *et al.* 1996).

The aim of this study was to evaluate the possibility of using texture analyser (TA-XT2i) to determine the cooking quality of lentils and to examine the relationship between lentil cooking quality with some physical and biochemical properties of lentils.

Chapter III
Preliminary Experiments

3.1 Introduction

Consistent experimental conditions significantly affect the quality of results. So, the aim of this chapter is to describe a pilot study to appraise factors that may affect the reliability and repeatability of the methodology and the outcome results and to finalise research methodology.

3.2 Pilot Study

3.2.1 Aim and Objectives

To evaluate and improve the experimental techniques to determine the cooking quality of lentils, by testing an existing methodology for texture analysis. Successful development and adaptation of the experimental techniques would improve the reliability and repeatability of methodology and the outcome of cooking quality results.

Objectives of this study were as follows;

- To evaluate an existing texture analysis methodology;
- To evaluate the methodology in terms of the following criteria: repeatability of data collection and limitations of the methodology.
- To consider the use of the results in evaluating the cooking quality of lentils;
- To make recommendations regarding the future development and implementation of the texture analysis methodology.

3.2.2 Samples

Seven samples harvested in 1997 comprised of five different cultivars grown at two different sites (Geraldton-GE and Merredin-ME) obtained from Agriculture Western Australia in 1999 were used as shown in Table 3.1.

Table 3.1 Lentil Samples and location

Cultivar	Location	Type
Adlinga	Merredin	Red
Cobber	Geraldton	Red
	Merredin	
Cumra	Geraldton	Red
Matilda	Geraldton	Green
	Merredin	
Northfield	Geraldton	Red

3.2.3 Methods

Ten grams of whole seed samples were cooked in a suitable teapot with 400-mL of boiling deionised water. The volume of water used for cooking was adapted from the methodology which was used normally for the analysis of chickpeas. The accessible rig base was originally designed for the texture measurement of chickpeas. The size of chickpeas is much larger than lentils, so a large amount of sample size was required for a proper texture measurement and it was decided to use10 grams of samples.

TA-XT2i was not able to measure samples with too high resistance force, so the measurement of compression force could only be started with 20 minutes cooking samples and increased at two minute cooking time intervals for each cultivar. After cooking at a set time period, water was drained and the hardness of samples were measured directly.

The compression force of the lentils was measured with a TA-XT2i texture analyser (Stable Micro Systems, England). The maximum compression force was measured by using A/BE40 back extrusion rig 40mm probe (Figure 3.1) and with specific TA-XT2i texture analyser settings (Table 3.2) is reported in grams (g).

Figure 3.1: The use of A/BE40 back extrusion rig 40mm probe attached to the load cell of TA-XT2i Texture Analyser (Stable Micro Systems, England)

Table 3.2 Test setting of TA-XT2i Texture Analyser (Stable Micro Systems, England):

Test mode and Option	Measure force in compression Return to start
Parameters	Pre test speed: 2mm/sec Test speed: 1 mm/sec Post test speed: 5 mm/sec Distance: 65% Load cell: 25-1
Trigger	Type: Auto Force: 0.05N Stop plot at: Final Auto tare: Selected
Units	Force: Newton Distance: %strain
Break	Detect: Off

* All other parameters are N/A

3.2.4 Methodology Evaluation and Recommendations

Methodology Evaluation

1. The available rig base volume was found to be too large and an inappropriate tool for lentil texture measurement. The accessible rig is too high (≈ 4 cm height) as the seed size of lentils is very small. Thus, a large sample volume (10g) is required to gain enough sample height for texture measurement. However, after cooking, it is very difficult to control a constant sample height for this sample-height dependent compression measurement.

   TA setting was designed to start measuring the compression force from distance O% (0.05N triggering force) to 65% of the sample height (peak compression force), and then measuring the back extrusion force after reaching the 65% travelling distance. So, the height of the samples significantly affected the outcome of compression force and varied the consistency of results.

   Recommendation

   A shorter rig with a smaller volume is recommended for texture measurement of lentils. A layer of lentil seeds, which allows a better control of sample thickness, should be used for measurement.

2. The moisture and excess water content in seeds might increase resistance force, thereby increasing the force of compression. It is important to control water content by removing the excess water content from seeds before measurement, to ensure the consistency of outcome results.

   Recommendation

   Samples should be sifted in a fine sieve after cooking and dried with tissue paper towels to ensure excess water content in seeds is removed.

3. The objective for this part of the study was to evaluate the proficiency of developing an objective measurement using TA-XT2i Texture analyser (Stable Micro Systems, England) to determine the cooking time of lentils and to replace the traditional subjective method ("Cooking time test"). However, before conducting the texture profile analysis, it was noted that some factors which might affect the accuracy and repeatability of the texture analysis result included the volume of boiling water used, the heat input from the hot plate and the effect of sample holding time.

   Recommendation

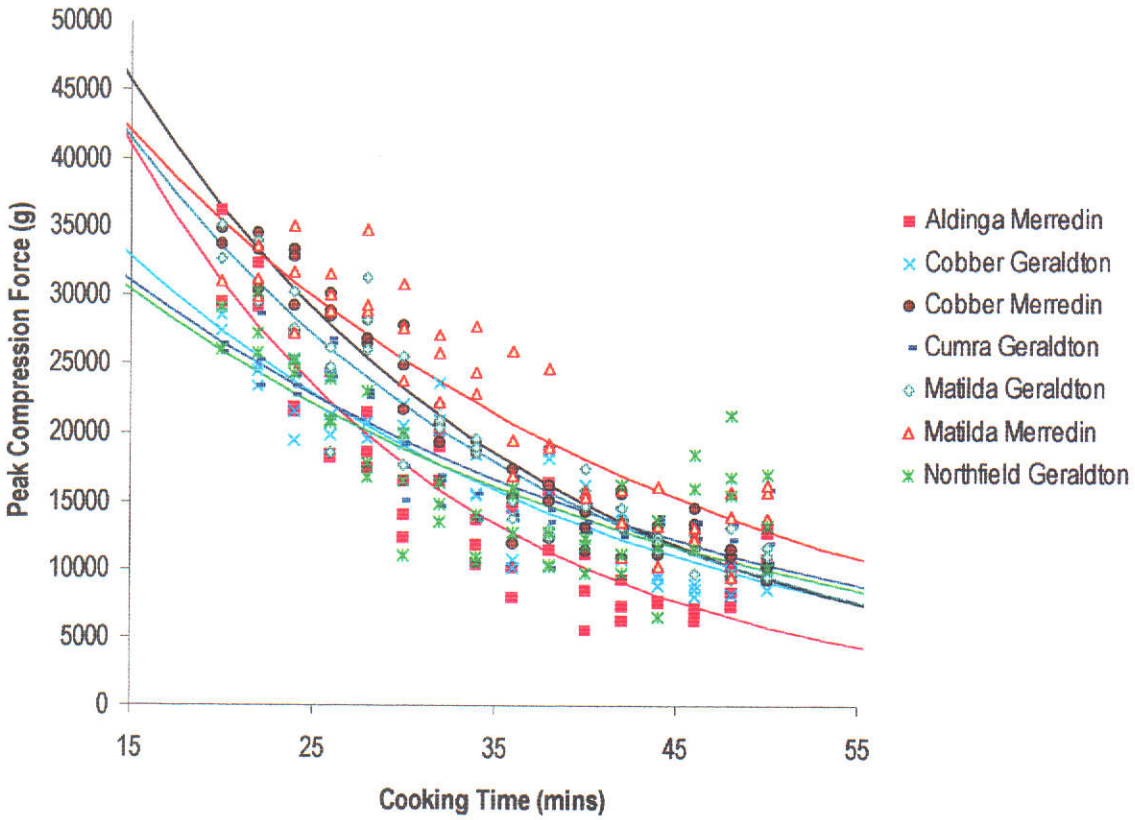
   Texture measurement conditions should be tested and standardised to ensure a good consistent result. These factors which should be tested and standardisation of the texture-analysed methodology details will be discussed in the preliminary experiment sections.

4. The measurement of peak compression force will only allow the evaluation of cooking quality (seed hardness) of lentils. Presently, there are many studies which have used Texture Profile Analysis (TPA) to evaluate the different texture parameters of the samples and correlate them with various sensory attributes. The use of TPA setting will broaden the understanding of the cooking texture of lentils and the valid result will be useful for future study. It will be discussed in the following section.

5. There were problems encountered and attention needed while running the texture measurement. For example, early triggering, force and probe calibration and sample selection. These specific details on texture analyser settings and techniques will be discussed in the following sections.

3.2.5 Result and Discussion

The results obtained are useful in several manners: to determine the effect of cooking on lentils, to determine the cooking time for different cultivars and to evaluate various cooking qualities among cultivars. Cooking reduced the hardness of seed in a curvilinear manner (Figure 3.1).



The reduced changes in hardness indicated that samples reached the cooked stage slowly. The significant difference in the mean seed hardness will be identified as the cooking time for different lentil samples. Aldinga is the hardest lentil variety, followed by Cobber, Matilda, Cumra and Northfield. This indicated that texture analyser is a useful equipment to evaluate the cooking quality of different lentil varieties (Table 3.3).

Data was collected in triplicate. The purpose of data collection in triplicate was to test the repeatability of the data and to improve the outcome results of the study. It was found that the highest standard deviation was around 30% and the lowest was 10%. This suggests that there is a need to improve the methodology and for this reason preliminary experiments were carried out.

Table 3.3 Exponential Regression Analysis of cooking quality for 1997 samples *(Aldinga, Cobber, Cumra, Matilda and Northfield).*

		Value	SD	CV	95%	95%	N
Cumra Geraldton	A	49570	3.6E+03	7.2E+00	42377	56763	48
	b	0.031	2.3E-03	7.3E+00	0.027	0.036	
	t50	42.25	1.6E+00	3.9E+00	38.97	45.53	
Northfield Geraldton	A	48885	6.2E+03	1.3E+01	36376	61394	48
	b	0.032	4.0E-03	1.3E+01	0.024	0.040	
	t50	41.83	2.8E+00	6.6E+00	36.25	47.41	
Matilda Merredin	A	69573	5.6E+03	8.1E+00	58251	80894	48
	b	0.034	2.6E-03	7.7E+00	0.029	0.039	
	t50	40.56	1.6E+00	3.9E+00	37.39	43.74	
Cobber Geraldton	A	56775	4.3E+03	7.6E+00	48104	65445	48
	b	0.036	2.5E-03	6.7E+00	0.031	0.041	
	t50	39.00	1.3E+00	3.3E+00	36.45	41.63	
Matilda Geraldton	A	77925	5.4E+03	7.0E+00	66994	88856	48
	b	0.042	2.3E-03	5.5E+00	0.037	0.047	
	t50	36.47	9.0E-01	2.5E+00	34.65	38.29	
Cobber Merredin	A	89841	5.0E+03	5.6E+00	79709	99973	48
	b	0.045	1.9E-03	4.2E+00	0.041	0.049	
	t50	35.37	6.4E-01	1.8E+00	34.08	36.66	
Aldinga Merredin	A	94580	1.1E+04	1.2E+01	72522	116638	48
	b	0.056	4.1E-03	7.3E+00	0.048	0.064	
	t50	32.44	9.1E-01	2.8E+00	30.61	34.27	

$y = A * e(-b*t)$
A (g) = initial value @ t = 0
b = exponent
t 50 = time to 50% of initial value (20 min)

3.3 Preliminary Experiments

3.3.1 Methodologies

i. *Seed: Water ratio*

Different quantities, 250, 300, 350 and 400 ml of deionised water were tested by cooking a standardized seed weight (≈20g) on the same hot plate for 50 minutes, to identify the impact of water used, on cooked seed texture. The procedures are described in Appendix 2E. Data was collected in triplicate. Results were evaluated with One-way ANOVA.

ii. *Heat input*

The testing of texture after cooking for a fixed cooking period on six hot plates with one heat input determined the effect of various heat inputs on texture. The procedures are prescribed in Appendix 2E. Data was collected in triplicate. One-way ANOVA was used for evaluation of results.

iii. *Standing Time*

The procedures are outlined in Appendix 2E. Data was collected in triplicate for the different samples. One-way ANOVA was used for evaluation of results.

3.3.2 Result of Preliminary Experiments

The results of the above experiments are shown in Table 3.4

Table 3.4 Preliminary Experiment Result

Preliminary Test	Sample	One-way ANOVA*	Sig.
Seed : Water ratio	Matilda	$P > 0.05$	NS
	Digger	$P < 0.05$	Sig.
Heat input	Digger	$P > 0.05$	NS
Standing time	Digger	$P > 0.05$	NS

* One-way ANOVA is significant at the 0.05 level (2-tailed).
NS = Non-significance, sig. = significance

3.3.3 Discussion of Preliminary Methodology

i. Seed: Water ratio

The volume of water used for cooking is suggested to play a significant role in affecting the cooking texture of lentils (Bhatty *et al.* 1983). The testing of seed to water ratio was to evaluate the effect of cooking water used on changing of seed hardness. A standardised ten grams of samples were cooked with 250 ml, 300 ml, 350 ml and 400 ml of boiling water.

Results suggested that the amount of water used for cooking significantly affected the cooking quality of Digger ($P < 0.05$), but not Matilda ($P > 0.05$). This result agreed with the preliminary test which was done by Bhatty *et al.* (1983). The use of 300 ml to 400 ml volume of cooking water provided a good consistent result. The average volume of 350 ml was chosen as the standard volume of water used to perform all the cooking analysis.

ii. *Heat input*

Six hot plates from one heat input may vary the cooking textures even with a standardised cooking time. The hot plate closer to the initial source of heat input has a stronger heat input than hot plates furthest away from the electrical energy source. Thus, this may increase the cooking rate even if the amount of samples, the volume of cooking water used and the period of cooking time are all standardised. It is important to test and standarise the heat input effect on lentils to improve the reliability of results.

iii. *Standing Time*

Standing time played a significant role in varying the result of texture analysis. Samples tended to be harder after cooling down. This may be due to the hardening of seed coat by evaporation and dehydration of seeds during standing.

This test aimed to test the effect of standing time on the cooking texture of lentils between measurements, thus to improve the validity and reliability of the results. Results showed that there is no significant hardness difference from two minutes to 30 minutes of standing. Thus indicating that the standing time from two to 30 minutes played a minor effect in changing the hardness of cooked samples. However, a 15 minutes standing time was chosen to standardise the analysis method.

3.4 Texture Analyser Settings

Several problems were encountered and needed attention while running the texture analysis.

Early triggering – The texture analyser started collecting data before it reached the test samples. According to the specialist from Stable Micro Systems, there are many reasons for this problem and these are listed as follows;

i Inadequate trigger force setting;
ii Location of texture analyser too near an open window or circulating fan;
iii Loose screws on the load cell plug;
iv Incorrectly fitted probes;
v Inertia – heavy probes;
vi Vibration of the table.

Specialists from Stable Micro Systems noted that texture analyser (TA-XT2i) is an extremely sensitive piece of equipment. The unwarranted 'vibration and noise' from the environment created false surface detection problems. However, the specialist suggested to avoid this early triggering problem by increased trigger force. Trigger force is defined as the setting force that the Texture Analyser will aim to detect before data collection. This is to ensure that samples with different sizes were accounted for during texture measurement.

The optimum trigger force should be the balance between a force that is high enough to avoid triggering in the air during the downstroke and low enough to be sensitive to the

true surface of samples. Trigger force depended on the size of the probe and/or the sample for testing, the hardness of the product and the irregularity of the product surface. In this study, 0.4kg was chosen as the setting trigger force as it provided the best consistency result for the lentil samples.

Force Calibration - Different load cell required different calibration weight to change the electrical resistance in proportion to the different load applied. Calibration of load cell is essential for different sample force measurement (Stable Micro Systems 1997). Calibration of force is required to "teach" the texture analyser about the relationship between the force and resistance (Stable Micro Systems 1997). It is important to run the force calibration after changing a new load cell. For this study, "25kg load cell" was used to measure the cooking quality of lentils. 5kg-calibration weight should be used for the force calibration.

Calibration of probe height – In this study, it was necessary to perform probe height calibration, as the mode "strain to base distance" was used. Calibration of probe height is necessary to perform after change of a new probe or using the stop button during the running of tests (Stable Micro Systems 1997). As pressing of the stop button freezes the movement by interrupting the microprocessor at any point, the probe position cannot be recalculated which leads to lost probe height calibration (Stable Micro Systems 1997); Re-calibration of probe height is required.

If partially cooked samples are too hard, overload occurs, the probe halts immediately and the texture analyser will refuse to perform further measurement. This stop condition must be removed by driving the probe away from the obstruction with the "up" or "down" buttons. This functioning interrupted the microprocessor. The calibrated probe position cannot be recalculated and the probe height calibration is lost.

Percentage of Strain - This is the travelling distance and is displayed as percentage of the trigger height (Stable Micro Systems 1997). The trigger height is the height of the product if the trigger force is set to auto. However, the trigger force was set at 0.4kg in this

study. The percentage of strain in this research is the travelling distance, from the point where the probe detected exceeding of trigger force (≥ 0.4kg), to the percentage of distance set in the TA setting. Calibration of probe height is critical. The higher the percentage of travelling distance, the higher the compression force obtained. In this study, a standardised 75% travelling distance was chosen for all the texture analysis.

3.5 *Cooked Samples Selections*

After samples had cooked for the fixed cooking period, only those samples that were firm, not mushy, with the hull attached to the seed and having pleasing appearance, were chosen for the texture measurement. However, for 45 to 50 minute-overcooked samples, it is difficult to choose samples that have the above criteria. So, in this case, samples that were relatively firm and had pleasing appearance were chosen to perform Texture Profile Analysis (TPA).

3.6 TPA

TPA is a double compression test. It imitates the action of the jaw to compress twice, 25% of the one-layer displayed sample height, in a reciprocating motion. The force-time curve portrays the entire force history of the simulated masticatory action plotted by the software programme. Analysis of the force-time curve (Figure 3.2) allowed the extraction of seven texture parameters and evaluated their potential use for the prediction of sensory attributes. They are hardness, cohesiveness, adhesiveness, springiness, gumminess, fracturability and chewiness. The definitions and calculations of all the sensory attributes are outlined in Appendix 2D.

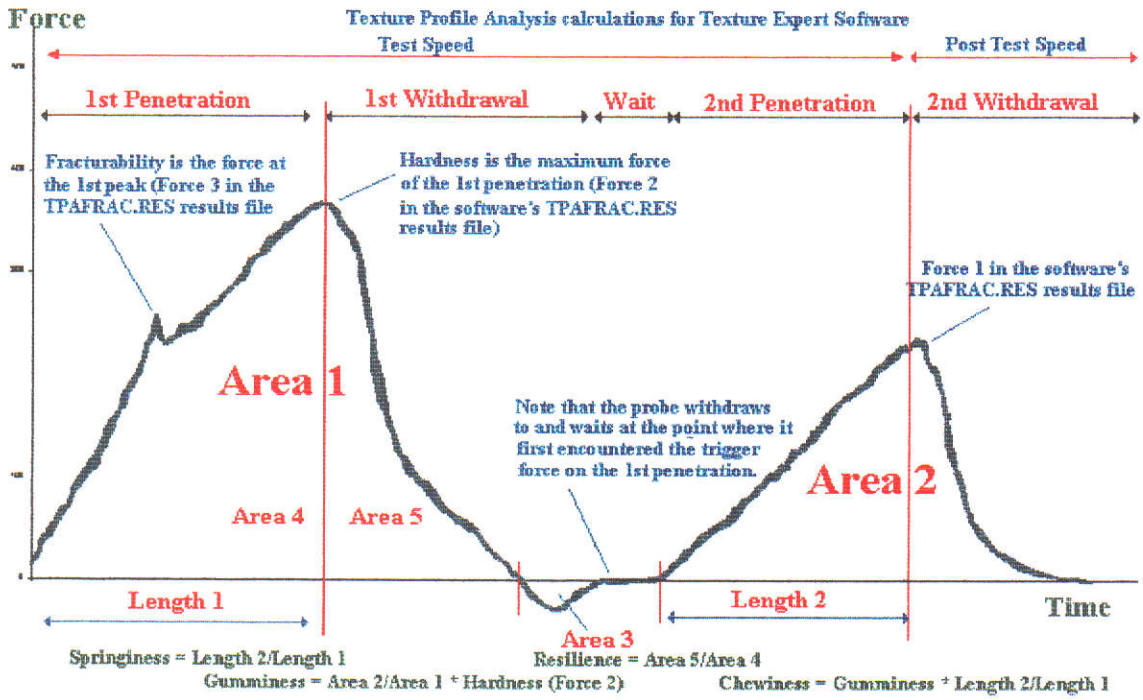


Figure 3.3 Texture Profile Analysis Curve

(Source: http://www.texturetechnologies.com/tpa.htm [2000, October 30])

3.7 Summary

From the above discussions, there are several conclusions that can be made to improve the reliability and repeatability of the methodology and the outcome results and to finalise research methodology.

A. Standarisation Conditions
   i. Seed : Water ratio is 1:17:5
   ii. Four out of six hot plates chosen as the standardised cooking equipment for each individual variety at each cooking time.
   iii. Fifteen minutes chosen as the standardised standing time

B. Texture Analyser settings
   i. Early triggering due to various reasons can be avoided by increasing the amount of trigger force. The standardised trigger force 0.4Kg was chosen as for the lentil texture analysis.
   ii. Calibration of force is essential after changing a different load cell
   iii. Calibration of probe height is necessary after the "stop" button is used and force overload occurs during measurement. It is recommended to calibrate the probe height before the beginning of any measurement.
   iv. The percentage of travelling distance is standardised at 75%.

C. Cooked samples selection
   Samples chosen for testing should be firm, not mushy, with the hull attached to the seed and having a pleasing appearance.

Chapter IV
Experimental Materials and Methods

4.1 Lentil Samples

Lentils harvested November 1999, comprised of four different cultivars grown at two different sites (Mullewa-MW and Pingaring-NE), obtained from Agricultural Western Australia (Pulse Productivity and Industry Development) were selected as samples for this study (Table 3.1).

Table 4.1 Lentil Samples and location

Cultivars	Location	Types	Commercialised or New released
Cassab	Mullewa Pingaring	Red	Commercialised
Digger	Mullewa Pingaring	Red	Commercialised
ILL 7180	Mullewa Pingaring	Red	New released
Matilda	Mullewa Pingaring	Green	Commercialised

4.2 Samples Handling

Samples were stored at room temperature in closed plastic boxes after collection, to minimise deterioration. Samples were used as whole seed: as no pre-treatment, such as soaking, was required before conducting the following series of experiment.

4.3 Physical Properties analysis of Lentil (Lens culimaris)

The physical property analysis in this study included testing cooking time, hydration coefficient and water absorption, hydration capacity and seed size. Cooking time was determined by both "Cooking Time test" and TA-XT2i Texture (Stable Micro Systems, England) analysing methods.

4.3.1 Cooking Time Test

The cooking time of lentils is determined by sequeezing seeds between thumb and finger and followed the procedures stated in Australian National Pulse Quality Manual (Petterson & Burridge 1996), as prescribed in Appendix 1A. Data was collected in duplicate.

4.3.2 TA.XT2i Texture analysis

The cooking quality (hardness) of lentils was measured with a TA-XT2i texture analyser (Stable Micro Systems, England) that was available from the laboratory in Curtin University of Technology, School of Public Health.

An A/BE40 back extrusions rig with 40mm probe was used for texture measurement. A specific texture analyser (TA) settings (Appendix 2A) with a micro program setting (Appendix 2B) as reported in Newton (N) was chosen to determine the texture profile analysis (TPA) of lentil cultivars.

Lentil samples (≈ 20g) cooked in boiling water for a fixed set of cooking periods (25, 30, 35, 40, 45 and 50 minutes) four times. For each set of cooking periods, 12 cooked samples were collected and placed in a layer on the rig for running through the texture profile analysis with a TA-XT2i texture analyser (Stable Micro Systems, England).

The first peak compression force which indicates seed hardness determined the cooking time. The time required for seeds to reach the "cooked" state was determined with statistical analysis The texture profile analysis of other texture quality was determined based on the calculation outlined in Appendix 2D. The procedures stated at Appendix 2F.

4.3.3 Hydration Coefficient and Water Absorption

Data was collected in duplicate for each individual 2, 4, 6, 8, 12, 16, 20 and 24 hour soaking period and followed the procedures outlined in Appendix 3A.

4.3.4 Hydration Capacity and unhydrated seeds

The procedures followed the Australian National Pulse Quality Manual and are attached in Appendix 3B. Data was collected in duplicate.

4.3.5 Seed size determination

Seed size was determined by counting and weighing 100 seeds, 26 times (Williams, 1988).

4.4 Proximate composition of phytate, ash, mineral and moisture content, on various lentil genotypes

4.4.1 Phytic acid analysis

Phytic acid content in seeds was analysed in the WA Chemistry Centre. Only uncooked samples were tested with duplicate. The procedures are outlined in Appendix 4A. Data was collected in duplicate.

4.4.2 Ash analysis

Ashing was conducted at the laboratory in Curtin University and followed the AOAC Official Methods procedures (Appendix 4B). The ash content in seed was determined in four replications for uncooked samples and samples that cooked in boiling water for 10, 20, 30 and 40 minutes. The calculation is based on the dry weight of samples.

4.4.3 Mineral analysis

Mineral content in seeds was determined by following the AOAC Official Methods procedures (Appendix 4C) and was conducted in the laboratory at Curtin University. Minerals are the inorganic part of food that is left after ashing. This method involved two parts;

i **Ashing** - the uncooked samples and samples that are cooked in boiling water for 10, 20, 30 and 40 minutes.

ii **Mineral Determination** - the organic matter was destroyed by digestion in acid, followed by determination of sodium, potassium, calcium, magnesium, manganese, iron, zinc and copper mineral concentration using Atomic Absorption Spectroscopy. The calculation was based on the dry weight of samples. Internal standards were used for all calibrations.

4.4.4 Moisture analysis

Moisture analysis of both uncooked and cooked at various stages seeds was carried out in the laboratory at Curtin University, following the AOAC Official Methods procedures (Appendix 4D). Moisture content was determined in duplicate, for uncooked samples and for samples cooked in boiling water for 10, 20, 30 and 40 minutes. Weight of samples reduced after being left overnight in an oven at 105 °C. The changes in weight, due to the loss of moisture through drying, determined the moisture content in samples.

4.5 *Microscopy analysis of structural changes with various cooking period*

Environmental Scanning Electron Microscopy (ESEM) allowed the examination of surfaces of any specimen, which included both wet and dry samples (Danilatos 1993). ESEM was chose to replace the traditional SEM, as it minimised the process of sample coating. Thereby, it will reduce the damage to samples due to pre-treatment process.

4.5.1 Sample Preparation

Uncooked samples were cross-sectioned into half, the thickness of the seed coat and the internal structure after sectioning were scanned and photographed with an Environmental Scanning Electron Microscopy (ESEM) at The Centre for Microscopy and Microanalysis located at the University of Western Australia.

4.5.2 ESEM Setting

ESEM was operated at 0.1 torr with low accelerating voltage (≈ 10-15 kV), low beam current (≈ 2.2 Amps), short working distance (≈ 5.5mm) and small spot size (50 – 60) to obtain the best resolution of the surface of images.

4.5.3 Digital Image Analysis

Freehand 7.0 was used for image layout and Adobe® Photoshop 4.01 was used for image manipulation by adjusting the contrast and brightness of images. The thickness of seed coat was measured manually after image layout and manipulation.

Chapter V
Analytical Methodology

5.1 Introduction

Data analysis was conducted using SPSS (1999) and Microsoft Excel (1997). Statistical analyses focused on examining the following hypotheses:

1. It is possible to use the TA-XT2i texture analyser to evaluate the cooking quality of lentils and to replace the old subjective method "The cooking time test".
2. There is no variation between cultivars of lentils in terms of cooking quality.
3. The chelating reaction of phytic acid with divalent ions affects the cooking quality of lentils.
4. Mineral retention in the lentil seeds after cooking has an effect on the cooking quality of lentils.
5. Cooking quality is related to the initial moisture content and water absorption of the lentil seeds.
6. Water absorption of lentil seeds is related to thickness of the seed coat.

The statistical analyses aimed to explore the relationships between the outcome and explanatory variables. The statistical models chosen to test the above hypotheses are included: One way ANOVA, correlation coefficient, analysis of covariance (ANCOVA) and randomised block factorial nested (hierarchical) design. The explanation on the use of each individual analysis and the assumptions needed to be addressed before conducting the analysis, will be described later.

5.2 *Data variable record for various experiments*

5.2.1 Texture Profile Analysis

Four cultivars from two locations were tested against individual cooking times (25, 30,35,40,45,50 minutes) for various texture profile analyses (hardness, cohesivenss, chewiness, gumminess, springiness, adhesiveness). The main outcome variables were the various texture attributes. The nested explanatory variables were cooking time, cultivar and location (Table 5.1).

Table 5.1 Texture Profile Analysis

Variable Name	Observation	Replications	O/E*	Value/Range
Block				
Cooking time	6 rep measure		E	25,30,35,40,45,50 mins
Cultivar			E	1 = Cassab 2 = Digger 3 = ILL 7180 4 = Matilda
Location			E	1 = Mullewa 2 = Pingaring
Hardness	6 rep measure	12	O	
Cohesiveness	6 rep measure	12	O	
Chewiness	6 rep measure	12	O	
Gumminess	6 rep measure	12	O	
Springiness	6 rep measure	12	O	
Adhesiveness	6 rep measure	12	O	

(O/E* - O= Outcome variables, E= Explanatory variables)

5.2.2 Phytic Acid content

The outcome variable was the baseline value / observations of phytic acid. The explanatory variables were cooking time, cultivar and location (Table 5.2).

Table 5.2 Data Variable Record for Phytic Acid

Variable Name	Observation	Replications	O/E*	Value/Range
Blocks				
Cultivar			E	1 = Cassab 2 = Digger 3 = ILL 7180 4 = Matilda
Location			E	1 = Mullewa 2 = Pingaring
Phytic acid	Baseline	2	O	

(O/E* - O = Outcome variables, E= Explanatory variables)

5.2.3 Seed Coat Thickness

The outcome variable was the baseline value / observations of seed coat thickness. The explanatory variables were cooking time, cultivar and location (Table 5.3).

Table 5.3 Data Variable Record for Seed Coat Thickness

Variable Name	Observation	Replications	O/E*	Value/Range
Block				
Cultivar			E	1 = Cassab 2 = Digger 3 = ILL 7180 4 = Matilda
Location			E	1 = Mullewa 2 = Pingaring
Thickness	Baseline	5	O	

(O/E* - O= Outcome variables, E= Explanatory variables)

5.2.4 Mineral Content

Four cultivars from two locations were tested against individual cooking times (0,10,20,30,40 minutes) for various mineral contents. The main outcome variables were the mineral contents. The nested explanatory variables were cooking time, cultivar and location (Table 5.4).

Table 5.4 Data Variable Record for Mineral content

Variable Name	Observation	Replications	O/E*	Value/Range
Block				
Cooking time	5 rep measure		E	0,10,20,30,40 mins
Cultivar			E	1 = Cassab 2 = Digger 3 = ILL 7180 4 = Matilda
Location			E	1 = Mullewa 2 = Pingaring
Mineral				
Na	5 rep measure		4 O	
K	5 rep measure		4 O	
Mg	5 rep measure		4 O	
Mn	5 rep measure		4 O	
Ca	5 rep measure		4 O	
Zn	5 rep measure		4 O	
Fe	5 rep measure		4 O	
Cu	5 rep measure		4 O	

(O/E* - O= Outcome variables, E= Explanatory variables)

5.2.5 Moisture Content

Four cultivars from two locations were tested against individual cooking times (0,10,20,30,40 minutes) for moisture content. The main outcome variables were the moisture content. The nested explanatory variables were cooking time, cultivar and location (Table 5.5).

Table 5.5 Data Variable Record for Moisture Content

Variable Name	Observation	Replications	O/E*	Value/Range
Block				
Cooking time	5 rep measure		E	0,10,20,30,40 mins
Cultivar			E	1 = Cassab 2 = Digger 3 = ILL 7180 4 = Matilda
Location			E	1 = Mullewa 2 = Pingaring
Moisture	5 rep measure	2	O	

(O/E* - O= Outcome variables, E= Explanatory variables)

5.2.6 Water Absorption and hydration coefficient

Four cultivars from two locations were tested against individual soaking times (2,4,6,8,12,16,20,24 hours) for both water absorption and hydration coefficient. The main outcome variables were water absorption and hydration coefficient. The nested explanatory variables were soaking time, cultivar and location (Table 5.6).

Table 5.6 Data Variable Record for Water Absorption and Hydration Coefficient

Variable Name	Observation	Replications	O/E*	Value/Range	
Block					
Soaking Time	8 rep. Measure		E	2,4,6,8,12,16,20,24 hrs	
Cultivar			E	1,2,3,4	label 1 = cassab label 2 = digger label 3 = ILL 9180 label 4 = Matilda
Location			E	1,2	label 1 = Mullewa label 2 = Pingaring
Water Absorption	8 rep. Measure		2 O		
Hydr. Coeff.	8 rep. Measure		2 O		
Solid lost	8 rep. Measure		2 O		

(O/E* - O= outcome variables, E= Explanatory variables)

5.3 Statistical Modeling

5.3.1 One way Analysis of Variance (ANOVA)

One way ANOVA compared the means of more than two groups or levels of an independent variable (Coakes 1999 & Pagano & Gauvreau 1993). The procedure is basically deriving two different estimates of population variance from the data. The first estimate (between-groups variance) is a measure of the effect of the independent variable combined with error variance. The second estimate (within-groups variance) is of error variance by itself.

F-ratio is the ratio of between-groups variance to within-group variance. A significant F-value shows that the population means are probably not all equal (Coakes 1999). Post-hoc analysis is used to locate where the significance lies by running through a multiple comparison on the entire set of data. However, this test planned to protect against type I errors and it is harder to obtain a significant result (Coakes 1999 & Pagano & Gauvreau 1993).

There are two necessary assumptions to meet before conducting the One Way ANOVA model; sample populations must be normal, and the scores in each group should have homogeneity of variance (Coakes 1999 & Pagano & Gauvreau 1993). Transformation of continuous data is required if assumptions are not met. In this research, One Way ANOVA model was used in evaluating the effect of cooking time on changing seed hardness.

5.3.2 Univariate Analysis Of Variance (UANOVA)

The main aim of the presently developed modelling was to model each individual outcome variable against various explanatory variables and to draw conclusions on the effects of each explanatory variable, after accounting for all other explanatory variables in the model. Analysis of variance is the most appropriate analytical method for examining continuous variables with multiple categorical explanatory variables. A single model is developed to combine all the explanatory variables and estimate the adjusted effect of each explanatory variable after the effects of other variables in the model are taken into account.

Univariate (UANOVA) allows examination of effects of several independent variables (factors) on a dependent variable. It allows conclusions to be drawn about the effects from different models and it also allows the testing of interactions between factors (Norusis 1993).

The assumptions for UANOVA are the same as One way ANOVA (namely normality / homogeneity of variance). This model evaluated the effect of variety, location and the interaction (variety*location) on phytic acid, thickness of seed coat, seed size and the initial moisture content in seeds.

Model specification:

y = constant + cooking time + variety + variety*location

5.3.3 Nested (hierarchical) Analysis of Variance

The presently established model is not a simple analysis of variance model, but a more complex nested (hierarchical) factorial design: varieties are naturally clustered within cooking time, location is naturally clustered within cooking time and the interaction of variety and location is naturally clustered within cooking time. In order to examine the potential effects in an analysis of variance, the variability between cooking times must be set apart from the variability among varieties, between location and between the interaction of location and variety so as to get the correct error term. These components are referred to as "components of variation".

The main effect of each location and variety level access variable on the outcome variable, is assessed by partial F-tests, in which the variation due to each access variable is divided by the unexplained variation. Partial F-tests are based on Type III sums of squares (also called partial sums of squares). Type III sum of squares for all effects do not sum to the model sums of squares but have the important property of being invariant to the ordering of effects in the model. This approach gives adjusted estimates of the variation due to a particular term in the model, simultaneously taking into account the variation due to all other terms in the model.

Table 5.7 Two-stage General Linear Modelling Template

Sources of Variation			Terms
I	Between Cooking Times (or Soaking Times)		Cooking Time (or Soaking Times)
	II	Between Varieties within Cooking Time (or Soaking Time)	Variety
		Unexplained within Variety within Cooking Time (or Soaking Times)	Error
	III	Between Locations within Cooking Time (or Soaking Times)	Location
		Unexplained within Location within Cooking Time (or Soaking Times)	Error
	IV	Location*Variety within Cooking Time (or Soaking Times)	Location*Variety
		Unexplained within Location *Variety within Cooking Time (or Soaking Times)	Error
	Unexplained within Cooking Times		Error

Model specification:

The hierarchical model used in the modelling strategy can generally be expressed as follows;

$$y = \text{constant} + \{\text{Cooking Time}^*\} + \{[\text{Variety (Cooking Time}^*)] + [\text{Location (Cooking Time}^*)] + [\text{Variety*Location (Cooking time}^*)]\}$$

(*Cooking time entered as a factor)

This model is used to determine the effect of cooking on seed hardness, mineral contents after cooking and moisture content after cooking. It is also used to determine the effect of soaking on water absorption.

5.3.4 Analysis of Covariance (ANCOVA)

ANCOVA is a combination of linear regression analysis and analysis of variance statistical techniques. The results of ANCOVA are adjusted for the linear relationships between dependant variables and the covariates (Norusis 1993). This model looked at the association between the cooking time with various dependent variables such as hardness and water absorption, but in this instance cooking time and soaking time were included in the model as covariate. Evaluating the plotted exponential line graph (cooking time vs. hardness) can compare the differences in cooking rate among cultivars.

Model specification:

The ANCOVA model used in the modelling strategy can generally be expressed as follows;

y = constant + cooking time + variety + variety*location (*Cooking time entered as a covariate)

5.4 Analysis Procedures

5.4.1 Data Screening

Data screening, which included several phases, is an important step before examination of the characteristics of the data and is listed as follows;

i. Graphical examination of the nature of the variables and the relationships that form the basis of multivariate analysis (Hair 1990).

5.4.2 Assumptions

Before conducting the statistical analysis for both the nested factorial model and ANCOVA, the data needs to be checked to ensure the underlying assumptions are being met.

1. **The distribution of each outcome variable in each model is normal**

   Kolmogorov - Smirnov statistics are used in SPSS to test the normality of the outcome variables. With a Lilliefors significance level, Kolmogorov-Smirnov statistics produced a normal probability and detrended probability plots. The normality is assumed, if the significance level is greater than 0.05, (Hair 1990).

   However, if the Kolmogorov -Smirnov statistics showed a significant result, by checking the shape of distribution from a histogram plot, the symmetry of the distribution can be assessed. For symmetric distributions and moderate sample sizes, normality of the sampling distribution can be assumed.

2. **All groups are subject to the same within-group variance (homoscedasticity)**

   Each outcome variable was examined for homoscedasticity by plotting the outcome variables against explanatory variables or plotting the standardised residuals against fitted values.

3. **The various explanatory variables in the model are not highly correlated**

   This is a designed experiment so the explanatory variables should not be correlated. For example, cooking time is not correlated with variety.

4. **The observations in the data are independent (Hair 1990)**

5.4.3 Descriptive Statistics

Descriptive statistics are used to explore the data and summarise and describe the observations (Coakes 1999). Sample mean, standard deviations, minimum and maximum summaries of descriptive statistics were obtained from the data set on all the physical and biochemical properties analysis.

5.4.3 Correlation

Pearson correlation is used to evaluate the strength and direction of the relationship between seed hardness and cooking time on individual cultivars. The correlation between cooking quality vs. seed size, cooking quality vs. seed coat thickness and water absorption vs. seed coat thickness are analysed by using the mean of various cultivars.

Chapter VI
Analytical Results

6.1 Cooking Time Determination

6.1.1 Descriptive Statistics

The descriptive results of hardness among various cultivars are listed in Table 6.1.

Table 6.1 Descriptive Summary of Mean, Range, Standard Deviation and % Cooked of various cooking times for 1999 samples (Cassab, Digger, ILL 7180 and Matilda from both Mullewa and Pingaring).

	Cassab-Mullewa				Cassab-Pingaring			
Cooking time	Mean Force[1]	Range	SD	% Cooked[2]	Mean Force[1]	Range	SD	% Cooked[2]
25 mins	296	348 - 211	46	35	284	345 - 236	28	35
30 mins	240	271 - 216	16	70	256	344 - 214	32	75
35 mins	197	224 - 169	18	95	204	221 - 167	16	90
40 mins	169	195 - 121	21	100	189	230 - 141	25	100
45 mins	172	248 - 116	37		192	224 - 158	20	
50 mins	153	176 - 126	14		172	195 - 112	24	
	ILL 7180-Mullewa				ILL 7180-Pingaring			
Cooking time	Mean Force[1]	Range	SD	% Cooked[2]	Mean Force[1]	Range	SD	% Cooked[2]
25 mins	321	348 - 266	26	50	322	345 - 276	21	50
30 mins	236	275 - 210	20	80	259	283 - 230	15	50
35 mins	192	223 - 157	21	100	215	245 - 164	24	100
40 mins	172	203 - 110	24		199	233 - 138	24	
45 mins	160	185 - 118	19		191	225 - 148	23	
50 mins	149	193 - 116	23		185	207 - 143	16	
	Digger-Mullewa				Digger-Pingaring			
Cooking time	Mean Force[1]	Range	SD	% Cooked[2]	Mean Force[1]	Range	SD	% Cooked[2]
25 mins	300	348 - 254	30	35	291	339 - 247	29	25
30 mins	223	263 - 174	30	50	254	285 - 217	20	65
35 mins	190	240 - 148	30	90	202	235 - 153	28	100
40 mins	185	224 - 160	19	100	209	239 - 182	19	
45 mins	184	208 - 156	14		203	235 - 168	22	
50 mins	173	194 - 156	12		187	223 - 167	17	
	Matilda-Mullewa				Matilda-Pingaring			
Cooking time	Mean Force[1]	Range	SD	% Cooked[2]	Mean Force[1]	Range	SD	% Cooked[2]
25 mins	341	346 - 311	10	5	322	345 - 297	18	10
30 mins	342	346 - 329	6	5	285	345 - 241	37	15
35 mins	272	347 - 215	53	50	258	294 - 203	30	50
40 mins	224	260 - 167	28	80	244	280 - 207	22	75
45 mins	200	239 - 173	24	90	219	243 - 168	27	90
50 mins	179	224 - 143	21		220	243 - 196	15	

[1] Mean Force (N, Newtons) determined with Texture analyser

[2] % cooked defined as the percentage of seeds that reached the cooked stage determined with "Cooking Time test"

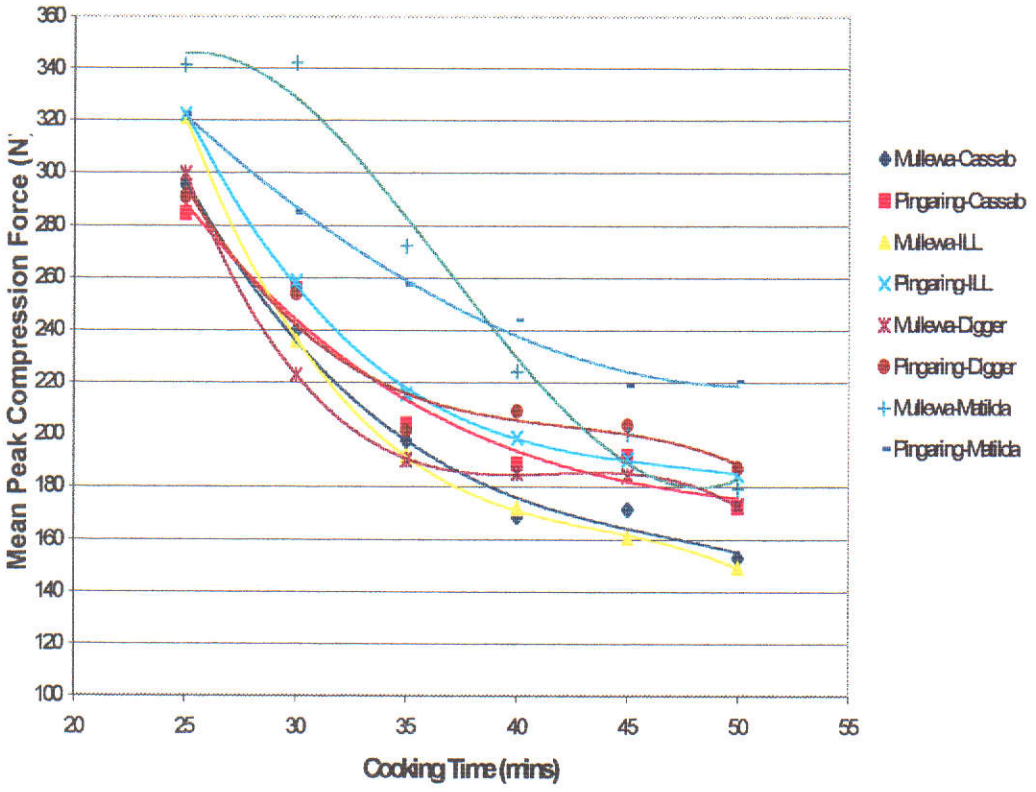


Figure 6.1: The relationship between seed hardness (N) determined with Texture Analyser (TA-XT2i), and cooking time in 1999 samples (Cassab, Digger, ILL 7180 and Matilda from both Mullewa and Pingaring).

The relationship between hardness and cooking time of lentils are shown in Figure 6.1. Hardness declined curvilinearly with increased cooking time and remained constant after 35 to 40 minutes cooking in all samples, except for Matilda (Mullewa).

6.1.2 One-way ANOVA

Hypothesis: The cooking qualities (seed hardness) of various cultivars do not vary after different periods of cooking

Outcome variable: Hardness (Peak Compression force N)

The hardness of various cultivars is computed into a log transformation to improve the skewness of distribution.

Explanatory variable: Cooking time (mins) (Entered as a factor)

i. Cassab - Mullewa

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($P < 0.05$). Cassab which was grown at Mullewa showed non-significant changes of mean hardness after 35 minutes or longer cooking time (Table 6.2).

Table 6.2: Post Hoc test for Cassab - Mullewa

		Hardness					
	Cooking Time	25	30	35	40	45	50
Hardness	25	--	*	*	*	*	*
	30	*	--	*	*	*	*
	35	*	*	--	NS	NS	*
	40	*	*	NS	--	NS	NS
	45	*	*	NS	NS	--	NS
	50	*	*	NS	NS	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

ii. Digger – Mullewa

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($p < 0.05$). Digger which was grown at Mullewa showed non-significant changes of mean hardness after 35 minutes or longer cooking time (Table 6.3).

Table 6.3: Post Hoc test for Digger - Mullewa

		Hardness					
	Cooking Time	25	30	35	40	45	50
	25	--	*	*	*	*	*
	30	*	--	*	*	*	*
Hardness	35	*	*	--	NS	NS	NS
	40	*	*	NS	--	NS	NS
	45	*	*	NS	NS	--	NS
	50	*	*	NS	NS	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

iii. ILL 7180 – Mullewa

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($p < 0.05$). ILL 7180 which was grown at Mullewa showed non-significant changes of mean hardness after 35 minutes or longer cooking time (Table 6.4).

Table 6.4: Post Hoc test for ILL 7180 - Mullewa

		Hardness					
	Cooking Time	25	30	35	40	45	50
	25	--	*	*	*	*	*
	30	*	--	*	*	*	*
Hardness	35	*	*	--	NS	*	*
	40	*	*	NS	--	NS	NS
	45	*	*	NS	NS	--	NS
	50	*	*	NS	NS	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

iv. Matilda – Mullewa

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($p < 0.05$). Matilda which was grown at Mullewa showed non-significant changes of mean hardness after 40 minutes or longer cooking time (Table 6.5).

Table 6.5: Post Hoc test for Matilda - Mullewa

		Hardness					
	Cooking Time	25	30	35	40	45	50
Hardness	25	--	*	*	*	*	*
	30	*	--	*	*	*	*
	35	*	*	--	*	*	*
	40	*	*		--	NS	*
	45	*	*	*	NS	--	NS
	50	*	*	*	*	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

v. Cassab – Pingaring

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($p < 0.05$). Cassab which was grown at Pingaring showed non-significant changes of mean hardness after 35 minutes or longer cooking time (Table 6.6).

Table 6.6: Post Hoc test for Cassab - Pingaring

		Hardness					
	Cooking Time	25	30	35	40	45	50
Hardness	25	--	*	*	*	*	*
	30	*	--	*	*	*	*
	35	*	*	--	NS	NS	*
	40	*	*	NS	--	NS	NS
	45	*	*	NS	NS	--	NS
	50	*	*	*	NS	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

vi. Digger – Pingaring

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($p < 0.05$). Digger which was grown at Pingaring showed non-significant changes of mean hardness after 35 minutes or longer cooking time (Table 6.7).

Table 6.7: Post Hoc test for Digger - Pingaring

		Hardness					
	Cooking Time	25	30	35	40	45	50
Hardness	25	--	*	*	*	*	*
	30	*	--	*	*	*	*
	35	*	*	--	NS	NS	*
	40	*	*	NS	--	NS	NS
	45	*	*	NS	NS	--	NS
	50	*	*	*	NS	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

vii. ILL 7180 – Pingaring

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($p < 0.05$). ILL 7180 which was grown at Pingaring showed non-significant changes of mean hardness after 35 minutes or longer cooking time (Table 6.8).

Table 6.8: Post Hoc test for ILL 7180 - Pingaring

		Hardness					
	Cooking Time	25	30	35	40	45	50
Hardness	25	--	*	*	*	*	*
	30	*	--	*	*	*	*
	35	*	*	--	NS	NS	NS
	40	*	*	NS	--	NS	NS
	45	*	*	NS	NS	--	NS
	50	*	*	NS	NS	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

viii. Matilda – Pingaring

One way ANOVA showed that there is a significant mean hardness difference between cooking time ($p < 0.05$). Matilda which was grown at Pingaring showed more complicated changes of mean hardness with cooking. There is no significant mean hardness difference between 25 and 30 minutes of cooking. The mean hardness for 30 minutes cooking seeds showed insignificant difference comparing with 25 and 35 minutes of cooking seeds. The mean hardness for 35 minutes cooking seeds showed insignificant difference comparing with 30 and 35 minutes of cooking seeds. Based on the post hoc test, the mean hardness started to show insignificant changes only after 40 minutes (Table 6.9).

Table 6.9: Post Hoc test for Matilda - Pingaring

		Hardness					
	Cooking Time	25	30	35	40	45	50
Hardness	25	--	NS	*	*	*	*
	30	NS	--	NS	*	*	*
	35	*	NS	--	NS	*	*
	40	*	*	NS	–	NS	NS
	45	*	*	*	NS	–	NS
	50	*	*	*	NS	NS	--

*One-way ANOVA is significant at the 0.05 level (2 -tailed)
NS (non-significant)

6.2 Cooking Quality (hardness)

6.2.1 Correlation and Correlation coefficient

Hypothesis: There is no correlation between seed hardness and cooking time

Outcome variable: Hardness (N)

Explanatory variable: Cooking time (mins)

Hardness of cooked samples is strongly negatively correlated with cooking time ($P < 0.01$). Correlation coefficient is between –0.75 to –0.9. Cooking time explained between 58 to 81% of the variability of hardness for all cultivars (Table 6.10).

Table 6.10: Pearson Correlation and R^2 of Seed hardness with cooking time among cultivars

	Cooking time							
	Cassab		Digger		ILL		Matilda	
	MW*	NE*	MW*	NE*	MW*	NE*	MW*	NE*
Pearson Correlation (R)	-0.831*	-0.805*	-0.752*	-0.760*	-0.869*	-0.845*	-0.899*	-0.793*
R^2	0.69	0.65	0.57	0.58	0.76	0.71	0.81	0.63

* MW- Mullewa; NE- Pingaring
* Transformation of data is performed before analysis

6.2.2 Nested Factorial Design

Hypothesis:

There is variation between cultivars of lentils for cooking quality (seed hardness).

Outcome variable: Peak compression Force (N)

The outcome variable (Peak compression force) was not normally distributed and a log transformation is required before conducting the statistical analysis.

Explanatory variables:

The nested explanatory variables are cooking time, variety and location.

Model Specification:

Cooking quality = {Cooking Time} + {[Variety (Cooking Time)] + [Location (Cooking Time)] + [Variety*Location (Cooking time)]}

Table 6.11 The effect of Cooking time, variety, location and variety*location on Cooking Quality in 1999 Lentil Samples (Cassab, Digger, ILL 7180 and Matilda).

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	29332789	1	29332789	1424.74	0
	Error	385448.3	19	20588.177 a		
COOKTIME	Hypothesis	1227049	5	245409.9	11.92	0
	Error	385448.3	19	20588.177 a		
VARIETY(COOKTIME)	Hypothesis	273306.1	18	15183.67	6.987	0
	Error	39117.19	18	2173.177 b		
LOCATION(COOKTIME)	Hypothesis	45466.09	6	7577.682	3.487	0.02
	Error	39117.19	18	2173.177 b		
VARIETY * LOCATION(COOKTIME)	Hypothesis	39117.19	18	2173.177	3.548	0
	Error	323441	528	612.578 c		

a MS(VARIETY(COOKTIME)) + MS(LOCATION(COOKTIME)) - MS(VARIETY * LOCATION(COOKTIME))

b MS(VARIETY * LOCATION(COOKTIME))

c MS(Error)

Effects of Cooking time, Cultivars and Location on cooking quality

In the nested model, there was a significant variation between cooking time in mean hardness ($P < 0.05$). After accounting for the variation between cooking time, there was significant variation in hardness between cultivars within cooking time ($P < 0.05$). There was also significant variation in hardness between location within cooking time after accounting for the factor of cooking time. There was significant hardness variation between location and cultivar interaction after the variation between cooking time was accounted ($P < 0.05$) (Table 6.11). ILL 7180 has the widest mean hardness variation between locations and conversely Matilda has the least (Figure 6.2).

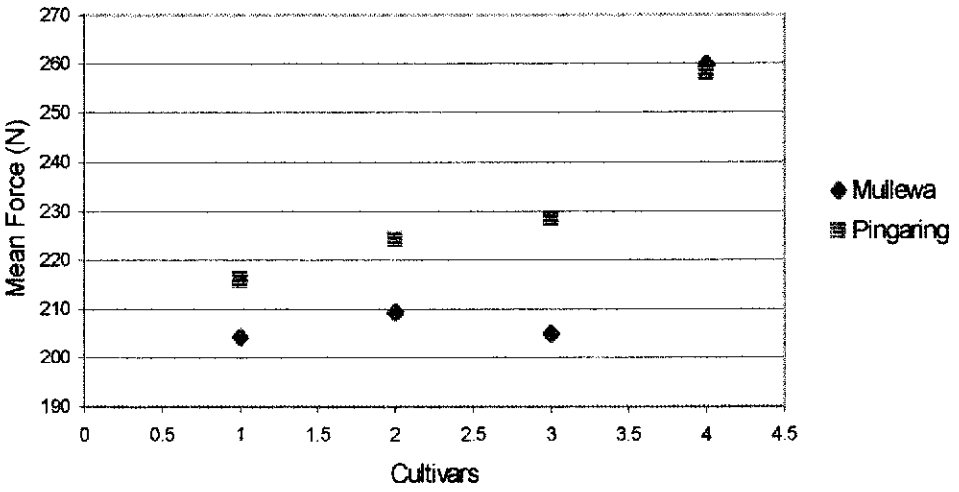


Figure 6.2 The Interaction Effect between Cultivars and Locations on mean hardness of 1999 Lentil Samples (1-Cassab, 2-Digger, 3-ILL 7180 and 4-Matilda)

6.3 Cooking Quality vs. Water Absorption, Seed coat thickness and Seed size

6.3.1 Univariate Factorial and Nested Factorial Analysis

Table 6.12: Summary of the Variety*Location interaction effect on seed size, initial moisture content and phytic acid.

Outcome variables	Statistical analysis	Significance [3]
Seed Size	Between Variety*Location[1]	P = 0.000
Thickness of Seed Coat	Between Variety*Location [1]	P = 0.000
Water Absorption	Between Variety*Location (within Soaking time) [2]	P = 0.206

[1] Model using: Univariate Factorial Analysis
[2] Model using: Nested Factorial Analysis
[3] Significant Different at P < 0.05

1. **Thickness of seed coat**

   The interaction of variety and location significantly affected the thickness of seed coat of different cultivars ($P < 0.05$).

2. **Seed Size**

   The interaction of variety and location significantly affected the seed size of different cultivars ($P < 0.05$).

3. **Water absorption**

   There was significant variation in water absorption between varieties within soaking time after accounting for the variation between soaking times ($P < 0.05$). However, there was no interaction effect on the water absorption between location and variety ($P > 0.05$).

6.3.2 Seed coat thickness vs. water absorption

Seed coat thickness does not affect the amount of water that is imbibed into seeds. Pearson correlation showed that there is positive, but weak correlation between the thickness of seed coat and water absorption ($r = 0.395$).

Table 6.13: Seed coat thickness vs. Water absorption % of Cassab, Digger, ILL and Matilda grown at Mullewa and Pingaring

Cultivars	Locations	Hardness (N) [1]	Seed coat thickness (um)	Water Absorption [2]
Cassab	Mullewa	169	45.29	118.87%
	Pingaring	189	49.04	101.48%
Digger	Mullewa	185	34.26	91.44%
	Pingaring	209	47.01	91.11%
ILL 7180	Mullewa	172	37.25	93.98%
	Pingaring	199	42.96	92.63%
Matilda	Mullewa	224	42.38	98.52%
	Pingaring	244	43.43	103.53%

[1] Hardness determined after 40 minutes cooking time.
[2] Water Absorption % indicated is samples soaked for 10 hours.

6.3.3 Hardness (Cooking Quality) vs. Seed size and Seed coat thickness

Seed size is weakly correlated to the hardness of seed (r = 0.202). Seed size marginally affected the hardness of seeds (P = 0.048) (Table 6.14).

Pingaring cultivars, which have a thicker seed coat, tended to have a harder cooked texture (Table 6.14). The thickness of seed coat does not affect the hardness of seeds. The thickness of seed coat is not correlated with the hardness of seed (r = 0.04).

Table 6.14: Seed Hardness (cooking quality), Seed size and Seed coat thickness of Cassab, Digger, ILL and Matilda grown at Mullewa and Pingaring

Cultivars	Locations	Hardness (N)*	Seed Size (g/100 seeds)	Seed coat thickness (um)
Cassab	Mullewa	169	4.294	45.29
	Pingaring	189	4.259	49.04
Digger	Mullewa	185	4.219	34.26
	Pingaring	209	4.488	47.01
ILL 7180	Mullewa	172	3.822	37.25
	Pingaring	199	4.186	42.96
Matilda	Mullewa	224	5.196	42.38
	Pingaring	244	3.847	43.43

* Hardness determined after 40 minutes cooking time

6.4 *Cooking Quality vs. Proximate Chemical Compositions*

6.4.1 Univariate Factorial and Nested Factorial Analysis

Table 6.15: Summary of the Variety*Location interaction effect on thickness of seed coat, water absorption, moisture content after cooking and mineral content after cooking.

Outcome variables	Statistical analysis	Significance*
Initial Moisture Content	Between Variety*Location [1]	P = 0.411
Phytic acid	Between Variety*Location [1]	P = 0.000
Moisture Content after cooking	Between Variety*Location (within Soaking time) [2]	P = 0.917
Minerals content		
Na	Between Variety*Location (within Soaking time) [2]	P = 0.000
K	Between Variety*Location (within Soaking time) [2]	P = 0.000
Mg	Between Variety*Location (within Soaking time) [2]	P = 0.000
Mn	Between Variety*Location (within Soaking time) [2]	P = 0.000
Ca	Between Variety*Location (within Soaking time) [2]	P = 0.000
Zn	Between Variety*Location (within Soaking time) [2]	P = 0.000
Fe	Between Variety*Location (within Soaking time) [2]	P = 0.000
Cu	Between Variety*Location (within Soaking time) [2]	P = 0.000

[1] Model using: Univariate Factorial Analysis
[2] Model using: Nested Factorial Analysis
[3] Significant Different at P < 0.05

a. Moisture

There is no significant difference in the initial moisture content among cultivars (P > 0.05). There is also no significant difference in the moisture content after cooking among cultivars (P > 0.05).

b. Phytic acid

There is significant location and cultivar interaction effect on the phytic acid content in seeds (P < 0.05).

c. Mineral

There is significant location and cultivar interaction effect on the sodium, potassium, magnesium, manganese, calcium, zinc, iron and copper content in seeds after cooking (P < 0.05).

6.4.2 Hardness (Cooking Quality) vs. Initial Proximate Chemical Composition

Cultivars grown at Pingaring generally have slightly more phytic acid and phosphorus content in seeds than Mullewa cultivars. Similarly, Pingaring cultivars are harder than Mullewa cultivars (Table 6.16).

Table 6.16 The relationship between Seed hardness and Proximate Chemical Compositions in 1999 Lentil Cultivars (uncooked samples)

SAMPLES / Mean		Force* (N)	Phytic acid %db	P (ICP) %db	K (ICP) %db	Na (ICP) %db	Ca (ICP) %db	Mg (ICP) %db	S (ICP) %db	B (ICP) mg/kg	Cu (ICP) mg/kg	Fe (ICP) mg/kg	Mn (ICP) mg/kg	Zn (ICP) mg/kg
Cassab	Mullewa	169	0.61	0.28	0.83	<0.01	0.08	0.09	0.16	5	12	70	23	29
	Pingaring	189	0.76	0.31	0.80	0.03	0.06	0.09	0.14	7	7.1	49	12	23
Digger	Mullewa	185	0.52	0.26	0.78	<0.01	0.08	0.08	0.15	4	13	68	20	27
	Pingaring	209	0.77	0.31	0.80	0.04	0.07	0.09	0.14	7	15	55	12	24
ILL 7180	Mullewa	172	0.78	0.31	0.90	<0.01	0.08	0.09	0.15	5	12	80	20	32
	Pingaring	199	0.76	0.31	0.80	0.02	0.07	0.09	0.13	5	7.7	55	11	23
Matilda	Mullewa	224	0.64	0.30	0.81	0.01	0.09	0.09	0.16	5	9.7	71	21	31
	Pingaring	244	0.70	0.32	0.76	0.04	0.07	0.10	0.14	5	7.5	60	12	24

* Force: Hardness determined after 40 minutes cooking time

6.4.3 Hardness (Cooking Quality) vs. Mineral retention

Cultivars grown at Pingaring generally have more minerals content retained in seeds than Mullewa cultivars after samples were cooked for forty-minutes. Similarly, Pingaring cultivars cooked for forty minutes are harder than Mullewa cultivars (Table 6.17).

Table 6.17: Seed Hardness (cooking quality), mineral content retention (%) of Cassab, Digger, ILL and Matilda grown at Mullewa and Pingaring after 40 minutes cooking.

Mean Mineral Content	Cassab		Digger		ILL		Matilda	
	MW♣	NE♣	MW♣	NE♣	MW♣	NE♣	MW♣	NE♣
Na*	2.4	7.2	3.1	5.9	2.5	4.4	2.4	6.5
K*	24.2	48.4	19.1	41.8	20.7	36.3	29.0	42.9
Ca*	14.5	22.1	16.5	20.0	12.8	16.8	18.8	20.4
Mg*	12.4	24.5	11.9	22.2	11.1	19.2	17.7	25.6
Mn*	0.7	0.6	0.7	0.6	0.6	0.5	0.8	0.6
Zn*	0.4	0.8	0.4	0.7	0.3	0.5	0.6	0.8
Cu*	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.2
Fe*	1.1	1.4	1.1	1.3	1.0	1.1	1.3	1.1
Mean Hardness (N)*	169	189	185	209	172	199	224	244

*Hardness and mineral content indicated are seeds after 40 minutes cooking time

♣ MW- Mullewa; NE- Pingaring

♣ Significant Different at $P < 0.05$

6.5 Summary of Results

1. **Cooking time determination**

   The length of cooking significantly varied with the hardness of seeds ($P < 0.05$). The hardness of lentil is strongly negatively correlated and declined curvilinearly with increased cooking time (Figure 6.1 and Table 6.10). Except for Matilda which was grown at both Mullewa and Pingaring, the rest of the cultivars showed reduced variation of hardness after 35 minutes of cooking (Table 6.2 – Table 6.9).

2. **Cooking quality (hardness) Comparison**

   The hardness of various cultivars is significantly different from each other. There is a significant hardness variation between the interaction of cultivar and location after the variation between cooking time was accounted for ($P < 0.05$). ILL 7180 had the widest range of mean hardness differences between locations and Matilda the least.

3. **Cooking Quality vs. Water Absorption, Seed coat thickness and Seed size**

   There is significant variety and location interaction effect on the thickness of seed coat, seed size and water absorption among cultivars ($P < 0.05$).

   i. **Seed coat thickness vs. water absorption**

      Seed coat thickness does not correlate with the rate of water absorption.

   ii. **Cooking Quality vs. seed size and seed coat thickness**

      Seed size and seed coat thickness does not correlate with the cooking quality of various cultivars.

4. Cooking Quality vs. Chemical Composition

There is significant variety and location interaction effect on the mineral content of lentil cultivars ($P < 0.05$).

i. Cooking Quality vs. initial proximate chemical composition

Lentils grown at Pingaring are generally had a higher in initial mineral content than those grown at Mullewa.

ii. Cooking Quality vs. Mineral retention

Lentils grown at Pingaring are generally had a higher in mineral retention rate and were harder than those grown at Mullewa.

Chapter VII
Discussion

7.1 Introduction

Lentils are used predominantly as food for human consumption, so cooking quality is a decisive factor to consumers. The "cooking quality" in this study is defined as peak compression force (N), after the completion of starch gelatinisation and break down of protein matrix during cooking. The aim of this research was to develop an objective measurement to evaluate the cooking quality of lentils and to investigate the relationship between lentil cooking quality with some of the physical and biochemical properties. The objectives of the study are listed as follows;

- To develop an objective measurement to determine the cooking quality of lentils;
- To develop an objective method to determine the cooking time of lentils;
- To investigate the interaction effect of environment and genotype on the variations of lentil cooking quality among cultivars
- To investigate the relationship between cooking quality and water absorption of seed
- To evaluate the effect of seed coat thickness on water absorption of seed
- To evaluate the relationship between cooking quality and the content of phytic acid and minerals.

7.2 Measurement of Cooking Quality

The first objective of the study was to develop an objective measurement technique to determine the cooking quality of lentils and replace the old subjective "Cooking Time Test" methods.

7.2.1 "The Cooking Time test" method

"*The Cooking time test*" method determines the cooking quality of lentils by testing the softness of seeds and evaluates the physical changes of seeds by visual observation. For every five minutes cooking, ten seeds are removed and tested for softness by compressing with fingers.

The seed coat for a partially cooked lentil was easily removed while pressing with fingers. The cooked portion is softer when compared to the hard and uncooked portion in the centre of the cotyledons. The uncooked portion in the centre of the cotyledons changed from white to whitish-brown and the cooked portion was greenish-brown in colour. The size of the white ungelatinised area relative to the total area of the cotyledons was clearly distinguishable.

One of the limitations of using "The cooking time test" to determine the cooking quality of lentils is that it does not acknowledge the effect of protein structures and linkages on cooking quality. Additionally, as this is a subjective measurement, minor variation on the cooking quality of lentils among cultivars could not be so easy to detect and erroneous conclusions might be drawn. Using TA-XT2i texture analyser can reduce the limitation of reliable subjective testing and generate more reproducible results.

7.2.2 Texture Analyser

TA-XT2i texture analyser determined the cooking quality of lentils by measuring the peak compression force (N) after samples were cooked for a certain period of time. This method unlike the "Cooking time test", measured the physical changes; the starch gelatinisation and the break down of protein linkages after cooking; by compressing the samples with a constant speed force (1 mm/sec). The peak compression force was measured when the probe traveled and reached the pre-set percentage of sample height (75%).

One layer of sample seeds, which consisted of approximately 20 seeds, was measured at the same time for one measurement. This improved the reliability of the test, as the characteristics of each lentil seed were different and seeds behaved differently in responding to the cooking process.

Bhatty *et al.* (1983) conducted an objective measurement by using Kramer shear press (Model TP-1) which measured the shear force of lentil samples under specific conditions. In this research, TA-XT2i Texture Analyser and measuring with compression force (N) with back extrusion is used instead, as it is a common method and widely practiced in Australia.

Using TA-XT2i Texture Analyser to determine the cooking quality of lentils allowed further investigation determining the relationship between cooking quality with the changes of other physical and biochemical properties after cooking. The cooking time determination, effect of cooking on texture and minerals, and water absorption will be discussed in the following sections.

7.3 Cooking Time Determination

The cooking time of various cultivars determined by the subjective method ("*The Cooking time test*") is used as a cross reference to indicate the specific amount of force required in order to consider whether seeds are soft enough for consumption (Table 7.1). "The cooking time test" determined the cooking time of lentils by observing the physical changes, rated the softness of the cooked seeds by compressing and recorded in a scale from one to five. Rating one was defined as cooked samples (easy to squash) and rating five was defined as uncooked samples (very firm). The time, at which 90% of the sample were cooked, was recorded as cooking time.

Table 7.1: Cooking Time Determined by Cooking time test

Cultivars	Locations	Cooking time (mins)[1]	Mean Force (N)[2]
Cassab	Mullewa	35	197.41
	Pingaring	35	203.83
Digger	Mullewa	35	190.17
	Pingaring	35	201.54
ILL 7180	Mullewa	35	191.82
	Pingaring	35	215.44
Matilda	Mullewa	45	199.94
	Pingaring	45	218.81

[1] Cooking time (mins, minutes) determined by The Cooking time test
[2] Mean Force (N, Newton) determined by Texture Analyser

Based on the "*The Cooking time test* " method, 35 minutes is an adequate cooking time for red lentil cultivars (Cassab, Digger and ILL 7180) grown in Mullewa and Pingaring. The green lentil cultivars (Matilda) grown in both locations required longer time to cook (45 minutes) (Table 7.1).

The cooking time determined by the Cooking time test was used as a reference time to draw some conclusive relationship between subjective and instrumental methods. As shown in Table 7.1, the mean peak compression force reveals a range of 190 to 215 N for the red lentil cultivars and 200 to 219 N for green lentil cultivars at the cooking time determined by *The Cooking time test* (Table 6.1).

By drawing a horizontal line at 220 N compression force (N) on Figure 7.1, the cooking time of various lentil samples could be determined. Matilda from both growing locations (Mullewa and Pingaring) took the longest time to cook. Cassab and ILL 7180 from Mullewa are the fastest cooking cultivars (Figure 7.1). In general, the cooking time of lentils is around 35 to 45 minutes for red and green lentil cultivars respectively (Table 7.2).

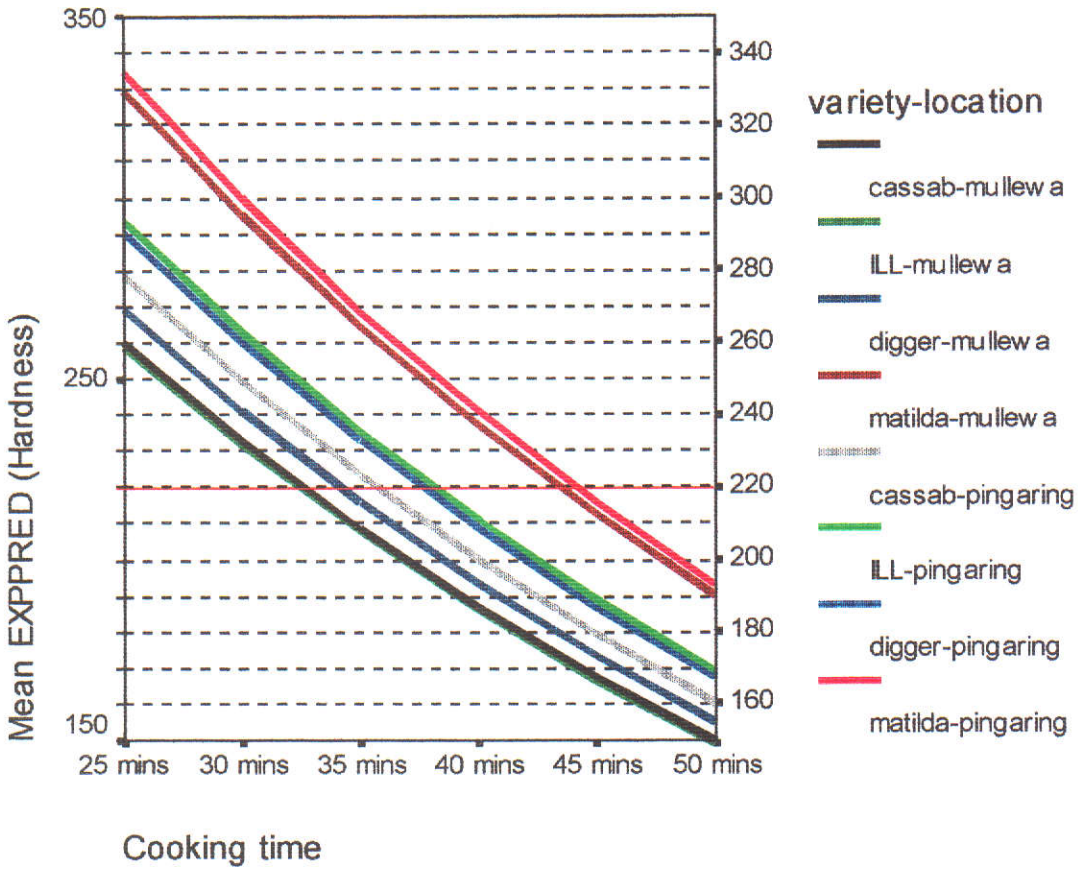


Figure 7.1: Comparison between cooking rates among different lentil cultivars with the interaction of locations

Table 7.2: Cooking Time Determination

Cultivars	Locations	Mean Force (N)	Cooking time (mins)	
			The Cooking time test	Texture Analyser
Cassab	Mullewa	197	35	35
	Pingaring	204	35	35
Digger	Mullewa	190	35	35
	Pingaring	202	35	35
ILL 7180	Mullewa	192	35	35
	Pingaring	215	35	35
Matilda	Mullewa	200	45	45
	Pingaring	219	45	45

Based on the above generalised result, 220 N is an optimal compression force. Below 220 N seeds are considered cooked and above 220 N seeds uncooked (Figure 7.1). Referring back to the One-way ANOVA result (Table 6.2 - Table 6.9), the mean compression force showed insignificant difference after 35 minutes of cooking for all the red lentil cultivars from both growing locations. For green lentils (Matilda), the mean compression force showed insignificant difference after 40 minutes of cooking. These results are closely related to the cooking time determined by "*The Cooking time test*". It also supports that 220 N can be used as an indicator to determine the seed cooking stage.

As concluded, 220 N is suggested as an optimal compression force for lentils to reach the cooked stage if TA-XT2i texture analyser is used for the determination of seed hardness. The time required to reach this force is considered as the optimal cooking time, if lentil samples having a peak compression force value of less than 220 N were considered cooked (good-cookers), and those greater than 220 N were considered undercooked (poor-cookers).

7.4 *Effect of Cooking*

Examination of the results shows cooking plays a significant role in changing the texture and mineral content in seeds. These changes were mainly due to the effect of cooking on the cell structure (Iyer *et al.* 1989). During the cooking process, there are four basic and distinct chemical and physical changes. They are:

- partial release of calcium, magnesium and other minerals into the cooking water,
- rapid starch gelatinisation,
- gradual plasticisation or partial solubilisation of components of the middle lamella and separation of bean cells along the planes of the cell wall without cell rupture, and
- progressive slow denaturation of proteins (Iyer *et al.* 1989).

All these physical and chemical changes are suggested to play important roles in varying the cooking quality of lentil cultivars. The details of how all these factors are related to the changes of cooking quality of lentil will be discussed later.

The results of the texture profile analysis showed that cooking played a significant role in varying the cooking texture of lentils (Appendix 2F: Figure 1-5). As cooking time increased, seeds became softer, less chewy, cohesive, springy and gummy, but more adhesive. After seeds reached the cooked state, seeds were softer and required less force for the first bite. The samples became less chewy; meaning the number of chews required before samples were ready to swallow lessened (Appendix 2F: Figure 2). The deformation of seeds (cohesiveness) reduced slightly and cooked seeds become less sticky (Appendix 2F: Figure 3).

Additionally, the cooked seeds became less springy, which means the ability for samples to return to original shapes after cooking reduced (Appendix 2F: Figure 5). Samples became more adhesive after cooking which means that the work required for overcoming the attractive forces between the surface of the food and other surfaces with which the food comes in contact increased (Appendix 2F: Figure 1).

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The results of the texture profile analysis showed that cooking played a significant role in varying the cooking texture of lentils (Appendix 2F: Figure 1-5). As cooking time increased, seeds became softer, less chewy, cohesive, springy and gummy, but more adhesive. After seeds reached the cooked state, seeds were softer and required less force for the first bite. The samples became less chewy; meaning the number of chews required before samples were ready to swallow lessened (Appendix 2F: Figure 2). The deformation of seeds (cohesiveness) reduced slightly and cooked seeds become less sticky (Appendix 2F: Figure 3).

Additionally, the cooked seeds became less springy, which means the ability for samples to return to original shapes after cooking reduced (Appendix 2F: Figure 5). Samples became more adhesive after cooking which means that the work required for overcoming the attractive forces between the surface of the food and other surfaces with which the food comes in contact increased (Appendix 2F: Figure 1).

The above phenomenon was mainly due to the gelatinisation of starch granules within the internal structure of seeds. Gelatinisation is the process that leads to the disruption of molecular order within starch granules during heating (BeMiller and Whistler 1996). During heating, starch granules swelled and leached some of the soluble components, primarily amylose. Compression force (N) was applied to the cooked seeds during the textural measurement, causing further disruption of the starch granules. This swelling and disruption of starch granules produced a viscous mass, which comprised a continuous phase of solubilisation of amylose and/or amylopectin and a discontinuous phase of granule remnants (granule ghosts and fragments) (BeMiller and Whistler 1996).

7.5 *Cooking Quality vs. Growing locations and Inheritance*

"Cooking quality" defined as the hardness of seed after cooking varied significantly with growing locations and varieties ($P < 0.05$)(Table 6.11). Red lentil cultivars (Cassab, Digger and ILL 7180) have a faster cooking rate than green lentil cultivars (Figure 6.1). Red lentil cultivars have a softer cooking texture in general as compared to green lentil cultivars (Table 6.2). This result agreed with studies which claim that locations and genetics play a significant role in varying the cooking quality and chemical composition of lentils (Bhatty *et al.* 1984, Chermick and Chernick 1962 and Iyer *et al.* 1989).

The growing location significantly contributed to the nutrient availability for the crop even though an additional fertilizer was used. In Saskatchewan, lentil growing on stubble under low moisture conditions did not promote phosphorus uptake although there was enough phosphate in the soil (Bhatty 1995). In this study, although the soil treatment, trial size, seeding rate and fertilizers used in all tested cultivars from both locations were identical, there was still an additional need to consider the effect of growing environment on the availability of nutrient to the lentil crops.

7.6 *Cooking Quality vs. Water absorption, Seed Coat Thickness and Seed Size*

7.6.1 Water Absorption and Seed Coat Thickness

Water absorption is defined as the amount of water imbibed into seeds after soaking, with consideration of solids lost from seeds during soaking. This is a test aimed to determine the optimum amount of water that the whole seed could absorb after 24 hours of soaking. Cassab and Matilda have faster water absorption rates than ILL 7180 and Digger (Figure 7.2). Statistical analysis showed that varieties significantly affect the water absorption after the soaking time is accounted for ($P < 0.05$). Lentils grown at Pingaring are generally had a faster water absorption rate than those grown at Mullewa (Figure 7.2).

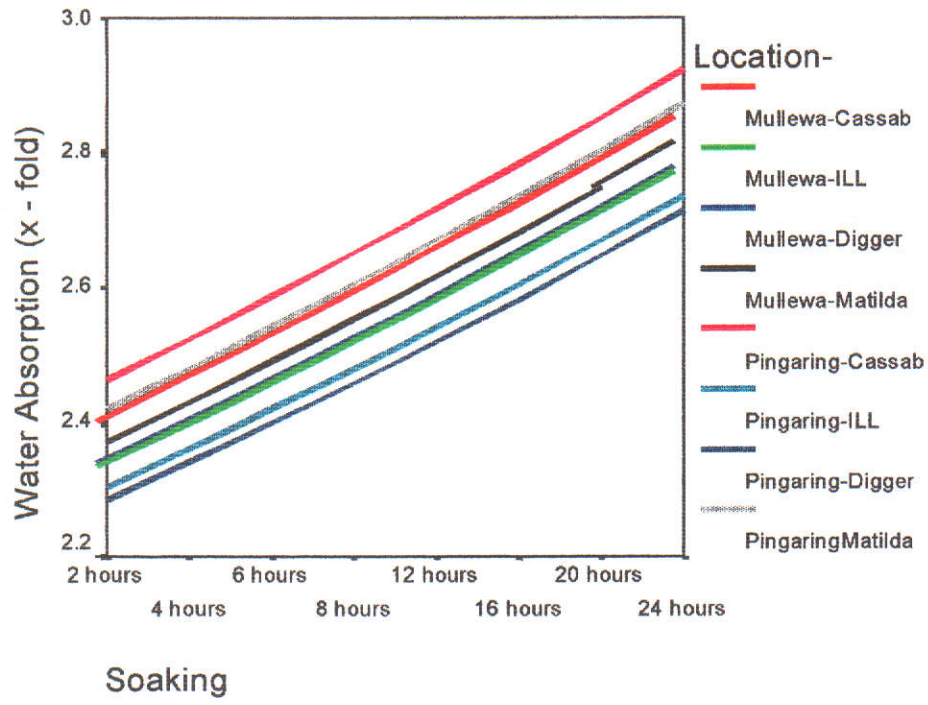


Figure 7.2: Comparison between water absorption rate among different lentil cultivars with the interaction of locations

The rate of water imbibed is mainly dependent on the permeability of the seed coat. The thickness of seed coat is a crucial factor that is suggested to be related to water permeability. Seeds with thick and smooth seed coats generally had a slow initial rate of water absorption compared to the thick seed coat varieties, hence faster cooking (Deshpande *et al.* 1989). However, according to the results obtained, the rate of water imbibition did not relate to the thickness of seed coat. Cassab from both locations had the thickest seed coat, and also had the faster water absorption rate. This suggested there are some other structural factors, which might affect the rate of water imbibition, rather than seed coat thickness.

Bhatty (1995) reported that poor cooking lentils have an irregular epidermis and more tightly packed epidermis and the nuclei are less elongated. This structure may be responsible for the impermeability of water in the epidermis. In addition, there may be a cuticle or waxy layer cover of the cellular layers in the hypothermis, which may act as a barrier to prevent the imbibition of water. This may explain why seeds with thin seed coats, still have a slow water absorption rate.

In addition, Cassab may have a wide hilar fissure, which provides a primary path for water to enter seeds and improve the water uptake. In conclusion, there are many seed coat structural factors responsible for the water absorption process. Excluding the seed coat thickness, other factors such as hilum and micropyle also contribute to the water absorption process. However, due to time limitations, the researcher did not attempt to further explore the significance of hilar fissure to the water absorption of seed.

Besides the seed coat structure, the internal structure of lentil seeds is also suggested to play a role in determining the water permeability in seeds. Starch molecules in cotyledons are mainly responsible for the water uptake in seeds (Reddy *et al.* 1989c). The starch allocation and molecular density in seeds may also play a part in water holding capacity and determine the water absorption rate.

7.6.2 Seed size and seed hardness

There is a significant cutlivar and location interaction effect on seed size ($P < 0.05$) (Table 6.17). This agreed with Singh *et al.* (1988) and Bhatty (1995) who suggested that inheritance and location determine the size of the lentil seed. Seed size determined the cooking time in lentils (Bhatty 1995). Erskine *et al.* (1985) reported that there is a +0.92 correlation between seed size and cooking time.

In this study, the mean weight per 100 seeds was used as the measure of seed size. Pingaring cultivars, are generally larger in size, have a harder cooking texture at 40 minutes cooking compared to Mullewa cultivars. Matilda, the largest seed, took the longest time to cook (40-45 minutes) compared with the rest of the cultivars (35 minutes). Digger, ILL and Cassab are comparatively similar in size, with similar cooking quality.

However, there is no relationship identified when comparing seed size among cultivars from both locations and relating back to the cooking quality. Matilda (Mullewa) is the biggest seed among all cultivars from both locations. It is harder to cook at the initial cooking stage. However, it turned significantly softer after 40 minutes cooking and it is comparatively similar in cooking quality with the rest of the cutlivars ($P < 0.05$). In contrast, Matilda (Pingaring), which is relatively smaller in size, is the hardest to cook cultivar compared to all ($P < 0.05$).

Digger and Cassab, both grown in Mullewa, are similar in size, but significantly different in cooking quality ($P < 0.05$). ILL (Mullewa), which is the smallest seed has a similar cooking quality to Cassab (Mullewa) which is significantly bigger ($P < 0.05$). All these observations suggested that cooking quality of lentils related to the internal structure and chemical composition of seeds rather than just seed size alone.

7.7 *Cooking Quality vs. Proximate Chemical Composition*

7.7.1 Cooking Quality vs. Moisture Content

The initial moisture content is suggested as a factor which varies the water absorption rate of lentils during the cooking process. The result showed no significant difference among varieties and between locations in the initial moisture content in seeds (Table 6.20). This suggested the variation of cooking quality among cultivars is insignificantly related to the moisture content in seeds.

7.7.2 Cooking Quality vs. Initial Proximate Chemical Composition

When comparing the initial proximate chemical composition among cultivars, there is a low level of variation. Statistical analysis showed that there is a significant interaction effect (variety*location) on phytic acid content in seeds ($P < 0.05$) (Table 6.22). Both location and cultivars have contributed to the variation of the phytic acid and phosphorus content in seeds. A significant result is shown as compared to the phytic acid content in uncooked seeds, among cultivars and between locations.

Phytic acid, constitutes the major portion of total phosphorus in seeds and can be used as an indirect estimate of phytic acid in seeds. Bhatty and Slinkard (1989) reported that phytic acid is highly and significantly correlated with phosphorus ($r = +0.98$). The results obtained from this study agreed with the above finding (Table 7.2).

Phytic acid is suggested as a main factor that contributes to seed hardening. Studies reported that seeds with lower phytic acid tended to be harder in texture. The reason is that phytic acid is a strong chelating agent. It chelated calcium and magnesium ions. This reaction prevented the formation of insoluble cross-linkage complexes between calcium and magnesium with the pectate molecules located in the middle of the lamella tissue. Consequently, higher phytate content in beans favours a rapid rate of softening and dissolution of pectic substances that would result in shorter cooking times (Reddy *et al.* 1989). However, the results of this study do not support the above findings. Lentils grown

at Pingaring are generally had a higher in phosphorus and phytic acid content than those grown at Mullewa.s (Table 6.16).

In Table 6.16, the faster cooking lentil samples (Cassab and ILL 7180) do not show much variation in initial proximate chemical composition compared to the slower cooking samples (Matilda). Digger from Mullewa, which has the lowest phytic acid content (Table 6.16), is not the slowest cooking lentil cultivar. Matilda showed to be the hardest to cook but had a comparatively similar amount of phytic acid content (Table 6.16). This again illustrated the disagreement with studies which claimed that higher phytic acid content in seeds results in better cooking lentils. Furthermore, Wassim et al. (1978) reported that seeds have a combination of high levels of K^+ and Na^+ which was associated with good cooking quality. However, compared to the locations, lentils grown at Pingaring are generally had a harder texture and lower Fe^{2+}, Mn^{2+}, Zn^{2+}, sulfur and Ca^{2+} content but higher phytic acid, phosphorus and Na^+ content than those grown at Mullewa (Table 6.16). It may also suggest that looking at the initial chemical composition alone is insufficient to draw any conclusion for determining the cooking quality of lentils.

7.7.3 Cooking Quality vs. Retention of Proximate Chemical Composition after cooking

i. Water and Mineral Imbibition

Heating seeds in boiling water promotes the leaching of minerals and reduces the water molecule holding capacity. When seeds reached the "cooked state" at around 35 to 40 minutes, the rate of leaching of mineral content and water holding capacity stayed constant (Appendix 3: Figure 1-9). This phenomenon suggested there are several stages during the cooking process. When seeds are first put into the boiling water, water diffused into seeds and an osmotic system was established. Starch, cellulose and protein imbibed water into the cell. Bursting of the cellular structure caused the leaching of minerals from seeds and reduced water-holding capacity within seeds.

During cooking, imbibition of water occurred as seed cells contained higher concentration of mineral content than the cooking water (deionised water) (Arnett and Bazinet, 1977). The differences in the concentration, promoted water molecules to move through their kinetic energy, from a region of greater concentration to a lower concentration. Water imbibition resulted.

The cell wall and plasma membrane of lentil cells formed a semi-permeable membrane which allowed the substances to pass into or out of cells (Arnett and Bazinet, 1977). This established the osmotic system between cooking water and solution within cells. This system allows the exchange of water and ions between the protoplasm and the surrounding medium (Arnett and Bazinet, 1977). According to the observations, Na^{+}, Mg^{2+}, Ca^{2+}, Mn^{2+}, Cu^{2+} and Fe^{2+} tended to imbibe into seeds, but not Zn^{2+} and K^{+}, during the first 10 minutes cooking (Appendix 3: Figure 1-8). Leaching of ions subsequently occurred with prolonged cooking.

The reasons for imbibitions of ions during the first ten minutes of cooking were summarised as followed. The pot used for cooking may be too old, some ion residues

may still remain on the surface of the pot or between the scratched surface. Heating provided extra kinetic energy for the movement of water molecules and ions to pass into or out of the cell. This may suggest reasons why even though deionized water is used for cooking, there is still some ions imbibed at the beginning of cooking.

Heating led to damage of cell membranes that caused an increase in permeability (Haard and Chism, 1996). Excessive cooking caused the destruction of cellular structure within seeds and hence mineral leaching. However, the rate of leaching slowed down and remained constant after 30 minutes cooking. This suggested that ion concentration between inside and outside cells might reach equilibrium when seeds reached the cooked state, and leaching stopped as a result.

ii. Cooking Quality vs. Leaching

The cooking process decreases phytic acid and ions in legumes, which is mainly due to leaching (Reddy *et al.* 1989). The ability for phytic acid and ions to be retained in seeds after cooking suggested that it might play a significant role in determining the cooking quality of lentils. Bernal-Lugo *et al.* (1991) reported that phytic acid diffused out of the seed once the beans were over cooked. However, the rate of leaching is unknown. Even though lentils that grown at Pingaring are generally had a better mineral retention than those grown at Mullewa did, it may not indicate they have a good phytic acid holding capacity.

Haard and Chism (1996) suggested the redistribution of calcium or other ions may lead to "hard to cook" problems of legumes. The reason is, cooking altered the permeability of cell membrane. This leads to Ca^{2+} or other ions, that bind with starch phosphate, to liberate and diffuse to the cell wall or middle of the lamella. At the same time, cooking also activated pectin methlyesterase to de-esterify pectin. This allowed free ions to form cross-linkages between carboxyl groups of pectin and the insoluble complex formed as a result (Haard and Chism 1996). This led to seed hardening during cooking.

In this study, lentils grown at Pingaring are generally had a higher mineral retention rate than those grown at Mullewa (Table 6.32). Bhatty (1990) reported that lentil pectin was low-ester molecule and contained enough sites in the natural state for binding Ca^{2+} and Mg^{2+} ions without the intervention of pectin methylesterase to de-esterify pectin (Bhatty 1990). This may increase the rate of insoluble complex molecule formation between ions and pectin as Pingaring cultivars contained free-ions in seeds. Consequently, lentils grown at Pingaring had a harder cooking texture than those grown at Mullewa.

7.8 Conclusion

In conclusion, these joint effects of both environmental and genetic factors suggested they play a significant role in determine the cooking quality of various lentil cultivars. These joint effects also vary the proximate chemical composition in seeds, seed coat thickness, seed size and water absorption. However, from all these factors it is not possible to draw any conclusive results to identify their role in varying the cooking quality of lentils.

7.9 Implications

1. The identification of 220 N as the indicator to determine the adequacy of cooking time for various lentil cultivars allowed the determination of lentil cooking time with an objective instrumental method. This reduced biases that may be drawn from the subjective method ("Cooking time test").

2. In this study, the method "Texture Profile Analysis" is used to determine not only the cooking quality of lentils, but also other cooking textures such as cohesiveness, chewiness, springiness, gumminess and adhesiveness. This allows food technologists to identify suitable lentil varieties to be used for newly developed lentil products and allows lentil breeders to select suitable cultivars.

3. The results of the study suggested that the interaction effect of variety and growing location significantly affect the cooking quality and chemical compositions of lentils. It suggested that breeders and agronomists need to pay specific attention to the effect of cultivars and environments not only on crop yield, but also the cooking quality of lentils.

4. The variation of proximate biochemical composition of phytic acid, minerals and the thickness of seed coat do not significantly affect the cooking quality of different lentil genotypes. However, the interaction effect of variety and growing location significantly affects the cooking quality of lentils. This provides an important assessment tool to allow food technologists to identify the functional lentil cultivars for added value food products. It also allows breeders to identify the potential differences in genotype and growing environment on the effect of the nutritional uptake among cultivars.

Chapter VIII
Conclusions and Recommendations

This current study was designed to investigate the physical or biochemical properties of lentils that may contribute to the variation in cooking quality of lentil. A pilot study, three preliminary experiments (seeds to water ratio, heat input and standing time) and methodology techniques were concluded and evaluated before the testing of the following hypotheses.

The first hypothesis was to test the possibility of using TA-XT2i texture analyser to evaluate the cooking quality of lentil and replacing the old subjective method "Cooking time test". Results support this hypothesis.

The second hypothesis is to test whether there is any variation in cooking quality among lentil cultivars. Results suggested that there is significant cooking quality difference among cultivars after cooking ($P < 0.05$). Matilda is harder in cooking texture and took longer time to cook (45 minutes). Cassab, Digger and ILL 7180 are conversely softer in texture and have a shorter cooking time (35 minutes).

The third and fourth hypotheses are to evaluate the effect of chelating reaction of phytic acid with divalent ions on cooking quality of lentils and to determine the effect of mineral retention ability in seeds after cooking, on cooking quality. Results suggested that there is a location and variety interaction effect on the phytic acid ($P < 0.05$) and mineral content ($P < 0.05$) in lentils. However, the initial phytic acid and mineral content in seeds do not significantly affect the cooking quality of lentils ($P > 0.05$). Mineral retention in seeds is suggested to play a role in determining the cooking quality of lentils.

The fifth and sixth hypotheses are to evaluate the relationship between moisture and water absorption with the thickness of seed coat and to determine their effect on cooking quality. Results do not support this hypothesis.

In conclusion, the interaction of growing location and variety suggest these are more important in effecting the cooking quality of lentils. They also significantly affect the proximate chemical composition in seeds, seed coat thickness, seed size and water absorption of lentils. However, there is no conclusive result to be drawn regarding the interrelationship between proximate chemical composition in seeds, seed coat thickness, seed size and water absorption in varying the cooking quality of lentils.

The following areas are recommended for future study;

1. Texture analyser (TA-XT2i) can be used to determine the cooking quality of lentils and it can also be used to determine the cooking time. However, a validation test needs to be done to evaluate the reliability of using 220N as the optimal peak compression force to indicate adequate cooking time for various lentil cultivars.

2. Although the present study cannot draw any conclusive results on the interrelationship between cooking quality of lentils and the chemical composition of seeds, seed coat thickness, seed size and water absorption, this does not suggest that they have no effect on variation of cooking quality of lentils. This may only suggest that a more comprehensive study design using a complex principal component analysis needs to be developed for the future to allow the evaluation of the inter-relationship among the above multiple factors on the effect of cooking quality.

3. The present study only tested four varieties grown at two growing sites. This limited the interpretation and conclusions to be drawn on the interaction effect of genotype and environment on various changes of cooking quality and mineral retention after cooking. This can be improved by increasing the scale of experiment with larger number of varieties and growing sites, e.g. 5 x 5 with both red and green lentil types reported as a minimum scale.

4. According to the research findings, the variation of cooking quality did not relate to the phytic acid content, mineral content, seed size and thickness of seed coats. It may indicate that there were some other chemical factors may significantly contribute to the variations. The density of starch and its location in seeds are suggested to play a role in varying the cooking

quality of lentils. Total dietary fibre content in cotyledon and seed coat may also contribute to the resistance of cell wall breakage and lead to seed hardening. Finally, as the cooking process involved the breakage of the protein matrix in the seed, so a simple protein profile tests is suggested for future study.

References

Adsule, R.N. and Kadam, S.S. 1989, 'Protein' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume I*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Adsule, R.N. 1996, 'Lentil (Lens culinaris Medik.)', in *Food and Feed from Legumes and Oilseeds*, E. Nwokolo, and J. Smartt, Chapman & Hall, London.

Adsule, R.N., Kadam, S.S. and Leung, H.K. 1989, 'Lentil' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume II*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Adsule, R.N., Kadam, S.S. and Reddy, N.R. 1989, 'Lectins' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume I*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Arnett, R. H. J. and Bazinet, G. F. J. 1977, 'The composition of living matter', in *Plant Biology A Concise Introduction*, The C.V. Mosby Company, Saint Louis.

Arthur, C. 1992, 'Meatless Menus Rising at Restaurants', *American Demographics*, vol. 14, no. 2, p. 18.

Australia New Zealand Food Authority, September 1998, *Folate Pilot*, (Online), Available: http://www.anzfa.gov.au/FolatePilot/Folatelist.htm#TOP [2000, October 30].

Australian Bureau of Agricultural & Resource Economics, 1999, 'Summary of Australian Statistics for Pulses', in *Australian Commodity Statistics 1999*, Commonwealth of Australia, Canberra, p. 190.

Australian Bureau of Agricultural & Resource Economics, 2000a, 'Pulses', in *Australian Commodity Statistics 2000*, Commonwealth of Australia, Canberra, p. 192.

Australian Bureau of Agricultural & Resource Economics, 2000b, Australian Crop Report, Commonwealth of Australia, Canberra.

Australian Bureau of Statistics 1993-1994, *Apparent Consumption of Foodstuffs and Nutrients*, Catalogue Number 4306.0 Commonwealth of Australia.

Australian Bureau of Statistics 1997, *Causes of Death*, Catalogue Number 3303.0 Commonwealth of Australia.

Ballentine, C. 1985, 'Protein: Getting the Complete Package', *FDA Consumer*, vol. 19, p. 24.

BeMiller, J. N. and Whistler, R. L. 1996, 'Carbohydrates', in *Food Chemistry*, 3rd ed., vol. ed. Fennema, O. R., Marcel Dekker, Inc, New York, pp. 158-221.

Bernal-Lugo, I., Castillo, A., Diaz De Leon, F., Moreno, E. and Ramirez, J. 1991, 'Does Phytic Acid Influence Cooking Rate in Common Beans?', *Journal of Food Biochemistry,* vol. 15, pp. 367-374.

Bhatty, R. S. 1988, 'Composition and Quality of Lentil (*Lens culinaris* Medik): A Review', *Canadian Institute of Food Science and Technology Journal,* vol. 21, no. 2, pp. 144-160.

Bhatty, R. S. and Slinkard, A. E. 1989, 'Relationship Between Phytic Acid and Cooking Quality in Lentil', *Canadian Institute of Food Science and Technology,* vol. 22, no. 2, pp. 137-142.

Bhatty, R. S., Nielsen, M. A. and Slinkard, A. E. 1984, 'Cooking Quality of Lentils grown in the Canadian Prair', *Canadian Journal of Plant Science,* vol. 64, pp. 17-24.

Bhatty, R. S. 1995, 'Lentils as a Dietary Cereal Complement', *Cereal Foods World*, vol. 40, no. 5, pp. 387-389, 391-392.

Bhatty, R. S. 1990, 'Cooking Quality of Lentils: The Role of Structure and Composition of Cell Walls', *Journal of the Science of Food and Agriculture*, vol. 38, no. 2, pp. 376-383.

Bhatty, R.S. 1995, 'Comparisons of Good- and Poor-Cooking Lentils', *Journal of the Science of Food and Agriculture*, vol. 68, no. 4, pp. 489-496.

Bhatty, R.S. and Christison G.I. 1984, 'Composition and nutritional quality of pea (*Pisum sativum* L.), faba bean (*Vicia faba L. spp.* minor) and lentil (*Lens culinaris* Medik) meals, protein concentrates and isolates'. *Qualities Planetarium Plant Foods for Human Nutrition*, vol. 34, no. 1, pp. 41-51.

Bhatty, R. S., Nielsen, M.A. and Slinkard, A.E. 1983, 'Comparison of the cooking quality of Laird and Commercial Chilean lentils grown in the Canadian Prairies', *Canadian Institute of Food Science and Technology Journal*, vol.16, no.2, pp.104-110.

Blahovec, J. 1991, 'Use of Denatured Radioalbumin for Determination of Trypsin and Chymotrypsin Inhibitors in Different Plant Seeds', *Journal of Agricultural and Food Chemistry*, vol. 39, no. 2, pp. 276-279.

Borget, M. 1992, *'Food Legumes'*, The Macmillan Press Ltd, London.

Bressani,R. and Elias, L.G. 1988, 'Seed quality and Nutritional Goals in Pea, Lentil, Faba, Bean and Chickpea Breeding', in *World Crops: Cool Season Food Legumes*, eds. R.J. Summerfield, Kluwer Academic Publishers, Dordrecht.

Bruyere, A. and Rapin, C.H. 1998, 'Association between Malmutrition, Poor General Health and Oral Dryness in Hospitalized Elderly Pateints', *Age and Ageing*, vol. 27, no. 2, pp. 123-128.

Carman, K. 1996, 'Some Physical Properties of Lentil Seeds', *Journal of Agricultural Engineering Research*, vol. 63, no. 2, pp. 87-92.

Chavan, J.K. Kadam, S.S. and Salunkhe, D.K. 1989, 'Storage in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume III*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Chernick, A. and Chernick, B. A. 1962, 'Studies of Factors affecting cooking quality of yellow peas', *Canadian Journal of Plant Science*, vol. 43, pp. 174-183

Coakes, S. J. 1999, *SPSS: analysis without anguish: versions 7.0, 7.5, 8.0 for Windows*, Jacaranda Wiley, Brisbane.

Coyle, L.P. 1982, *'The World Encyclopedia of Food'*, Facts on File, Inc., New York.

Danilatos, G.D. 1993, 'Introduction to the ESEM Instrument', *Microscopy Research and Technique*, vol. 25, pp.354-361.

Deshpande, S.S., Sathe, S.K. and Salunkhe, D.K. 1989, 'Soaking' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume III*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Dowsley, K.J., Carter, J.M. and Materne, M.A. 1996, *Lentil Growers Guide "A Guide for the Production of Lentils in Victoria"*, Agriculture Victoria, Victoria.

Duke, J.A. 1981, *'Handbook of Legumes of World Economic Importance'*, Plenum Press, New York.

English, R. and Lewis, J. 1991, *Nutritional Values of Australian Foods*, Australia New Zealand Food Authority, Canberra.

Ensminger, A.H., Ensinger, M.E., Eonlande, J.E. and Robson, J.R.K. 1994, *Foods and Nutrition Encyclopedia*, CRC Press , Inc., USA.

Erskine, W., Williams P.C. and Nakkoul H. 1991a, 'Splitting and Dehulling Lentil (Lens culinaris): Effects of Seed Size and Different Pretreatments', *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 77-84.

Erskine, W., Williams P.C. and Nakkoul H. 1991b, 'Splitting and Dehulling Lentil (Lens culinaris): Effects of Genotype and Location, *Journal of Agricultural and Food Chemistry*, vol. 57, pp. 85-92.

Erskine, W., Williams, P.C. and Nakkoul, H. 1985, 'Genetic and Environmental Variation in the Seed Size, Protein, Yield and Cooking Quality of Lentils', *Field Crops Research*, vol. 12, pp. 153-161.

Everelt, T.H. 1981, 'Lens', in *The New York Botanical Garden Illustrated Encyclopedia of Horticulture Volume 6 (Id-Ma)*, Garland Publishing, Inc., New York.

FAO 1991, Protein quality evaluation: report of the Joint FAO/WHO Expert Consultation, Bethesda, Md., USA 4-8 December 1989, Food and Agriculture Organisation of the United Nations, Rome.

FAO 1995, *Production Year Book Volume 49*, Food and Agriculture Organisation of the United Nations, Rome.

FAO 1997, *Production Year Book Volume 51*, Food and Agriculture Organisation of the United Nations, Rome.

Frias, J. Diaz-Pollan, C, Hedley, C.L and Vidal-Valverde 1995, 'Evolution of Trypsin Inhibitor Activity during Germination of Lentils', *Journal of Agricultural and Food Chemistry*, vol. 43, no. 8, pp. 2231-2234.

Freeland-Graves & Peckham 1996, *Foundations of Food Preparation* (6th ed.), Prentic Hall Inc., New Jersey.

Haard, N. F. and Chism, G. W. 1996, 'Characteristics of Edible Plant Tissues', in *Food Chemistry*, ed. Fennema, O. R., Marcel Dekker, Inc., New York, pp. 944-1007.

Hair, J. F. 1990, *Multivariate data analysis: with readings, 2nd* ed., Macmillan, New York.

Halstead, R. L. and Gfeller, F. 1964, 'The Cooking Quality of Field Peas', *Canadian Journal of Plant Science*, vol. 44, no. 3, pp. 221-228.

Hättenschwiler, S. a. and Vitousek, P. M. 2000, 'The role of polyphenols in terrestrial ecosystem nutrient cycling', *Tree*, vol. 15, no. 6, pp. 238-243.

Hentges, D.L., Weaver, C.M., and Nielsen, S.S. 1991. 'Changes of Selected Physical and Chemical Components in the Development of the Hard-to-Cook Bean Defect', *Journal of Food Science, vol.* 56, no. 2, pp.436-442.

Hincks, M.J. and Stanley, D.W. 1986. 'Multiple mechanism for bean hardening', *Journal of Food Technology*, vol. 21, pp.731-750.

Hincks, M.J. and Stanley, D.W. 1988. 'Lignification: evidence for a role in hard-to-cook beans', *Journal of Food Biochemistry*, vol.11, no. 1, pp. 41-58.

Hohlberg, A.I. and Stanley, D.W. 1987. 'Hard-to-Cook Defect in Black Bean. Protein and Starch Consideratons', *Journal of Agricultural Food Chemistry*, vol. 35, no. 4, pp.571-576.

Hughes, J. S. and Swanson, B. G. 1986, 'Microstructure of Lentil Seeds (Lens Culinaris)', *Food Microstructure*, vol. 5, pp. 241-246.

Iyer, V., Kadam, S.S. and Salunkhe, D.K. 1989, 'Cooking' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume III*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Jadhav, S.J. Reddy, N.R. and Deshpande, S.S. 1989, 'Polyphenols' *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume I*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Jindal, S., Soni, G.L. and Singh, R. 1983, Effect of feeding of lectins from lentils and peas on the intestinal and hepatic enzymes of Albino rats. *Journal of Plant Foods*, vol. 4, no. 2, pp. 95-103.

Jones, P.M.B. and Boulter, D. 1983, 'The cause of reduced cooking rate in *Phaseolus vulgaris* following adverse storage conditions, *Journal of Food Science*, vol. 48, no. 2, pp. 623-626, 649. 623.

Kadam, S. S., Deshpande, S. S. and Jambhale, N. D. 1989, 'Seed Structure and Composition', in *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume I*, I eds. Salunkhe, D. K. a. and Kadam, S. S., CRC Press, Inc., Florida, pp. 23-50.

Kadam, S.S. and Salunkhe, D.K. 1989, 'Milling' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume III,* D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Kozlowsak, H., Honke, J. Sadowska, J. Frias, J. Vidal-Valverde, C. 1996, 'Natural Fermentation of Lentils: Influence of time, concentration and temperature on the kinetics of hydrolysis of Inositol phosphates', *Journal of the Science of Food and Agriculture*, vol. 71, no. 3, pp. 367-375.

Liu, K. 1997, 'Storage protein and Hard-to-cook Phenomenon in Legume Seeds', Food Technology, vol. 51, no. 5. pp. 58-61.

Loss, S. and Siddique, K. 1996, *'Pulse Industry Development, Production Science, Selection and Breeding. Summary of Experimental Results Season 1995 (1st ed.).* Grains Research and Development Corporation, Camberra.

Loss, S. and Siddique, K. 1997, *Pulses Research and Industry Development in Western Australia, 1997,* Grains Research and Development Corporation, Camberra.

Luo, Q. a. and Andrade, J. D. 2000, 'Phytic acid as an efficient low-molecular-mass displacer for anion-exchange displacement chromatography of proteins', *Journal of Chromatography B,* vol. 41, pp. 23-29.

Mattson, S., 1946, 'The cookability of yellow peas: a colloid chemical and biochemical study', Acta Agricultural Suecana II, vol. 2, pp.185.

Matthews, R.H. 1989, *Legumes Chemistry, Technology, and Human Nutrition',* Marcel Dekker, Inc., New York.

Microsoft Excel 97 (version 97) 1997, Microsoft Co., UK

Mohamed-Yasseen, Y., Barringer, S. A., Splittstoesser, W. E. and Costanza, S. 1994, 'The Role of Seed Coats in Seed Viability', *The Botanical Review,* vol. 60, no. 4, pp. 426-439.

Moscoso, W., Bourne, M.C. and Hood, L.F. 1984. Relationship between the hard to cook phenomenon in red kidney beans and water absorption, puncture force, pectin, phytic acid and minerals. *Journal of Food Science.* vol. 49, no. 6, pp.1557-1583.

National Farmer Federation 1993/94a, 'Agribusiness', Australian Agriculture, in *The Complete Reference on Rural Industry* (4th ed.), Morescope Publishing Pty. Ltd., Camberwell.

National Farmer Federation 1993/94b, 'Grain Legume Industry', in *Australian Agriculture. The Complete Reference on Rural Industry* (4th ed.), Morescope Publishing Pty. Ltd., Camberwell.

Norusis, M. J. 1993, *SPSS for Windows Advanced Statistics Release 6.0,* SPSS Inc., Chicago

Nwokolo, E., 1996, 'The Need to Increase Consumption of Pulses in the Developing World', in *Food and Feed from Legumes and Oilseeds*, E. Nwokolo, and J. Smartt, Chapman & Hall, London.

Nygaard, D.F. and Hawtin, G.C., 1981 'Production, Trade and Uses' in *Lentils*, C. Webb, and G. Hawtin, Commonwealth Agricultural Bureaux, England.

Pagano, M. & Gauvreau, K. 1993, *Principles of Biostatistics.* Belmont, California.

Petterson, D.S., Sipsas, S. and Mackintosh, J.B. 1997, The *Chemical Composition and Nutritive Value of Australian Pulses* (2nd ed.), Grains Research and Development Corporation, Camberra, pp. 387-389 and 391-392.

Petterson, D.S. and Burridge, P.M. 1996, *Australian National Pulse Quality Manual,* Grains Research and Development Corporation, Camberra.

Pulse Australia Homepage, (Online), and Available: http://www.pulseaus.com.au [2001, February 22]

Quesada, C., Bartolome, B., Nieto, O., Gomez-Cordoves, C., Hernandez, T. and Estrella, I., 1996, 'Phenolic Inhibitors of α-Amylase and Trypsin Enzymes by Extracts from Pears, Lentils and Cocoa', *Journal of Food Protection*, vol. 59, no. 2, pp. 185-192.

Rao, P.U. and Belavady, B., 1978, 'Oligosaccharides in Pulses: Varietal Differences and Effects of Cooking and Germination', *Journal of Agricultural and Food Chemistry*, vol.28, no. 2, pp.316-319.

Reddy, N. R., Pierson, M. D., Sathe, S. K. and Salunkhe, D. K. (eds.) 1989a, *CRC Phytates in Cereals and Legumes,* CRC Press, Inc, United States.

Reddy, N. R., Sathe, S. K. and Salumkhe, D. K. 1989b, 'Phytates', in *CRC Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization. Volume I,* Salunkhe, D. K. and Kadam, S. S., CRC Press, Inc, United States, pp. 163-187.

Reddy, R. R., Sathe, S. K. and Salunkhe, D. K. 1989c, 'Carbohydrates', in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization Volume I,* D.K. Salunkhe and Kadam, S. S., CRC Press, Inc., United States.

Rees, R.O., Brouwer, J.B., Mahoney, J.E., Walton, G.H., Brinsmead, R.B. Knights, E.J. and Beech, D.E. 1994, 'Pea and Chickpea production in Australia', in *Expanding the Production and Use of Cool Season Food Legumes*, F.J. Muehlbauer and W.J. Kaiser, Kluwer Academic Publishers, Dordrecht.

Regan, K.L., Siddique, K.H.M., Brandon, N. and Beermier, R. 2000. Details of Cipal Interstate Variety Trails – WA, Agriculture WA, Perth.

Roberts, E. H. 1972, 'Loss of Viability and Crop yields', in *Viability of Seeds*, E.H. Robers, Chapman and Hall, London, pp. 307-359.

Rockland, L.b. and Jones, F.T. 1974. Scanning electron microscope studies on dry beans. Effect of cooking in the cellular structure of cotyledons in rehydrated large lima beans. *Journal of Food Science*, vol. 39, no. 2, pp.342-346.

Ruiz, R.G., Price, K.R., Rose, M.E. and Fenwick G.R., 1997, 'Effect of Seed Size and Testa Colour on Saponin Content of Spanish Lentil Seed', *Food Chemistry*, vol. 58, no. 3, pp. 223-226.

Salunkhe, D.K. & Kadam, S.S. 1989, 'Production, Distribution and Consumption' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume I*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Salunkhe, D.K., Sathe, S.K. and Reddy, N.R. 1989, 'Lipids' in *Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology, and Utilization Volume I*, D.K. Salunkhe, & S.S. Kadam, CRC Press, Inc., United States.

Sathe, S.K., 1996, 'The Nutritional Value of Selected Asiatic Pulses: Chickpea, Black gram, Mung bean and Pigeon pea', in *Food and Feed from Legumes and Oilseeds*, E. Nwokolo, and J. Smartt, Chapman & Hall, London.

Saura-Calixto, F., Goni. I., Braavo, L. and Manas, E. 1993, 'Resistant Starch in Foods: Modified Method for Dietary Fiber Residues', *Journal of Food Science*, vol. 58, no. 3, pp. 642-643.

Scanlon, M.G., Malcolmson, L.J., Arntfield, S.D., Watts, B., Ryland, D. and Prolopowich, D.J. 1998, 'Micronization Pretreatments for Reducing the Cooking Time of Lentils', *Journal of the Science of Food and Agriculture*, vol. 76, no.1, pp. 23-30.

Schweizer, T. F., Horman, I. and Wiirsch, P. 1978, 'Low Molecular Weight Carbohydrates from Leguminous Seeds; a New Disccharide: Galactopinitol', *Journal of Science of Food and Agriculture*, vol. 1978, no. 29, pp. 148-154.

Siddique, K. 1993, *Grain Legume (Pulse) Markets in the Indian Sub-Continent: Production, Consumption and Trade*, Co-operative Research Centre for Legumes in Mediterranean Agriculture, Perth.

Siddique, K. and Sykes, J. 1997, 'Pulse production in Australia past, present and future', *Australian Journal of Experimental Agriculture*, vol. 37, pp. 103-111.

Siddique, K., Loss, S. and Pritchard, I. 1995, 'Pulses profitable new crops for the wheatbelt', *Western Australian Journal of Agriculture*, vol. 36, pp. 112-115.

Siddique, K. 2000a, 'Registration of Cassab Lentil', *Crop Science*, vol. 40, pp. 1198-1999.

Siddique, K. 2000b, 'Registration of Cumra Lentil', *Crop Science*, vol. 40, pp. 1199-2000.

Sievwright, C.A. and Shipe, W.F. 1986. Effect of storage conditions and chemical treatments on firmness, in vitro protein digestibility, condensed tannins, phytic acid and divalent cations of cooked black beans (*Phaseolus vulgaris*). *Journal of Food Science*. vol. 51, no. 4, pp. 982-987.

Singh, S., Singh, H. D. and Sikka, K. C. 1968, 'Distribution of nutrients in the anatomical parts of common Indian pulses', *Cereal Chemistry*, vol. 45, pp. 13.

Slee, F. 1987, *The Potential for Lentil Production in Australia*, Muresk Institute of Agriculture, Perth.

SPSS for Windows (version 8) 1999, John Wiley & Sons, Brisbane.

Stable Micro Systems 1997, *User Manual - TA-XT2i Texture Analyser,* Stable Micro Systems, England

Stanley, D.W. and Aguilera, J.M. 1985. 'A review of textural defects in cooked reconstituted legumes - the influence of structure and composition', *Journal of Food Biochemistry,* vol. 9, no. 4, pp. 277-323.

Stoddard, F.L., Marshall, D.R. and Ali, S.M., 1993, 'Variability in grain protein concentration of peas and lentil grown in Australia,' *Australian Journal of Agricultural Research*, vol. 44, pp. 1415-1419.

Tang, J., Sokhansanhj, S., Sosulski, F.W. and Saskatoon, S. 1990, 'Effect of swathing and moisture content on seed properties of Laird lentil', *Canadian Journal of Plant Science*, vol. 70, no. 4, pp.1173-1178.

Texture Technologies Corp. Homepage, (Online), and Available: http://www.texturetechnologies.com/tpa.htm [2000, October 30].

The Lentil Company Homepage, (Online), and Available: http://www.thelentilcompany.com.au/ [2000, October 30].

USA Dry Pea and Lentil Council Homepage, Nutritional and Preparation Information, (Online), (1999, April 4-last update) Available: http://www.wa.gov/ofm/fund97/100/fund13r.htm [2000, October 30].

Varriano-Marston, E. and Omana, E.D. 1979. Effects of sodium salt solutions on the chemical composition and morphology of black beans (*Phaseolus vulgaris*). *Journal of Food Science.* vol. 44, no. 2, pp. 531-536.

Vidal-Valverde, C., Frias, J. Estrella, I., Gorospe, M.J. Ruize, R. and Bacon, J. 1994, 'Effect of Processing on Some Antinutritional Factors of Lentils', *Journal of Agricultural and Food Chemistry*, vol. 42, no. 10, pp. 2291-2295.

Wassim, N., Abu-Shakra, S., Tannous, R. and Hallab, A. H. 1977, 'Effect of Mineral Nutrition on Cooking Quality of Lentils', *Canadian Journal of Plant Science*, vol. 58, pp. 165-168

Webb, C. and Hawtin, G. 1981, *Lentils*, Commonwealth Agricultural Bureaux, Farnham Royal, pp. 49.

Williams, P. and El-Haramein, F. J. 1988, 'Food Legumes (Pulses)', in *Crop Quality Evaluation Methods and Guidelines Technical Manual 14 (Rev. 1) En*, International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, pp. 96-125

Williams, P.C, Hanni N. and Singh, K.B. 1983, 'Relationship Between Cooking Time and Some Physical Characteristics in Chickpeas (Cicer arietinum L.)', *Journal of the Science of Food and Agriculture*, vol. 34, pp. 492-496.

Williams, P.C., Erskine, W. and Singh, U. 1993. *Lens Newsletter*, vol. 20, pp.3-13.

Appendices

Appendix 1
Thumb and Finger Method

Appendix 1A: Procedures

1. Whole seeds of lentil (20g) were cooked in a suitable teapot with 350mL deionised boiling water.

2. At a two minutes time interval, 5 representative seeds were removed and tested the cookability by squeezing each one between thumb and index finger and cutting them into half.

3. Samples were rate from hard, large core, medium core, small core, and very small core to soft. A sample of recording sheet used is given following.

4. Samples were continued to remove at every 5 minutes time intervals until 9 out of 10 (90%) were rating soft.

5. The time at which 90% were cooked was recorded as the cooking time. The final cooking time determined as the mean of duplicate tests and was reported in minutes

(Source: Petterson and Burridge, 1998).

Appendix 1B. Results – Raw Data

Cultivars	Cooking time (mins)	
	Test 1	Test 2
Cassab-Pingaring	40	35
Cassab-Mullewa	40	35
Digger-Pingaring	35	35
Digger-Mullewa	40	40
ILL-Pingaring	40	35
ILL-Mullewa	35	35
Matilda-Pingaring	45	45
Matilda-Mullewa	45	45

Appendix 2
TA-XT2i-Texture Analysis

Appendix 2A: Test setting of TA-XT2i texture analyser (Stable Micro Systems, England)

Test mode and Option	TPA
Parameters	Pre test speed: 2mm/sec
	Test speed: 1 mm/sec
	Post test speed: 1 mm/sec
	Distance: 75%
	Load cell: 25-1
Trigger	Type: Auto
	Force: 0.4 kg
	Stop plot at: Final
	Auto tare: Selected
Units	Force: Newton
	Distance: %strain
Break	Detect: Off

All other parameters are N/A

Appendix 2b: Micro Setting of Texture Profile analysis of lentil (Stable Micro Systems, England)

Program	
Clear Graph Results	
Got to time	0,000s
Drop Anchor	1
Search Forwards	
Peak Force +ve	
Mark Force	
Drop Anchor	2
Mark Distance	
Mark Time	
Area	
Search Forwards	
% of Max. +ve Force	100%
Drop Anchor	3
Mark Force	
Mark Distance	
Mark Time	
Got to force	400.0g
Drop Anchor	4
Area	
Select Anchor	1
Select Anchor	2
Area	
Search Forwards	
Go to Force	0.0g
Drop Anchor	5
Search Forwards	
Peak Force	
Search Forwards	
Go to Force	0.0g
Drop Anchor	6
Area	
Search Forwards	
Go to Force	400.0g
Drop Anchor	7
Mark Time	
Mark Distance	
Search Forwards	
Peak Force +ve	
Mark Force	
Mark Time	
Mark Distance	
Search Forwards	
Go to Force	400.0g
Drop Anchor	8
Area	

Appendix 2C: Texture Profile Analysis Definitions and Calculations

Terms	Definitions
Hardness	The **peak force** during the first compression cycle (" first bite ").
Cohesiveness	Cohesiveness is how well the product withstands a second deformation relative to how it behaved under the first deformation. It is measured as the area of work during the second penetration divided by the area of work during the first penetration. **Calculation: Area 2/Area 1**
Springiness	Springiness is how well a product physically springs back after it have been deformed during the first penetration. **Calculation: Length 2/ Length 1**
Chewiness	Chewiness only applies for solid products and is calculated as Gumminess*Springiness **Calculation: Length1/Length2**
Gumminess	Gumminess only applies to semi-solid products and is Hardness *Cohesiveness **Calculation: Area 2/Area1**
Adhesiveness	The stickiness of cooked product **Calculation: Area 5/Area4**

(Source: http://www.texturetechnologies.com/tpa.htm, 2000 October 30)

Appendix 2D: Preliminary Tests

Seed:Water Ratio

Procedures:

1. 20g lentil samples weighted and cooked in a series of 250-mL, 300-mL, 350-mL and 400-mL boiled de-ionised water for 50 min.

2. After 10 min standing time, volume of drained water, weight and hardness of cooked samples measured with TA-TXT2i texture analyser.

Raw Data Results:

Matilda (MW) 50 mins cooking, 15 mins standing				
	250	300	350	400
Test 1	240.809	238.902	255.961	248.465
Test 2	216.173	248.281	241.506	254.129
Test 3	274.313	221.486	264.582	243.953
SD (mm)	29.1825	13.5969	11.66026	5.098856
Mean	243.765	236.223	254.0163	248.849

Digger (NE) 50 mins cooking, 15 mins standing				
	250	300	350	400
Test 1	194.311	228.605	220.91	233.264
Test 2	253.931	233.092	202.253	215.046
Test 3	256.546	209.738	212.256	217.229
Test 4	270.654	187.327	216.267	174.558
SD (mm)	33.83973	20.86078	7.942885	25.00045
Mean	201.8563	175.9246	171.9258	173.0195

Effect of various heat input from stove on texture

Procedures:

i. 20g lentil samples weighted and cooked in 350mL boiled de-ionised water for 50 min.

ii. After 10 min standing time, hardness of cooked samples measured.

Raw Data Results:

Stove	1	2	3	4	5
Test 1	215.028	190.369	157.962	231.28	203.513
Test 2	216.96	226.692	224.337	219.203	202.29
Test 3	226.386	212.094	216.392	198.693	216.326
Mean	219.458	209.7183	199.5637	216.392	207.3763
SD	6.077092	18.27766	36.24644	16.47436	7.774724

Effect of Standing Time on Texture

Procedures:

1. 20g lentil samples weighted and cooked in 350mL boiled de-ionised water for 50 min.

2. Hardness of sample measured starting from 2 min standing time to 80 min standing time.

Raw Data Results:

Standing Time (Mins.Secs)	Test 1	Test 2	Test 3
1.53	182.747	260.165	250.199
4.00	212.355	273.701	195.3
5.13	227.696	260.551	n.a
7.00	n.a	247.46	217.576
8.00	215.187	232.748	n.a
10.32	232.748	238.759	260.327
15.00	n.a	243.292	296.56
20.00	236.728	243.199	268.457
25.00	243.898	255.337	311.699
30.32	255.026	253.834	n.a
38.23	n.a	259.103	259.075
40.08	294.671	246.735	299.238
80.18	296.479	291.221	n.a
100.26	287.358	270.758	282.185

Appendix 2E: TA-XT2i Texture Analysis

Procedures:

1. Twenty grams of whole seed samples were cooked in a suitable teapot with 350-mL boiling deionised water. (The cooking time started from 25 minutes and increased at every five minutes cooking time intervals for each cultivar).

2. After cooking at a set time period, water drained.

3. Samples placed on tissue paper to absorb additional water.

4. Samples cover and allow 10 minutes standing time.

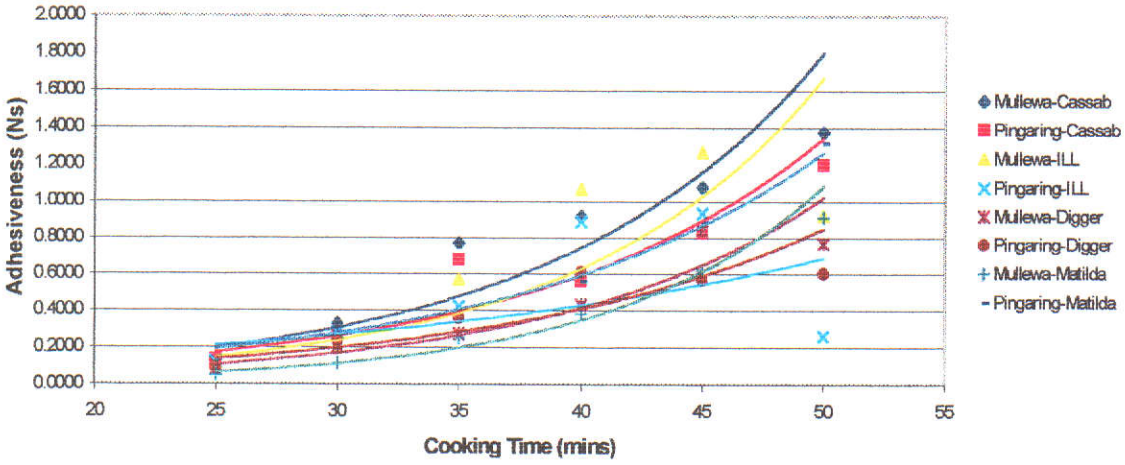
5. Texture measurement.

Raw Data Results on Texture Profile Analysis

Texture Result of Mean Adhesiveness with different cooking time:

	25	30	35	40	45	50
Mullewa-Cassab	0.1313	0.3298	0.7683	0.9183	1.0763	1.3763
Pingaring-Cassab	0.1319	0.2578	0.6784	0.5569	0.8271	1.1977
Mullewa-ILL	0.1052	0.1926	0.5671	1.0616	1.2628	0.9169
Pingaring-ILL	0.1201	0.2812	0.4246	0.8888	0.9347	0.2619
Mullewa-Digger	0.0827	0.2087	0.2753	0.4305	0.8382	0.7656
Pingaring-Digger	0.0885	0.2327	0.3584	0.6135	0.5663	0.6024
Mullewa-Matilda	0.0514	0.1160	0.2529	0.3861	0.6202	0.9118
Pingaring-Matilda	0.2017	0.2747	0.3938	0.5583	0.8846	1.3154

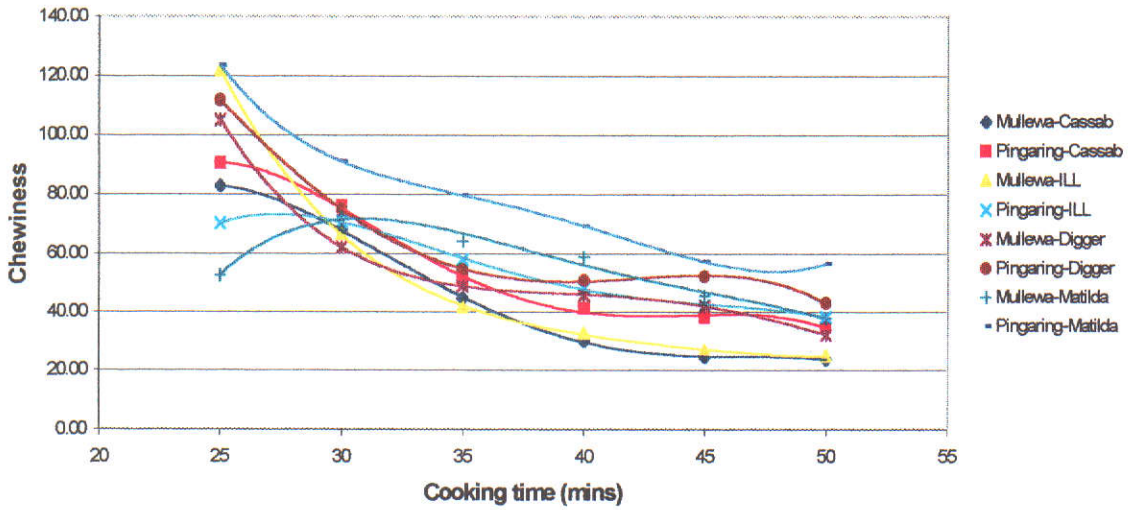
Figure 1: Various Changes of Texture Adhesiveness with increasing cooking time among cultivars



Texture Result of Mean Chewiness with different cooking time:

	25	30	35	40	45	50
Mullewa-Cassab	82.66	67.98	44.71	29.94	24.50	23.67
Pingaring-Cassab	90.57	75.89	50.85	41.13	38.05	34.50
Mullewa-ILL	121.76	66.52	41.41	32.59	26.57	24.79
Pingaring-ILL	70.21	70.94	57.78	48.38	42.21	38.23
Mullewa-Digger	105.13	62.05	48.82	45.67	41.91	32.08
Pingaring-Digger	111.74	75.12	54.29	50.84	52.22	42.89
Mullewa-Matilda	52.51	72.78	64.12	58.82	45.46	37.90
Pingaring-Matilda	123.34	91.12	79.72	69.37	57.25	56.49

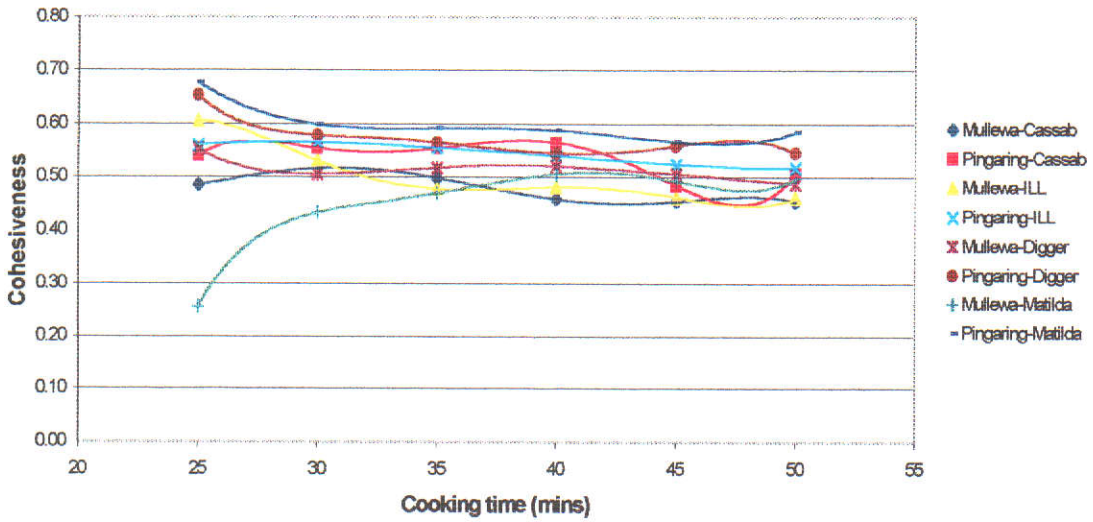
Figure 2: Various change of texture Chewiness after cooking among cultivars



Texture Result of Mean Cohesiveness with different cooking time:

	25	30	35	40	45	50
Mullewa-Cassab	0.49	0.52	0.50	0.46	0.45	0.45
Pingaring-Cassab	0.54	0.55	0.55	0.56	0.48	0.51
Mullewa-ILL	0.60	0.53	0.48	0.48	0.46	0.46
Pingaring-ILL	0.56	0.57	0.56	0.54	0.52	0.52
Mullewa-Digger	0.55	0.51	0.52	0.52	0.51	0.49
Pingaring-Digger	0.65	0.58	0.56	0.54	0.56	0.55
Mullewa-Matilda	0.26	0.43	0.47	0.51	0.49	0.50
Pingaring-Matilda	0.68	0.60	0.59	0.59	0.57	0.58

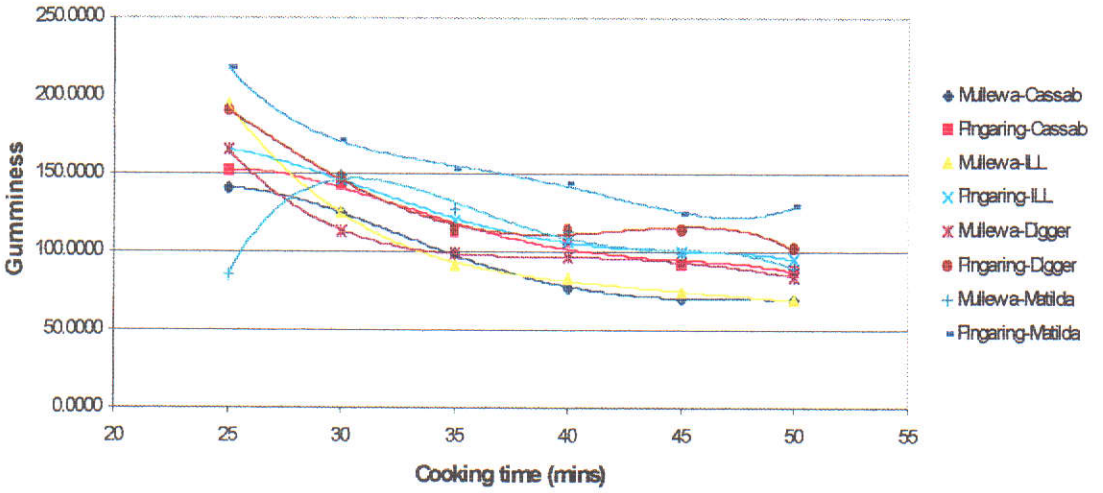
Figure 3: Various Change of texture Cohesiveness after cooking among cultivars



Texture Result of Mean Gumminess with different cooking time:

	25	30	35	40	45	50
Mullewa-Cassab	140.61	124.15	98.77	77.62	71.22	69.67
Pingaring-Cassab	151.53	142.97	113.12	106.91	92.60	87.96
Mullewa-ILL	194.25	125.00	91.84	82.91	74.10	69.33
Pingaring-ILL	165.44	146.35	119.93	107.66	100.24	95.81
Mullewa-Digger	165.07	113.58	99.07	96.71	93.74	84.64
Pingaring-Digger	190.50	147.11	114.17	114.17	114.39	102.23
Mullewa-Matilda	85.48	148.72	126.77	113.46	99.16	89.67
Pingaring-Matilda	218.02	171.24	153.20	143.40	124.36	129.02

Figure 4: Various Change of texture Gumminess after cooking among cultivars



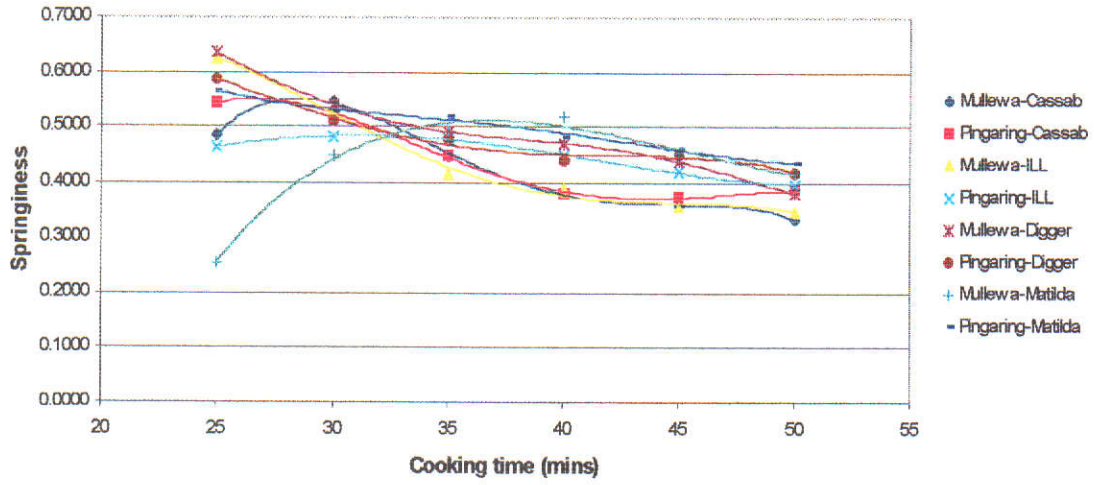
Texture Result of Mean Hardness with different cooking time:

	25	30	35	40	45	50
Mullewa-Cassab	295.62	239.61	197.41	168.75	171.61	152.97
Pingaring-Cassab	284.45	255.68	203.83	188.70	191.84	172.39
Mullewa-ILL	321.11	235.62	191.82	171.70	160.38	149.24
Pingaring-ILL	322.47	258.80	215.44	198.85	190.62	184.60
Mullewa-Digger	299.69	222.85	190.17	185.44	184.47	172.99
Pingaring-Digger	291.10	253.92	201.54	209.02	203.35	187.07
Mullewa-Matilda	341.37	342.08	272.04	224.08	199.94	179.32
Pingaring-Matilda	322.28	285.26	257.74	243.76	218.81	220.17

Texture Result of Mean Spinginness with different cooking time:

	25	30	35	40	45	50
Mullewa-Cassab	0.4852	0.5452	0.4484	0.3838	0.3580	0.3351
Pingaring-Cassab	0.5428	0.5232	0.4474	0.3815	0.3739	0.3854
Mullewa-ILL	0.6233	0.5304	0.4140	0.3885	0.3565	0.3483
Pingaring-ILL	0.4636	0.4822	0.4799	0.4481	0.4194	0.3971
Mullewa-Digger	0.6343	0.5403	0.4912	0.4706	0.4380	0.3811
Pingaring-Digger	0.5875	0.5105	0.4774	0.4401	0.4508	0.4180
Mullewa-Matilda	0.2557	0.4495	0.4912	0.5182	0.4517	0.4164
Pingaring-Matilda	0.5660	0.5285	0.5195	0.4828	0.4618	0.4358

Figure 5: Various Changes of texture springiness after cooking among cultivars



Appendix 3
Hydration Coefficient, Water Aborption, Hydration Capacity and Seed size

Appendix 3A: Hydration coefficient and Water Absorption

Procedures:

1. Count and weigh 100 seeds into a 200-250 ml breaker.

2. Add 100 ml deionised water, cover and allow stand at 18°C to 24°C for 2, 4, 6, 8, 12, 16, 20 and 24 hours individually.

3. Drain the water and put into a 100ml breaker; and followed the procedures stated for the determination of solid lost.

4. Seeds blots dry and re weight

Procedures for Sold lost:

1. Evaporate excess water by heating on hot plate with low heat input,

2. Leave overnight in oven at 105°C.

3. Re weight

Calculations:

$$\text{Hydration Coefficient \%} = \frac{(\text{Initial wt (g)} + \text{wt of imbibed water (g)}) \times 100}{\text{Initial wt (g)}}$$

$$\text{Water Absorption \%} = \frac{[\text{wet wt (g)} - (\text{dry wt (g)} - \text{Solid lost (g)})] \times 100}{\text{Initial dry wt (g)} - \text{Solid lost (g)}}$$

Raw Data Results

Hydration Coefficient %

Soaking hours	Digger Pingaring	Digger Mullewa	ILL Pingaring	ILL Mullewa	Cassab Pingaring	Cassab Mullewa	Matilda Pingaring	Matilda Mullewa
2	157.3547%	162.7069%	146.0935%	158.3070%	181.2668%	176.8047%	167.0745%	164.3139%
2	142.0824%	162.7659%	148.7399%	156.8712%	179.6857%	183.1678%	161.1325%	171.7701%
4	169.6579%	171.6698%	155.4600%	173.6433%	229.8375%	182.3569%	182.1997%	181.7429%
4	171.3538%	188.2723%	186.9018%	171.4013%	185.7425%	180.9761%	177.7430%	182.3738%
6	181.1552%	184.0345%	178.7765%	181.5053%	190.0605%	190.4722%	191.0520%	189.2591%
6	179.2779%	183.4690%	177.4914%	184.4707%	189.1995%	193.8307%	186.2863%	190.5128%
8	185.0884%	186.7888%	183.2481%	188.5739%	193.1286%	190.1261%	195.7120%	194.3283%
8	181.8277%	187.6617%	183.4166%	185.5885%	190.7802%	191.6229%	194.7648%	190.8252%
12	188.6584%	190.7942%	191.3024%	192.8038%	197.3091%	201.8019%	198.4314%	195.8464%
12	189.7375%	187.2322%	188.5764%	188.6926%	197.5581%	226.5943%	196.5764%	193.9867%
16	191.2183%	193.2988%	193.4029%	188.8613%	171.6603%	170.2752%	185.6439%	170.6443%
16	189.1800%	190.9166%	190.4760%	189.0271%	186.7429%	171.8513%	171.3578%	175.8411%
20	194.2738%	194.5132%	210.5293%	218.6377%	199.7687%	194.3075%	209.0474%	199.5891%
20	198.3801%	193.3276%	209.6129%	197.2904%	197.7365%	195.0080%	203.5725%	201.1530%
24	194.7908%	194.3740%	199.8834%	192.8312%	200.2332%	194.8319%	206.4476%	199.0651%
24	198.8466%	200.3845%	195.5834%	193.8805%	199.2799%	191.5982%	213.9109%	197.6409%

Water Absorption (%)

Soaking hours	Digger Pingaring	Digger Mullewa	ILL Pingaring	ILL Mullewa	Cassab Pingaring	Cassab Mullewa	Matilda Pingaring	Matilda Mullewa
2	57.8709%	63.5031%	46.7195%	58.8965%	82.2933%	77.5466%	68.4486%	65.4789%
2	42.5761%	63.4407%	49.4028%	57.6901%	81.1277%	84.5955%	62.6991%	73.1168%
4	70.6251%	72.7837%	56.3552%	74.7952%	157.0431%	84.3743%	85.2970%	84.2898%
4	72.3883%	89.7650%	88.5024%	72.9785%	87.6796%	83.3673%	80.8345%	84.3640%
6	82.5040%	86.3059%	80.5141%	83.5675%	93.0555%	93.3280%	95.3409%	91.8747%
6	80.9592%	85.7109%	79.4123%	86.8603%	92.3128%	96.6758%	89.5767%	93.3194%
8	87.0124%	89.2518%	85.4219%	91.4189%	96.8321%	93.7104%	101.0031%	97.9516%
8	83.6981%	90.0244%	86.0440%	88.2938%	94.1141%	95.1998%	100.0000%	94.3232%
12	90.5991%	93.1512%	93.7747%	96.1536%	100.9850%	106.0442%	104.3388%	99.7614%
12	91.6197%	89.7375%	91.4829%	91.8157%	101.9729%	131.6886%	102.7256%	97.2722%
16	95.6713%	96.9025%	96.4279%	92.6049%	75.7162%	73.8236%	91.2055%	74.1563%
16	91.7623%	93.9710%	93.8673%	92.5434%	90.7910%	75.7806%	76.2580%	79.5863%
20	97.2303%	97.2172%	113.8145%	123.1918%	104.4025%	98.7369%	116.4505%	104.0041%
20	101.4831%	96.6305%	113.3745%	101.4963%	102.4364%	99.5234%	110.1061%	105.7022%
24	97.8146%	97.6235%	103.4114%	97.5555%	104.9297%	99.9257%	112.8839%	103.0636%
24	101.7895%	103.4065%	98.2115%	97.4957%	104.4869%	96.5328%	119.9084%	101.7983%

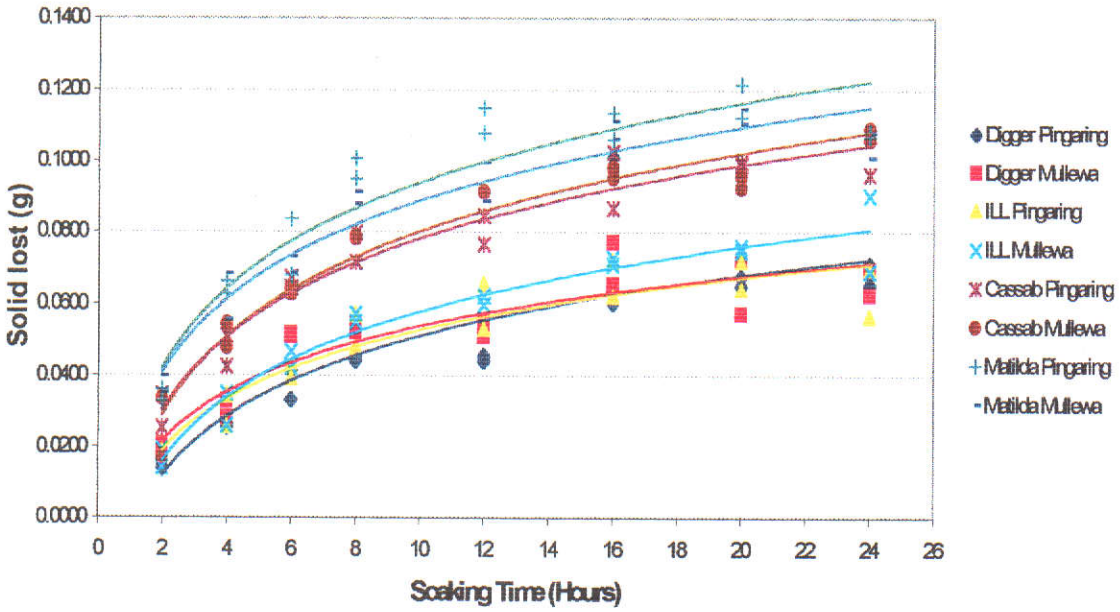


Figure: The increased of solid lost with increasing soaking time

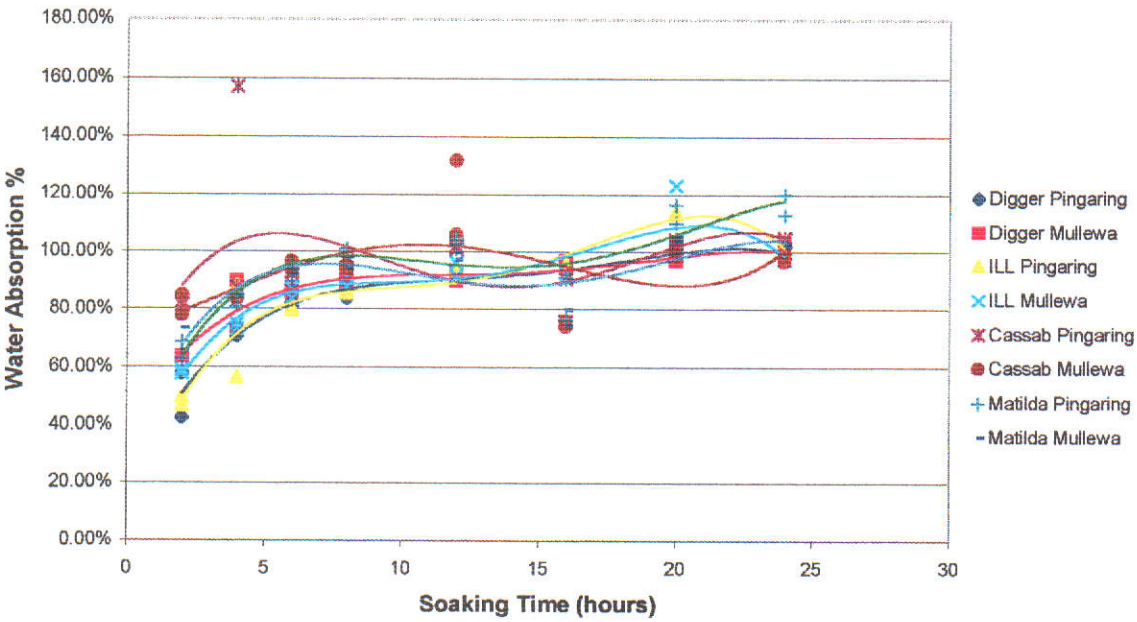


Figure: Water Absorption % of various cultivars after 24 hours soaking time

Appendix 3B: Hydration Capacity and unhydrated seeds

Procedures:

1. Count and weigh 100 seeds into a 200-250 ml breaker.

2. Add 100 ml deionised water and cover and stand at 18°C to 24°C for 16 hours.

3. Drain the water and blots dry the seeds.

4. Remove and count any unswollen seeds (hard seed coatedness).

5. Weigh the swollen seeds and the unswollen seeds.

Calculations:

$$\text{Hydration Capacity} = \frac{\text{weight of swollen seeds X 100}}{\text{(Weight of seeds before soaking – Weight of presumed unswollen seeds)}}$$

$$\text{Unhydratable seeds \%} = \frac{\text{Number of unswollen seeds X 100}}{\text{Original number of seeds}}$$

(Source: Petterson and Burridge, 1998).

Raw Data Results

Hydration Capacity after 16 hrs soaking

	Hydration Capacity %	Unhydrated seeds %
Digger-Pingaring	193.78%	2.00%
Digger-Pingaring	189.81%	3.00%
Digger-Mullewa	194.35%	1.00%
Digger-Mullewa	191.93%	1.00%
ILL- Pingaring	201.94%	6.00%
ILL- Pingaring	191.42%	1.00%
ILL-Mullewa	189.99%	2.00%
ILL-Mullewa	189.03%	0.00%
Cassab-Pingaring	182.80%	2.00%
Cassab-Pingaring	215.31%	0.00%
Cassab-Mullewa	229.77%	5.00%
Cassab-Mullewa	213.45%	5.00%
Matilda- Pingaring	197.04%	0.00%
Matilda- Pingaring	198.60%	0.00%
Matilda-Mullewa	195.94%	0.00%
Matilda-Mullewa	196.69%	0.00%

Appendix 3C: Seed Size

Procedures:

1. Count 100 seeds.

2. Weight

Raw Data Results

Cassab-MW	Cassab-NE	Digger-MW	Digger-(NE)	ILL-MW	ILL NE	Matilda-MW	Matilda-NE
4.3173	4.3333	4.1342	4.3315	3.9054	4.1456	5.3695	3.9926
4.1476	4.5387	4.1986	4.6387	3.9333	4.1434	5.2693	3.9880
4.3095	4.3813	4.2839	4.5031	3.9416	4.2063	5.3198	3.8513
4.4847	4.4963	4.1898	4.5664	3.9799	4.1812	5.1651	4.0151
4.2331	4.5104	4.2193	4.4055	3.8362	4.3048	5.3919	3.9326
4.4005	4.4350	4.2690	4.5291	3.9397	4.4029	5.4065	4.0472
4.2397	4.3991	4.2668	4.4776	3.9749	4.3183	5.2785	3.9445
4.2750	4.3663	4.1902	4.7191	3.9291	4.3783	5.4497	3.8248
4.2433	4.2953	4.1783	4.6466	3.8303	4.3674	5.3660	3.8978
4.2791	4.3522	4.3384	4.7605	3.8916	4.2687	5.2081	4.0057
4.1405	4.4931	4.2300	4.2816	3.8275	4.3824	5.0140	4.0209
4.3185	4.3334	4.1658	4.5342	3.6973	3.9442	5.1162	3.7597
4.3592	3.3163	4.3586	4.4809	3.9451	4.4890	4.9493	3.9724
4.1716	4.1080	4.1696	4.4163	3.8278	4.0395	5.1412	3.6793
4.3326	4.1511	4.2241	4.4787	3.7654	4.0620	5.0396	3.8031
4.3344	4.1572	4.2248	4.4344	3.6518	3.9696	5.0352	3.9063
4.2263	4.2306	4.2184	4.2866	3.8552	4.0688	4.9879	3.8293
4.3058	4.1747	4.1902	4.4689	3.8282	4.0432	4.8900	3.6293
4.4343	4.1882	4.1713	4.4588	3.6422	4.1540	5.0871	3.7357
4.1569	3.8658	4.1957	4.4794	3.6728	4.2955	5.3199	3.7913
4.6685	4.4450	4.2127	4.4513	3.7455	4.0260	5.2712	3.8987
4.3931	4.0816	4.1405	4.4778	3.8823	4.1159	5.3322	3.8164
4.1458	4.3670	4.1864	4.3362	3.7247	4.1655	5.1105	3.5524
4.2288	4.2987	4.2923	4.3829	3.5835	4.0841	5.1864	3.6081
4.2820	4.2020	4.2268	4.6514	3.7761	4.0301	5.1344	3.5424
4.2217	4.2217	4.2135	4.4912	3.7748	4.2512	5.2519	3.9710

Appendix 4
Chemical Composition

Appendix 4A – Phytic Acid

Procedures:

1. Sample (1 g) was weighed into a plastic centrifuge tube, 20 ml 2.4% HCl/10% Na_2SO_4 added and phytate extracted for 2 hr with slow shaking in a water bath.

2. The suspension was centrifuged at 15, 000 g for 40 mins. The supernatant was transferred to a 50 ml volumetric flask.

3. The precipitate was rewashed with 10 l 2.4% HCl/10% Na_2SO_4 added for 30 min and centrifuged at the same speed (15,000g) for 20 min.

4. The supernatant as combined with the flask containing the first extract and diluted to volume with deionised water. An aliquot (2-5ml) of the phytate solution was transferred to a 25 ml volumetric flask and made up to volume with deionised water.

5. 10 ml of the diluted solution was passed through an anion exchange resin column (AGI-XB).

6. Phytate was eluted by washing the resin column with 15 ml deionised water followed by 15 ml 0.1 M NaCl and then 25 ml of 0.7 M NaCl.

7. A 3 ml aliquot of the phytate solution was reacted with 1 ml modified Wade reagent (0.03% $FeCl_3$.6 $6H_20$ and 0.3% sulfosalicylic acid), within 30 min of elution,, and mixed in a Vortex for 10 sec.

8. Optical density (OD) of samples was read in a spectrophotometer (Varian) at 500 nm after allowing them to stand for 30 mins.

9. The OD of a reagent blank was also determined and used to correct the OD of samples.

Calculations:

1. The phytate content of a sample was calculated by relating the value to a standard curve produced using a purified sodium phytate (0-50 Ag phytate/ml)

2. Phytate P was calculated by assuming 6 moles of P per mole of phytate.

Appendix 4B – Ash

Procedures:

1. Dishes removed after drying and put into a desiccator for at least 1 hour to allow them to cool to the room temperature, after that each dish weighted to the nearest mg.

2. 2.5g of lentil samples weighted accurately into each dish and their position was recorded on a sheet of paper.

3. Dishes were placed inside the cool muffle furnace, as near to the centre as possible and ash overnight at 550°C.

4. Dishes remove from the muffle in accordance with the recorded plan and place in a desiccator for at least 1 hour to allow cooling.

5. Dishes re-weight to the nearest mg.

Calculation:

$$\% \text{ Ash} = \frac{\text{Weight residue in crucible after ashing X 100}}{\text{Weight wet sample X (100 - \% moisture)}}$$

Raw Data Results

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Mullewa	Matilda-Pingaring
0	2.22%	3.69%	2.20%	2.20%	2.36%	2.21%	2.32%	2.32%
0	2.28%	2.40%	2.09%	2.36%	2.59%	2.26%	2.14%	1.93%
0	2.04%	2.17%	2.19%	2.20%	2.44%	2.37%	2.28%	2.09%
0	2.29%	2.26%	2.21%	2.16%	2.52%	2.13%	2.38%	2.14%
10	1.01%	0.99%	0.91%	1.13%	1.27%	1.03%	1.06%	1.06%
10	0.49%	1.07%	0.98%	1.19%	1.17%	1.11%	0.88%	0.96%
10	1.12%	1.13%	0.97%	1.15%	1.32%	1.16%	0.97%	0.98%
10	1.03%	1.07%	0.96%	0.76%	1.27%	1.07%	0.96%	1.00%
20	0.63%	0.85%	0.59%	0.81%	0.76%	0.81%	0.76%	0.72%
20	1.43%	0.74%	0.57%	0.77%	0.71%	0.88%	0.78%	0.76%
20	0.70%	0.81%	0.60%	0.77%	0.69%	0.91%	0.71%	0.75%
20	0.63%	0.70%	0.63%	0.79%	0.68%	0.77%	0.73%	0.77%
30	0.35%	0.49%	0.34%	0.47%	0.42%	0.48%	0.48%	0.47%
30	0.36%	0.51%	0.39%	0.51%	0.40%	0.51%	0.47%	0.47%
30	0.39%	0.50%	0.36%	0.54%	0.41%	0.52%	0.54%	0.50%
30	0.40%	0.48%	0.35%	0.52%	0.46%	0.44%	0.54%	0.55%
40	0.24%	0.48%	0.24%	0.42%	0.29%	0.45%	0.31%	0.40%
40	0.27%	0.47%	0.23%	0.48%	0.25%	0.42%	0.34%	0.39%
40	0.22%	0.47%	0.24%	0.42%	0.26%	0.48%	0.35%	0.46%
40	0.22%	0.44%	0.18%	0.44%	0.23%	0.45%	0.29%	0.45%

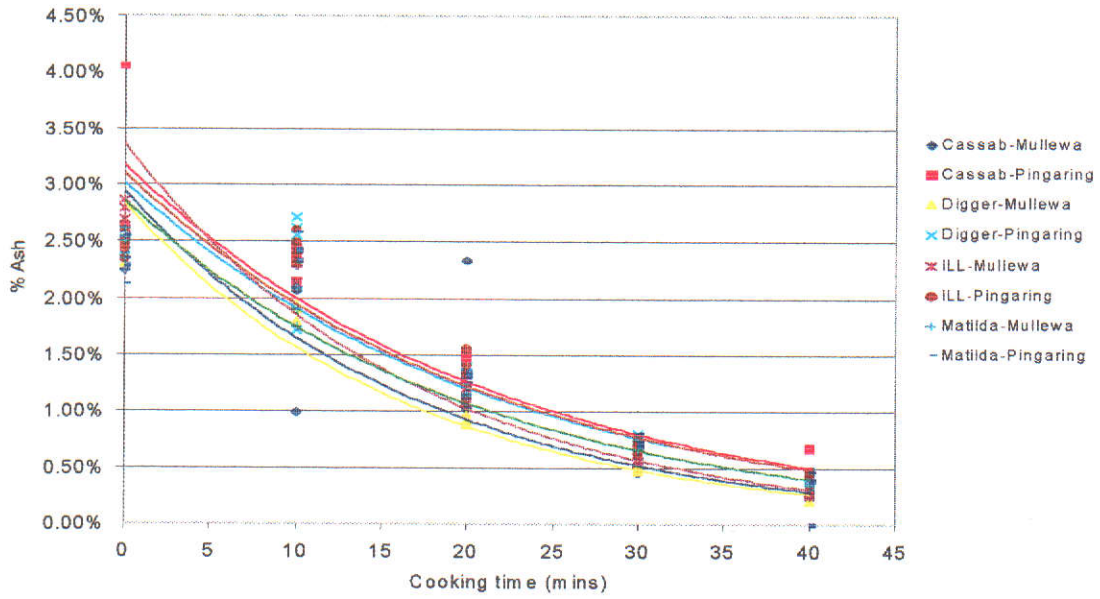


Figure: Change of % of Ash during cooking process

Table: Pearson Correlation and R^2 between ash and cooking time among cultivars

Cultivars	Locations	Cooking time (mins)	
		Correlations	R^2
ILL	Pingaring	-0.9623*	0.9261
	Mullewa	-0.9755*	0.9516
Cassab	Pingaring	-0.9298*	0.8646
	Mullewa	-0.9132*	0.8340
Matilda	Pingaring	-0.9462*	0.8954
	Mullewa	-0.9877*	0.9756
Digger	Pingaring	-0.9470*	0.8968
	Mullewa	-0.9761*	0.9528

*Correlation is significant at the 0.01 level (2-tailed)

Appendix 4C: Minerals

Procedures:

Part I: Analysis of Ash

1. Dishes removed after drying and put into a desiccator for at least 1 hour to allow them to cool to the room temperature, after that each dish weighted to the nearest mg.
2. 2.5g of lentil samples weighted accurately into each dish and their position was recorded on a sheet of paper.
3. Dishes were placed inside the cool muffle furnace, as near to the centre as possible and ash overnight at 550°C.
4. Dishes remove from the muffle in accordance with the recorded plan and place in a desiccator for at least 1 hour to allow cooling.
5. Dishes re-weight to the nearest mg.

Part II: Treatment of Ash

1. Treat the ash with 5-10 ml of 6N hydrochloric acid to wet it completely, and carefully take to dryness on a low temperature hot plate.
2. Add 15 ml of 3N hydrochloric acid and heat the dish on the hot plate until the solution just boils.
3. Cool and filter through a filter paper into a graduated flask, retaining as much of the solids as possible in the dish.
4. Add 10ml of 3N hydrochloric acid to the dish and heat until the solution just boils.
5. Cool and filter into the graduated flask.
6. Wash the dish at least 3 times with water; filter the washings into the flask.
7. If calcium is to be determined add 5 ml of lanthanum chloride per 100ml of solution.
8. Cool and dilute the contents of the flask to the mark with water.

Calculation:

$$\text{Metal content (mg/100g)} = \frac{(a-b) \times V}{10W}$$

w: weight (g) of Dry samples
v: volume (ml) of extract
a: concentration (ug/ml) of sample solution
b: concentration (ug/ml) of blank solution

Raw Data Results

Sodium

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Mullewa	Matilda-Pingaring
0	19.16	10.62	16.14	2.15	14.05	9.84	14.34	7.46
0	41.03	10.60	44.12	11.41	26.03	7.21	24.33	10.05
0	24.21	10.25	13.90	4.06	30.47	10.61	53.33	11.09
0	44.57	5.69	38.09	4.98	31.50	6.41	57.03	14.10
10	6.08	26.48	13.52	28.43	13.71	15.41	7.59	27.24
10	4.68	19.64	7.27	28.04	12.16	19.76	2.92	31.75
10	7.57	22.31	9.02	24.22	6.71	19.41	5.27	25.82
10	5.28	17.45	5.34	20.57	4.95	21.47	5.57	35.09
20	4.09	10.28	0.98	22.36	10.06	4.82	6.07	12.73
20	4.37	16.72	3.16	21.17	5.38	11.53	5.56	20.45
20	4.51	16.42	4.29	13.15	4.82	11.87	7.04	14.15
20	4.76	10.93	2.30	15.04	5.19	11.87	3.53	16.31
30	5.14	8.51	4.26	8.58	4.58	7.50	2.25	7.97
30	2.93	5.54	3.32	10.20	3.86	6.66	3.69	6.65
30	1.42	6.14	3.51	9.16	5.13	6.56	4.46	7.89
30	3.90	7.56	3.43	8.61	0.95	9.06	3.98	8.13
40	2.33	9.75	3.81	5.51	3.49	4.08	2.97	6.69
40	1.78	7.00	2.78	5.50	2.90	6.91	2.38	6.53
40	0.95	6.32	2.54	6.56	3.24	5.50	1.57	7.13
40	3.14	5.89	3.42	6.07	0.37	1.28	2.52	5.52

Potassium

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Mullewa	Matilda-Pingaring
0	333.40	300.83	315.61	298.38	394.18	336.89	377.53	312.09
0	402.59	266.07	303.41	232.88	365.73	332.74	368.47	316.14
0	264.05	345.93	432.62	116.91	318.77	322.98	350.03	220.07
0	271.88	246.00	300.08	245.86	295.24	296.61	328.92	322.02
10	284.25	300.00	240.70	366.75	555.43	283.46	276.24	334.26
10	116.99	310.55	257.96	390.50	403.50	352.66	248.75	313.23
10	262.09	296.97	227.68	308.29	225.32	342.09	253.37	280.58
10	266.74	310.75	248.66	286.68	170.76	298.26	228.18	309.67
20	107.95	158.55	102.49	175.48	154.49	143.39	146.27	151.89
20	119.05	165.27	94.00	167.85	148.89	195.44	149.80	164.27
20	131.57	171.73	108.96	132.17	127.22	197.70	121.27	156.64
20	119.79	157.52	109.92	188.79	122.48	175.66	130.87	154.27
30	40.45	68.46	36.42	62.44	49.51	54.87	66.47	56.34
30	36.86	64.41	40.65	76.90	45.63	69.07	48.38	58.57
30	22.46	72.07	37.43	78.46	53.16	69.88	68.59	69.33
30	42.10	63.42	38.59	77.02	62.64	59.88	70.35	70.65
40	23.87	46.89	18.95	41.80	29.97	45.25	29.89	44.02
40	27.31	51.42	18.90	37.18	23.57	40.98	29.59	37.89
40	25.41	46.99	18.21	43.70	26.49	47.15	30.14	49.16
40	20.23	48.42	20.27	44.40	2.78	11.98	26.28	40.56

Calcium

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Mullewa	Matilda-Mullewa
0	33.74	30.93	30.35	31.80	33.64	29.94	33.07	36.05
0	25.06	27.12	32.89	31.58	32.94	36.61	27.38	38.13
0	25.61	35.32	32.03	21.21	30.05	30.66	26.08	30.27
0	23.11	36.00	29.60	30.30	30.27	28.41	23.49	29.07
10	44.12	46.27	53.97	47.07	75.48	45.34	48.65	44.36
10	23.55	41.91	47.34	48.48	55.27	48.42	45.55	42.16
10	61.09	37.32	53.15	42.79	43.87	41.68	43.74	38.28
10	47.92	35.87	44.67	37.34	29.45	44.20	42.97	31.36
20	27.96	41.70	27.42	38.84	28.53	31.58	37.30	29.82
20	30.77	32.85	27.20	29.54	25.16	22.86	35.67	25.90
20	33.51	33.42	31.70	25.74	29.23	26.92	27.34	23.25
20	22.19	25.94	29.12	29.08	24.43	27.21	25.42	24.73
30	21.51	21.61	20.80	24.13	24.25	20.24	22.54	20.73
30	18.70	21.81	23.38	18.73	21.29	23.22	21.96	16.53
30	13.60	21.22	24.78	25.02	21.78	23.00	30.57	18.90
30	21.55	21.53	23.45	21.53	23.28	21.22	27.26	24.21
40	15.43	26.65	18.86	18.92	17.48	20.69	21.14	20.67
40	13.74	22.74	14.59	20.48	14.76	19.22	18.60	20.08
40	15.19	19.69	15.85	20.56	17.53	22.01	18.93	21.19
40	13.48	19.45	16.56	20.12	1.48	5.37	16.71	19.72

Magesium

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Pingaring	Matilda-Mullewa
0	73.55	67.28	79.08	67.74	80.49	74.43	89.68	75.41
0	88.76	62.65	78.71	56.17	81.54	72.42	82.41	80.01
0	67.09	81.28	90.22	28.67	72.03	71.14	97.34	53.86
0	65.10	56.05	84.69	61.71	78.82	67.32	91.11	82.74
10	73.58	82.35	73.63	98.81	133.20	77.14	79.91	102.93
10	35.01	75.42	70.62	104.17	95.63	92.24	71.36	99.00
10	79.08	72.89	73.26	86.43	63.69	88.90	69.20	104.31
10	71.49	80.55	68.37	80.72	47.48	87.33	66.77	93.54
20	57.25	48.38	35.34	52.97	46.40	42.77	47.54	56.49
20	37.13	48.99	31.47	48.73	42.90	59.71	46.86	60.72
20	40.09	47.47	38.02	35.59	38.43	54.47	41.30	54.90
20	35.53	49.91	37.98	48.30	38.21	50.31	43.34	56.97
30	20.93	28.34	21.81	29.83	26.10	24.10	30.10	29.56
30	17.00	26.80	23.57	32.41	22.93	25.96	24.43	28.73
30	10.92	30.20	21.77	34.86	26.73	30.83	34.75	35.34
30	20.50	31.26	19.91	34.04	27.53	30.38	35.29	36.63
40	11.47	25.58	11.45	21.54	16.74	24.46	17.38	25.68
40	14.94	24.72	11.79	19.95	12.16	23.72	19.09	22.94
40	12.95	22.65	10.94	22.73	14.12	23.52	18.78	27.84
40	10.11	24.84	13.47	24.41	1.20	5.29	15.45	25.79

Manganese

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Pingaring	Matilda-Mullewa
0	0.88	1.72	0.90	1.51	1.14	1.58	0.88	1.57
0	1.23	1.32	1.12	1.30	1.15	1.63	1.02	1.53
0	0.99	2.11	1.16	0.52	0.97	1.33	1.13	1.48
0	0.89	1.61	1.10	1.41	1.08	1.45	1.04	1.66
10	2.39	1.52	2.36	1.69	3.39	1.46	2.04	1.54
10	1.03	1.38	2.12	1.63	2.50	1.48	2.12	1.50
10	2.31	1.22	2.29	1.34	1.88	1.51	1.91	1.42
10	2.13	1.37	2.04	1.37	1.43	1.45	2.00	1.33
20	1.27	0.85	1.14	2.37	0.82	1.55	1.60	0.95
20	1.35	0.92	1.09	0.71	0.96	0.66	2.14	0.91
20	1.35	1.73	1.31	1.31	1.36	0.85	1.29	0.84
20	1.13	0.37	1.24	1.01	0.98	0.78	0.46	0.91
30	0.87	0.69	0.86	0.72	0.92	0.56	0.91	0.46
30	0.71	0.55	0.92	0.67	0.95	0.49	0.84	0.59
30	0.57	0.66	0.83	0.79	0.84	0.68	1.22	0.71
30	0.74	0.72	0.90	0.78	0.88	0.50	1.19	0.79
40	0.74	0.57	0.77	0.56	0.69	0.47	0.86	0.61
40 Fail		0.65	0.57	0.57	0.65	0.55	0.92	0.62
40	0.64	0.68	0.58	0.61	0.65	0.68	0.83	0.64
40	0.63	0.57	0.70	0.56	0.45	0.21	0.57	0.38

Zinc

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Mullewa	Matilda-Pingaring
0	1.62	2.33	1.51	2.16	2.52	2.54	1.99	2.91
0	2.36	2.09	2.93	1.88	2.03	2.66	3.12	2.83
0	2.13	2.80	2.95	0.69	2.01	2.48	2.47	2.18
0	2.18	2.09	2.38	2.12	2.27	2.35	2.50	2.67
10	3.82	2.02	6.33	2.38	9.63	1.82	3.04	2.28
10	1.92	1.97	2.82	2.55	5.22	2.32	2.34	2.12
10	2.70	1.82	2.08	2.20	5.54	2.37	2.68	1.94
10	2.92	2.01	2.47	2.17	1.59	2.15	2.45	2.08
20	1.62	1.81	1.45	2.63	1.59	1.79	1.91	1.86
20	1.85	1.56	1.19	1.76	1.75	1.78	1.82	2.08
20	1.79	1.77	1.83	1.24	1.75	1.88	1.63	1.60
20	1.51	1.60	1.49	1.44	1.57	1.85	1.66	1.94
30	0.83	0.88	0.78	0.99	0.96	0.62	1.11	0.87
30	0.72	0.86	0.80	1.05	1.08	0.86	0.94	0.79
30	0.45	0.92	0.78	1.23	1.09	1.08	1.25	1.01
30	0.88	0.99	0.76	1.17	1.16	1.02	1.43	1.16
40	0.60	0.84	0.51	0.69	0.69	0.83	0.71	0.78
40	0.61	0.83	0.44	0.75	0.54	0.83	0.79	0.70
40	0.43	0.79	0.35	0.86	0.59	0.85	0.77	0.84
40	0.35	0.82	0.44	0.67	0.09	0.23	0.52	0.74

Copper

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Mullewa	Matilda-Pingaring
0	0.69	0.72	0.66	0.63	1.16	0.72	0.52	0.68
0	0.78	0.71	0.88	0.52	0.59	0.82	0.53	0.95
0	0.48	0.83	0.73	0.27	1.26	0.76	0.75	0.54
0	0.49	0.57	0.85	0.57	0.60	0.66	0.51	0.82
10	1.11	1.11	0.94	0.77	1.67	0.67	0.89	0.94
10	0.55	0.75	0.85	0.74	1.25	0.76	0.85	0.97
10	0.96	0.59	1.17	0.86	0.90	0.67	0.77	0.68
10	1.15	0.70	0.80	0.54	0.61	0.70	0.95	0.75
20	0.63	0.57	0.38	0.67	0.60	0.81	0.69	0.63
20	0.56	0.52	0.43	0.49	0.62	0.68	0.67	0.56
20	0.57	0.50	0.64	0.40	0.59	0.59	0.68	0.55
20	0.47	0.52	0.58	0.40	0.53	0.48	0.88	0.58
30	0.43	0.43	0.36	0.43	0.47	0.60	0.38	0.56
30	0.34	0.49	0.40	0.35	0.41	0.41	0.33	0.35
30	0.25	0.43	0.40	0.56	0.46	0.49	0.50	0.44
30	0.40	0.42	0.47	0.47	0.40	0.41	0.54	0.44
40	0.13	0.25	0.13	0.22	0.17	0.24	0.15	0.25
40	0.16	0.24	0.11	0.20	0.11	0.27	0.14	0.22
40	0.11	0.23	0.11	0.22	0.13	0.27	0.20	0.25
40	0.10	0.20	0.14	0.20	0.01	0.07	0.13	0.20

Iron

Cooking time	Cassab-Mullewa	Cassab-Pingaring	Digger-Mullewa	Digger-Pingaring	ILL-Mullewa	ILL-Pingaring	Matilda-Mullewa	Matilda-Mullewa
0	3.80	5.49	4.26	6.42	5.22	6.54	3.19	5.69
0	4.91	5.51	4.78	4.85	5.29	6.13	4.67	Fail
0	4.01	6.92	6.89	2.15	8.71	6.07	3.89	4.50
0	3.54	5.07	4.89	5.45	4.19	5.31	3.26	5.74
10	5.83	4.22	6.07	5.02		4.27	5.49	3.89
10	4.09	4.10	5.80	5.63	7.81	5.20	5.40	3.72
10	5.87	3.85	5.41	4.66	5.27	4.88	5.54	3.52
10	6.02	3.90	5.69	4.26	3.81	4.56	5.22	3.58
20	3.53	3.54	3.30	3.66	3.03	4.13	4.32	2.88
20	4.18	3.72	3.03	3.09	4.21	3.67	3.96	2.71
20	3.75	3.33	3.61	2.29	4.10	3.53	3.42	2.71
20	3.46	3.08	3.41	2.91	3.65	3.36	3.61	2.87
30	2.03	1.65	1.83	1.65	2.28	1.31	2.23	1.12
30	1.62	1.68	1.79	1.83	2.43	1.45	1.61	1.18
30	0.99	1.77	1.99	1.96	2.40	1.72	2.52	1.38
30	2.01	1.84	1.97	1.84	2.58	1.67	2.59	1.51
40	1.11	1.38	1.17	1.23	1.48	1.31	1.39	1.10
40	1.28	1.37	1.00	1.10	1.15	1.27	1.38	0.95
40	1.11	1.44	0.95	1.41	1.30	1.50	1.39	1.10
40	1.00	1.38	1.09	1.37	0.10	0.24	1.08	1.04

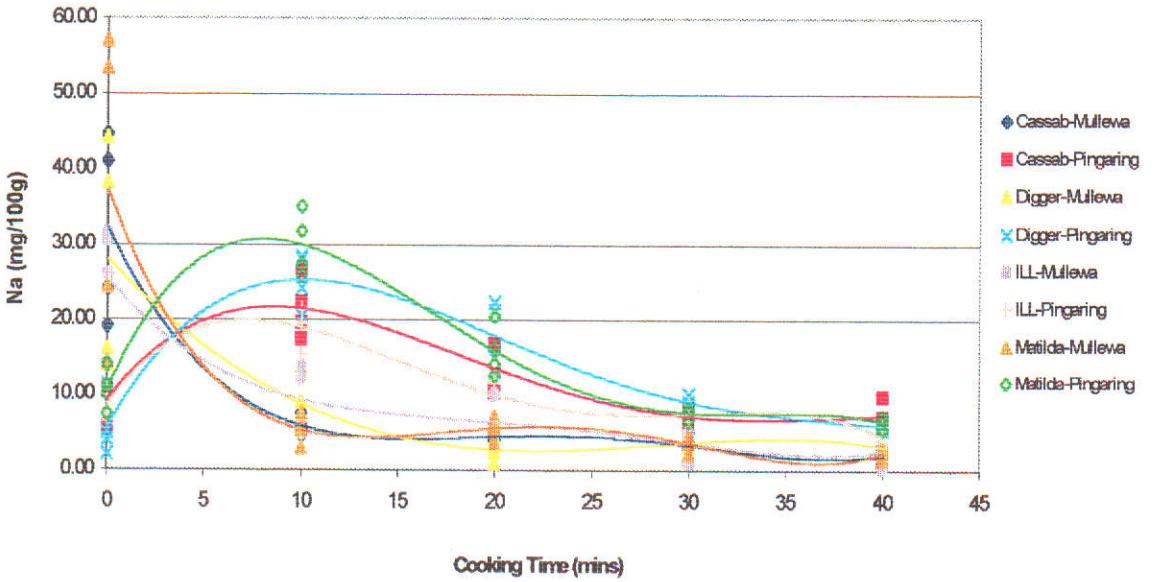


Figure: Effect of cooking on Sodium content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Sodium* and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R^2
Cassab	Mullewa	-0.8741*	0.7641
	Pingaring	-0.4777*	0.2282
Digger	Mullewa	-0.7545*	0.5693
	Pingaring	-0.1150	0.0132
ILL	Mullewa	-0.8298*	0.6885
	Pingaring	-0.5696*	0.3245
Matilda	Mullewa	-0.8228*	0.6769
	Pingaring	-0.5762*	0.3320

*Correlation is significant at the 0.01 level (2-tailed)

*Transformation is performed

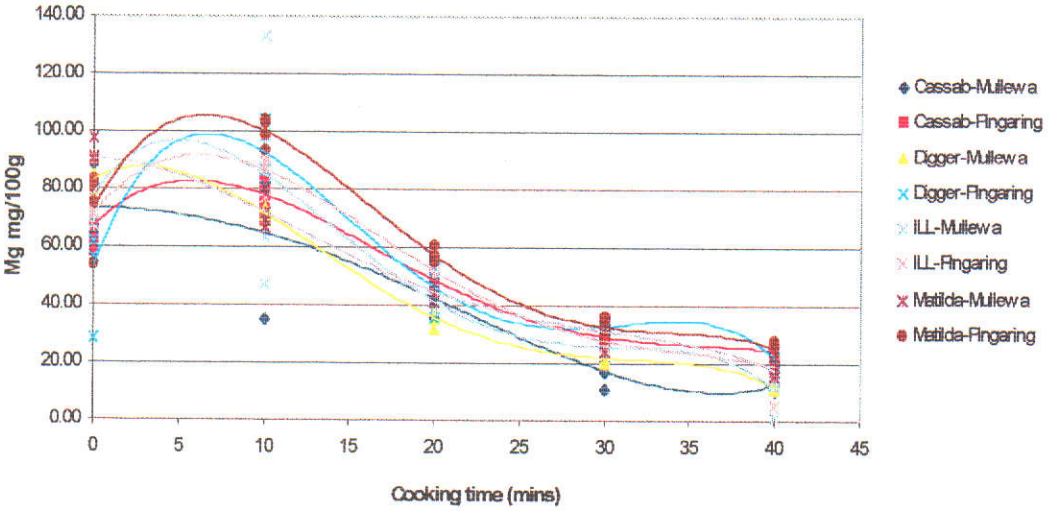


Figure: Effect of cooking on Magnesium content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Magnesium$^{\clubsuit}$ and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R^2
Cassab	Mullewa	-0.8888*	0.7900
	Pingaring	-0.9129*	0.8335
Digger	Mullewa	-0.8588*	0.7376
	Pingaring	-0.7205*	0.5192
ILL	Mullewa	-0.8930*	0.7975
	Pingaring	-0.9253*	0.8563
Matilda	Mullewa	-0.9867*	0.9736
	Pingaring	-0.9835*	0.9673

*Correlation is significant at the 0.01 level (2-tailed)

$^{\clubsuit}$ Transformation is performed

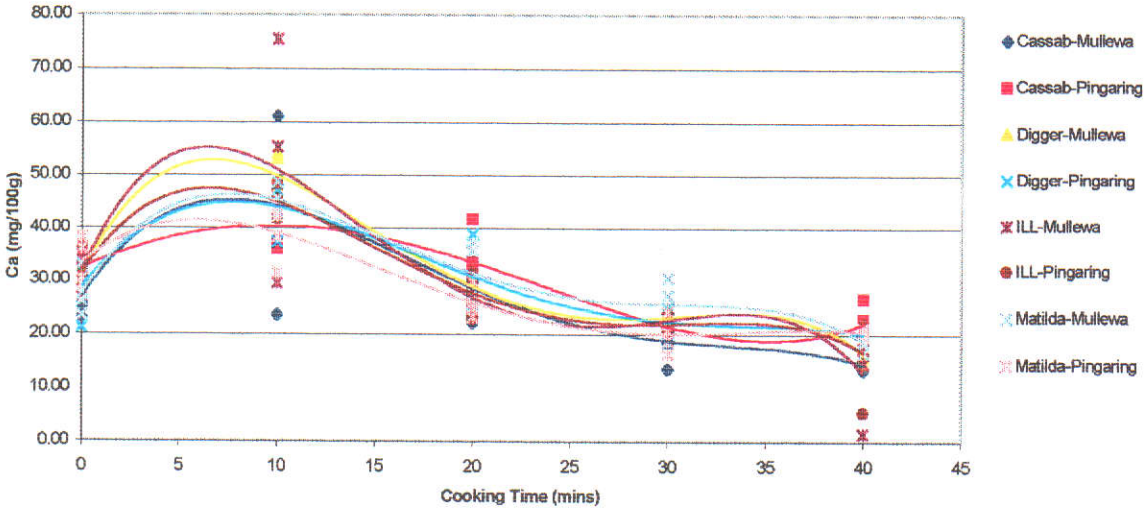


Figure: Effect of cooking on Calcium content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Calcium* and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R^2
Cassab	Mullewa	-0.6877*	0.4729
	Pingaring	-0.7233*	0.5232
Digger	Mullewa	-0.7823*	0.6120
	Pingaring	-0.6495*	0.4219
ILL	Mullewa	-0.7164*	0.5132
	Pingaring	-0.6837*	0.4674
Matilda	Mullewa	-0.6055*	0.3666
	Pingaring	-0.8144*	0.6632

*Correlation is significant at the 0.01 level (2-tailed)

* Transformation is performed

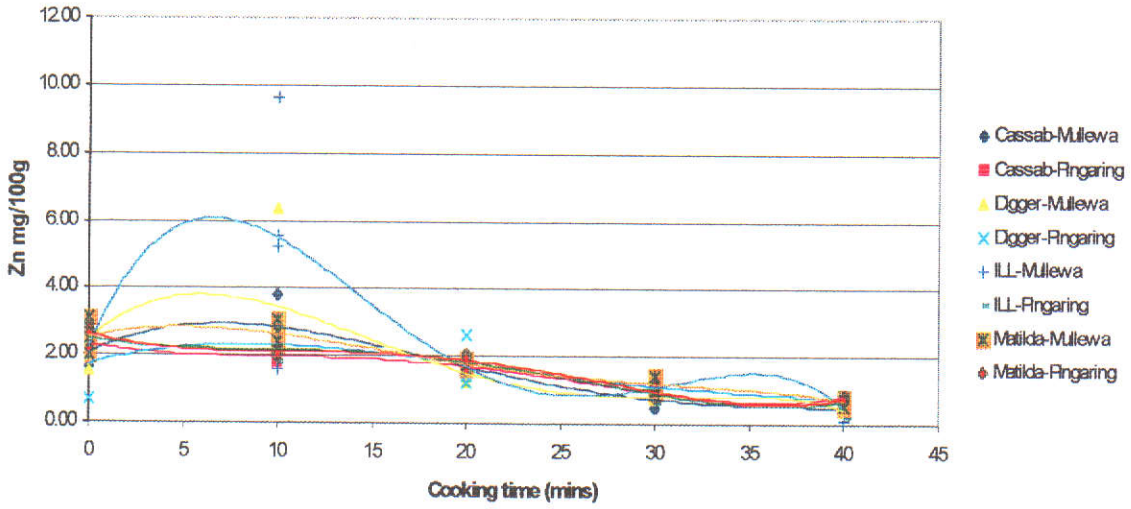


Figure: Effect of cooking on Zinc content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Zinc♣ and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R^2
Cassab	Mullewa	-0.8681*	0.7535
	Pingaring	-0.9506*	0.9037
Digger	Mullewa	-0.8873*	0.7873
	Pingaring	-0.6768*	0.4580
ILL	Mullewa	-0.7534*	0.5677
	Pingaring	-0.8604*	0.7402
Matilda	Mullewa	-0.9214*	0.8490
	Pingaring	-0.9465*	0.8959

*Correlation is significant at the 0.01 level (2-tailed)

♣ Transformation is performed

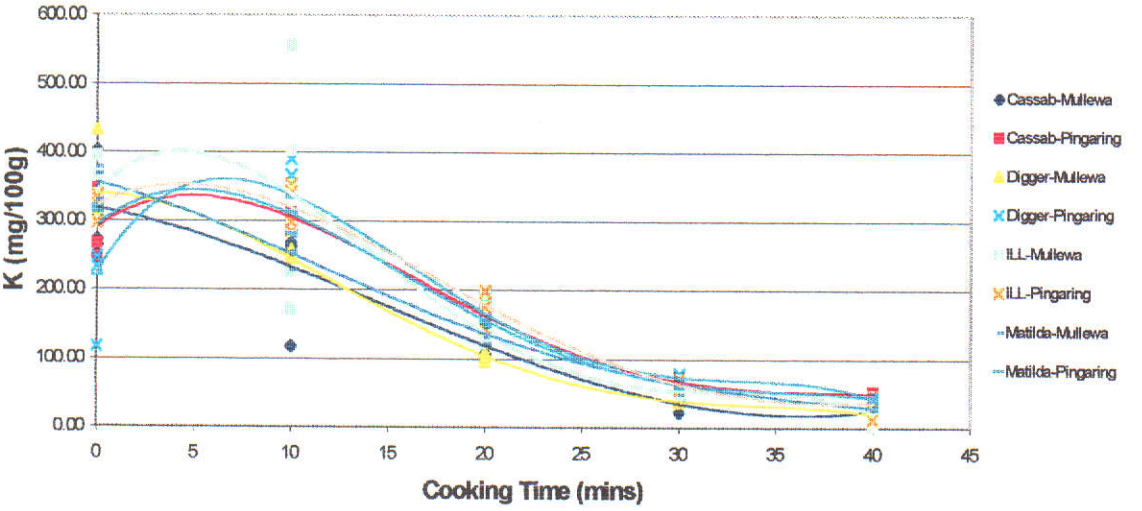


Figure: Effect of cooking on Potassium content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Potassium* and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R^2
Cassab	Mullewa	-0.9548*	0.9116
	Pingaring	-0.9493*	0.9012
Digger	Mullewa	-0.9469*	0.8966
	Pingaring	-0.8387*	0.7034
ILL	Mullewa	-0.9269*	0.8591
	Pingaring	-0.9512*	0.9048
Matilda	Mullewa	-0.9926*	0.9853
	Pingaring	-0.9805*	0.9613

*Correlation is significant at the 0.01 level (2-tailed)

* Transformation is performed

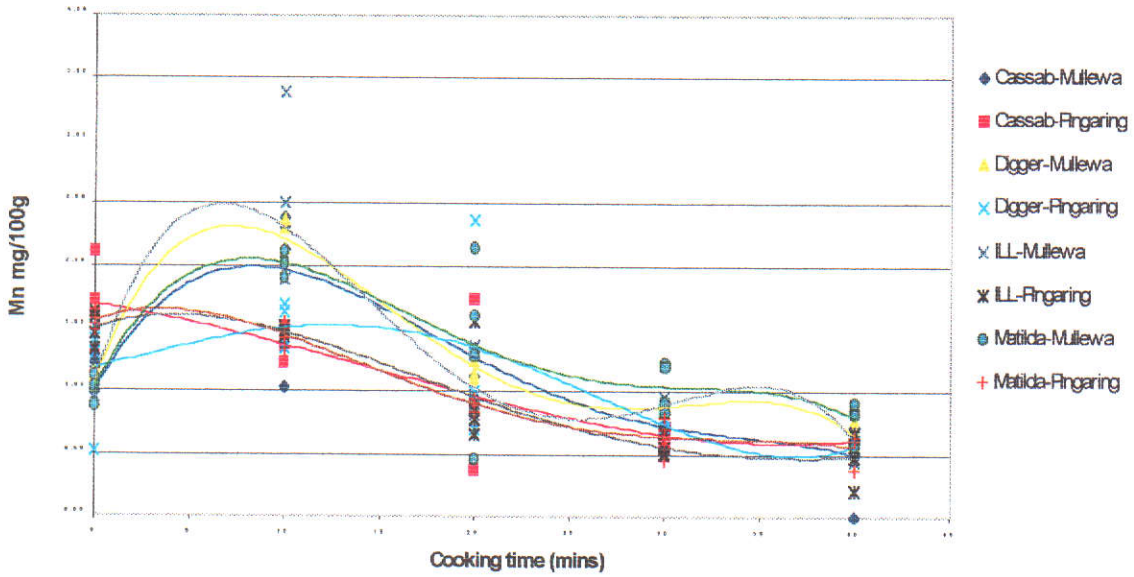


Figure: Effect of cooking on Manganese content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Manganese$^{\clubsuit}$ and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R2
Cassab	Mullewa	-0.5533*	0.3062
	Pingaring	-0.8076*	0.6523
Digger	Mullewa	-0.6536*	0.4272
	Pingaring	-0.6308*	0.3979
ILL	Mullewa	-0.6760*	0.4570
	Pingaring	-0.8656*	0.7493
Matilda	Mullewa	-0.3977*	0.1581
	Pingaring	-0.9278*	0.8609

*Correlation is significant at the 0.01 level (2-tailed)

$^{\clubsuit}$ Transformation is performed

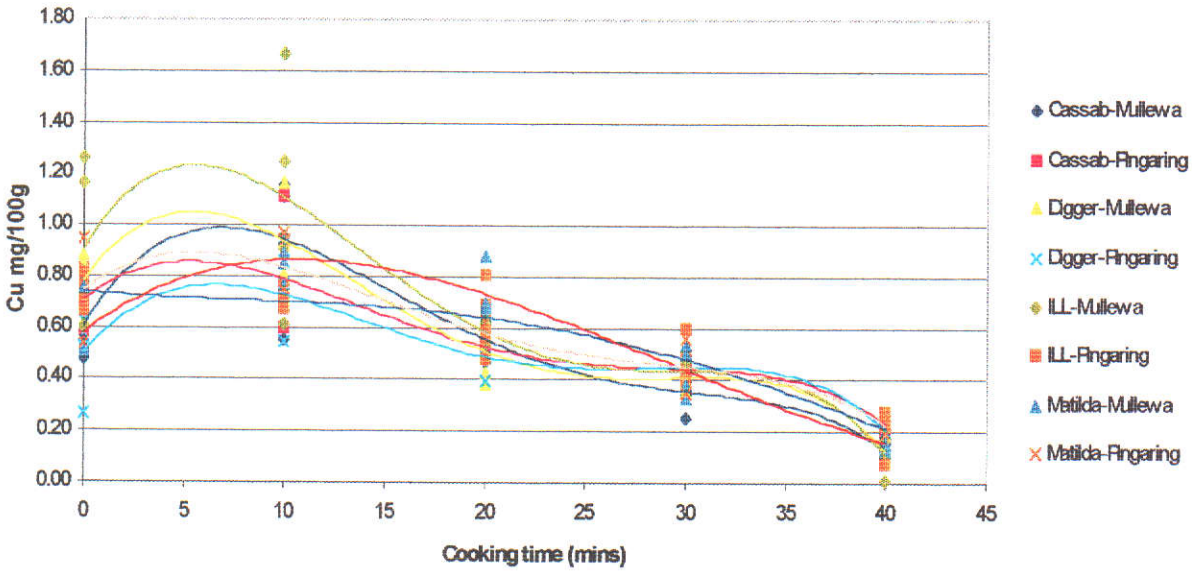


Figure: Effect of cooking on Copper content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Copper* and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R^2
Cassab	Mullewa	-0.8143*	0.6631
	Pingaring	-0.8787*	0.7722
Digger	Mullewa	-0.8801*	0.7745
	Pingaring	-0.6608*	0.4366
ILL	Mullewa	-0.7473*	0.5584
	Pingaring	-0.7658*	0.5864
Matilda	Mullewa	-0.7495*	0.5617
	Pingaring	-0.8708*	0.7584

*Correlation is significant at the 0.01 level (2-tailed)

* Transformation is performed

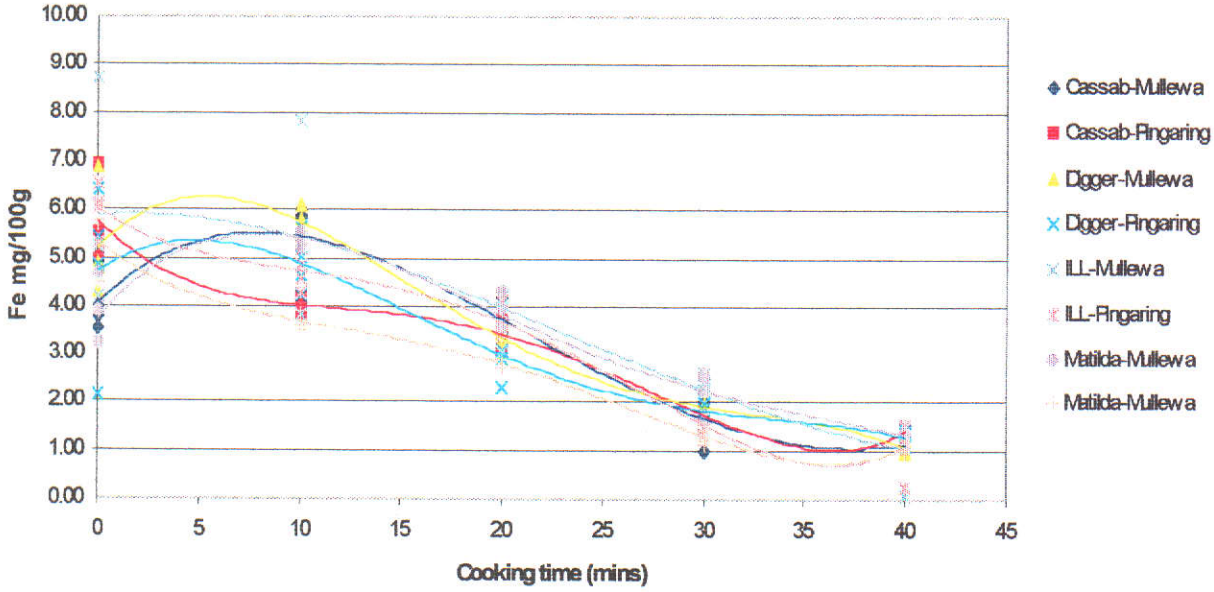


Figure: Effect of cooking on Sodium content in seeds among cultivars.

Table: Pearson Correlation and R^2 between Iron$^{\clubsuit}$ and cooking time among cultivars

Cultivars	Locations	Cooking Time (mins)	
		Correlation	R^2
Cassab	Mullewa	-0.8603*	0.7401
	Pingaring	-0.9736*	0.9480
Digger	Mullewa	-0.9406*	0.8848
	Pingaring	-0.8802*	0.7747
ILL	Mullewa	-0.7511*	0.5642
	Pingaring	-0.8754*	0.7663
Matilda	Mullewa	-0.8236*	0.6783
	Pingaring	-0.9731*	0.9470

*Correlation is significant at the 0.01 level (2-tailed)

$^{\clubsuit}$ Transformation is performed

Appendix 4D: Moisture

Procedures:

1. Aluminum dishes with lids dried overnight at 105°C and cold in desiccators.
2. Approximately 10g of sample added and weighed accurately.
3. Dishes placed with lids underneath and leave overnight in oven at 105°C.
4. Dishes removed and stored in desiccators until room temperature is reached
5. Re weight.

Calculation:

$$\% \text{ Moisture} = \frac{\text{weight after drying (g)} \times 100}{\text{weight of sample (g)}}$$

Raw Data Results

Cooking time	Pingaring-ILL	Mullewa-ILL	Pingaring-Cassab	Mullewa-Cassab	Pingaring-Matilda	Mullewa-Matilda	Pingaring-Digger	Mullewa-Digger
0	0.0898	0.0953	0.0850	Fail	0.0904	0.0903	0.0869	0.0913
0	0.0896	0.0998	0.0886	0.0897	0.0877	0.0887	0.0908	0.0903
0	0.0879	0.0953	0.0895	0.0906	0.0878	0.0867	0.0877	0.0929
0	0.0894	0.0972	0.0886	0.0918	0.0916	0.0905	0.0906	0.0917
10	0.5605	0.4427	0.5446	0.4939	0.5596	0.4773	0.5601	0.5044
10	0.5447	0.4618	0.5345	0.5254	0.6214	0.5129	0.5575	0.4808
20	0.4190	0.4190	0.4365	0.4365	0.4245	0.4245	0.4147	0.4147
20	0.4257	0.4257	0.4361	0.4361	0.4392	0.4392	0.4235	0.4235
30	0.3017	0.2726	0.3103	0.1850	0.3205	0.2813	0.3518	0.2670
30	0.3069	0.2758	0.3047	0.2176	0.3164	0.2659	0.3028	0.2518
40	0.2610	0.2610	0.2919	0.2919	0.2696	0.2696	0.2603	0.2603
40	0.2568	0.2568	0.2798	0.2798	0.2571	0.2571	0.2649	0.2649

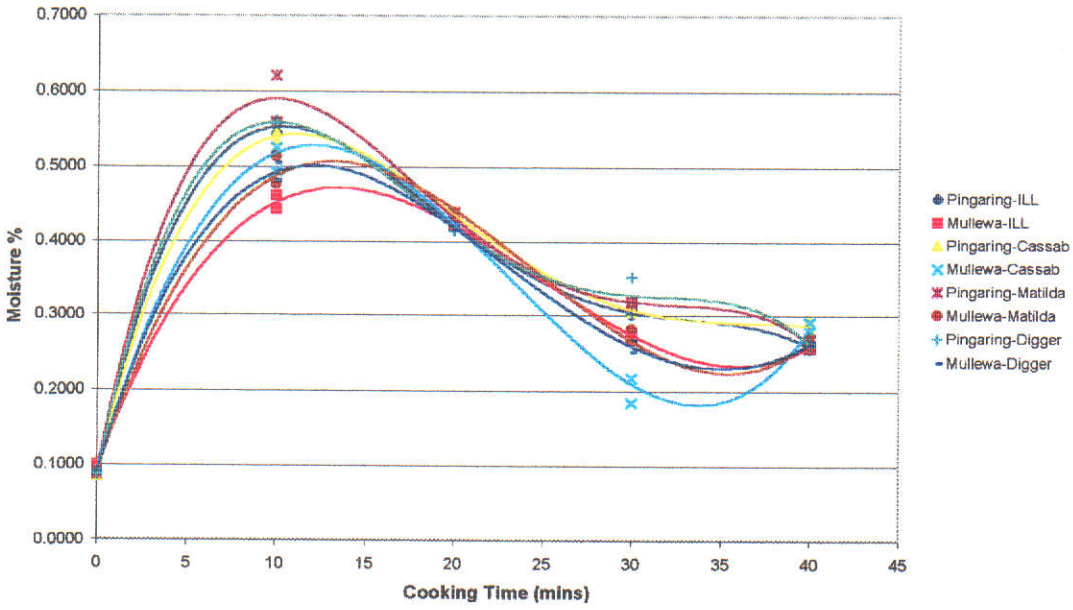


Figure: Change of moisture content with increasing Cooking time