School of Public Health

The Effects of Heat Treatment and Processing Techniques on the Quality of Australian Sweet Lupin (*Lupinus Angustifolius*) Flour

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

SHERIFAH BAHARUDIN
31/10/2016

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Abstract

Australian Sweet Lupin (ASL) is an important legume crop in Australia, with Western Australia being the world's largest producer of lupin. Lupin has promising food application potential due to its impressive nutritional values, including high protein and dietary fibre contents. Lupin has unique human health benefits such as reducing the incidence of obesity and type 2 diabetes, lowering blood pressure and cholesterol levels, and improving bowel health. However, lupin is underutilised as a human food source and mainly used as animal feed. Attempts to incorporate lupin into food products have resulted in limited commercial success due to lower consumer acceptability, mainly due to its beany flavour. The enzymatic reaction of Lipoxygenase (LOX) enzyme on the polyunsaturated fatty acids (PUFA) contributes to the beany flavour. The elimination or reduction of LOX activity is likely to enhance the acceptability of lupin-incorporated food. One such technique of reducing the LOX activity in lupin is by heat treatment of the lupin seed or the flour prior to use in food applications. Hence, the main aim of this study was to eliminate or reduce the LOX in ASL flour to increase consumer acceptability, when applied to a food product.

The LOX activity of 25 lupin varieties grown in Western Australia in 2009, 2010 and 2011 period were evaluated. This was to understand the LOX variations in different lupin varieties and the possibility of determining seasonal variations that may impact the flavour of the end product. The proximate composition and colour of the seed samples were also measured. There were significant differences among the 25 varieties of lupin in terms of LOX activity. Coromup, Mandelup and Moonah have the highest LOX activity and Warrah has the lowest LOX activity. The LOX activities of lupin varieties grown in 2010 were significantly different ($p<0.05$) from those of the two harvesting years of 2009 and 2011, which indicates environmental conditions have a significant effect on LOX activity in lupin. There are significant differences between the 25 lupin varieties for yield percentages, ash, protein and fat contents as well as colour measurements (L*, a*, b*). Yellowness (b*) is the most relevant in this study due to the yellow colour imparted in lupin incorporated foods. There could also be apossible correlation between the yellowness of the lupin flour with the level of LOX

activity, whereby many varieties with high LOX activity have a more pronounced yellow colour.

Based on the LOX results of the 25 varieties, Belara (medium LOX), Danja (medium LOX) and Mandelup (high LOX) were selected for further studies. The critical time and temperature combinations and three types of heat treatments that can remove or reduce the beany flavour of lupin flour were also determined. These were conventional oven, microwave oven and electric pressure cooker processing of lupin seeds, dehulled lupin and flour. The three selected varieties were subjected to these heat treatments. All three methods, at certain stages, were able to significantly reduced the LOX activity. However, the heat treatment method best used to reduce LOX activity level was the conventional oven method with the time – temperature combination of 5 minutes at 80
°C as the microwave oven causes uneven burnt on samples and the pressure cooker causes sample to be moist and damp. There was no significant difference ($p>0.05$) in LOX activity when compared to heat treated lupin at different processing stages and time in oven heat treatment for each temperature.

The effects of using heat treated lupin flour in chapatti were also determined. Heat-treated lupin flour samples were incorporated into a chapatti formulation at 20%, 30% and 40% levels of substitution of wheat flour with lupin kernel flour and its effects on texture and colour were measured instrumentally and overall acceptability was determined using a consumer panel. Mandelup variety was chosen to be incorporated into chapatti production due to its high LOX activity. It was found that up to 40% of heat-treated and non-heat treated lupin flour could be incorporated into the chapatti without affecting its overall acceptability ($p<0.05$). As for chapatti texture measured using a texture analyser, the heat-treated lupin chapatti at 40% inclusion needed a significantly lesser amount of force to tear the chapatti compared to the control and all non-heat treated lupin incorporated chapatti. This could be due to the higher inclusion of heat -treated lupin flour, which interacts and reduces the formation of gluten matrix, resulting in an easier to tear chapatti. There were also changes in fibre profile when the lupin flour was subjected to the heat treatment. This change in fibre profile affects

its water binding capacity thus impacting the texture of chapatti at a high inclusion rate of 40%.

The results demonstrate that LOX activity is affected by variety and it can be reduced by heat treatment. Future lupin researchers will find the determined LOX activity of the different varieties in this study valuable when shortlisting varieties to be incorporated into food products.

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1.0 Introduction

Australia is the world's largest producer of Australian Sweet Lupin (ASL) (*Lupinus angustifolius*), also known as narrow-leafed lupin. Lupin plays a significant role in Australia's agriculture as it is highly feasible in crop rotations. This grain legume has been proven to support the sustainability of wheat and barley production in Australia (Gladstones, 1998). Lupin shows promising potential to be applied in staple food due to its high protein and dietary fibre contents which may help resolve the hunger predicament that are prevalent in the third world nations (Jayasena & Quail, 2004). Off late, the increasing evidence of unique human health benefits as functional food and nutraceutical opportunities has triggered more commercial interests in applying lupins in food products. ASL is reported to be able to combat obesity and other health related complications namely diabetes and cardiovascular diseases that are normally common in occidental countries (Sipsas, 2008b).

Lupin can be used as a cost-effective substitute for soybeans in many food formulations such as bread, biscuits, tempe, miso and tofu. In fact, many researchers emphasize that lupin should be incorporated extensively into human diets due to its impressive nutritional composition (Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson, 2016). At present however, lupin is underutilized and its main use only concentrates on animal feed (Jayasena, Chih, & Dods, 2006; Jayasena, Kartawinata, Coorey, & Dods, 2006). There have been research attempts to incorporate lupin in food formulation but most has resulted in limited commercial success. One of the main reasons is the beany flavour from this nutritious grain which is so unappealing to consumers. The undesirable beany flavour or off-flavour is caused by Lipoxygenase (LOX) enzyme reaction on polyunsaturated fatty acids (PUFA). (Dahuja & Madaan, 2004; Malekian et al., 2000).

There is a growing interest in improving flavour of legumes by removing the undesirable "beany", "green" and "grassy" flavours during processing and storage (Clemente, 2000). Lipoxygenase was found to be the cause of off-flavours in legumes such as green peas, green beans, soybeans and corn during storage.(Yoshie-Stark &

Wäsche, 2004). It is suggested that to remove the beany flavour, the enzyme lipoxygenase should be destroyed by using heat treatments. Research by Ha et al (1992) on soybean showed that heat treatment is an effective method in removing 'beany' off-flavour in soymilk.

This research is aimed at finding the right combination of time, temperature and method of heat treatment that can eliminate or significantly reduce the beany flavour of lupin improving the consumer acceptability of foods made from lupin flour. The emphasis of the design of this study is not to compare temperature but to compare the heat treatments combination.

1.1 Objectives

- To eliminate or reduce the 'beany flavour' of ASL flour in order to increase consumer acceptability.
- To determine the critical time and temperature combination and types of heat treatment that can remove or reduce the 'beany flavour' of lupin flour.
- To determine the effects of using heat treated lupin flour in chapatti.

2.0 Literature Review

2.1 Lupin

2.1.1 Overview of Lupin

Lupin is a member of the genus *Lupinus* from the family *Leguminosae*, subfamily Papilioniodeae (Gladstones, 1998). *Lupinus* is a large and diverse genus comprising 200–500 annual and perennial herbaceous species, mostly with a height of 0.3–1.5m and attractive long flowers (Ainouche & Bayer, 1999). Lupins are found widespread around the world, mainly in South America and western North America, in the Mediterranean region, and Africa. It grows well in cool to moderately warm climates and can be planted early due to its tolerance to frost (Waldroup & Smith, 1989).

There are around 12 species which are native to the Europe and Mediterranean region. Botanical features and geographical distributions of the 12 species of lupins are shown in Table 1. The three species that were brought into Australia during the mid-19th century are: *Lupinus albus, Lupinus angustifolius, and Lupinus luteus* (Cowling, Buirchell, & Tapia, 1998). According to Waldroup and Smith (1989), these three species have significant nutritional values for livestock feedings, as compared to the other nine species.

The first species to be commercially cultivated in Australia was the Mediterranean white lupin, *Lupinus albus*. The common name for *Lupinus albus* which is "white lupin" comes from a direct translation from its Latin botanical name, and this is the name known in most English-speaking countries. However, in Western Australia, *Lupinus angustifolius*, which is the narrow-leafed lupin variety, is incorrectly called "white lupin" due to its white flower and seeds (Gladstones, 1976).

Table 1: Botanical features and geographical distributions of the 12 Lupinus species

Species	Synonyms	Common names	Characteristics	Distributions
New World (Americas & Oceania) L. mutabilis sweet		Tarwi Pearl lupin Chocho	Large, permeable white seeds, indehiscent pods, high alkaloid, attractive scent	South America: Andean Highlands
Old World (Europe, Asia, Africa) smooth-seeded:				
L. albus L. Var. Albus var. graecus	*L. termis* Forsk. *L. graecus* Boiss. and Sprun. *L.jugoslavicus* Kazim. and Now. *L. vavilovi* Atab. and Maiss.	European white lupin, Albus lupin	Permeable seeds, indehiscent pods, high alkaloid content, bluish white flower, wild species has blue flowers, shattering pods, smaller dark-coloured impermeable seeds	Mediterranean, Nile valley
L. angustifolius. L.	L. varius L., L. linifolius Roth., L. reticulates Desv. L. opsianthus Atab. and Maiss.	Narrow-leafed lupin, blue lupine (USA)	Hard medium size seed, flowers are white (domesticated), blue, and occasionally pink in wild species.	Pan-Mediterranean. Australia
L. micranthus Guss.	L. hirsutus L.	Lesser hairy blue lupin,	Individual seed size of 100 mg.	Pan - Mediterranean

Species	Synonyms	Common names	Characteristics	Distributions
L. luteus L.	-	Yellow lupin	Golden yellow flowers, sweet scent, high nitrogen green production, bitter.	Iberia, Pan - Mediterranean
L. hispanic Boiss. and Reut.	L. rothmaleri Klink	-	Pale violet flowers, unscented, seeds slightly rough, plain olive brown/white.	Spain, Algeria, Greece, Turkey, Portugal
Old World, rough- seeded:				
L. pilosus Murr.	L. hirsutus L. L. anatolicus Swiec.	Greater hairy blue lupin	Pink or occasionally purple or white flowers, early flowering, large to very large seeds.	North East Mediterranean
L. cosentinii Guss.	-	Sandplain lupin, Western Australian blue lupin	Brown seeded (north of Perth), grey seeded (south of Perth).	West Mediterranean, Morocco, Australia
L. digitatus Forsk.	L. semiverticillatus Desr. L. tassilicus Maire	-	-	Sahara
L. atlanticus Gladst.	-	Morocco lupin	-	Morocco

Species	Synonyms	Common names	Characteristics	Distributions
L. prince Harms.	-	-	Wild lupin having white flowers, large seeded	East Africa
L. somaliensis Baker.	-	-	-	East Africa

Source: Gladstones (1998)

2.1.1.1 History of Lupin

Lupin has been a traditional food in the Mediterranean region and the Andean highlands for thousands of years. Figure 1 shows the distribution of *Lupinus angustifolius* L. in the Mediterranean region from which it can be concluded that this lupin variety thrives in a Mediterranean temperature similar to the Western Australia (WA) climate, hence the reason why *Lupinus angustifolius* can thrive in WA. Besides using lupin for human consumption, the Greeks, Romans, Egyptians, and the Andean people cultivated lupins to improve their soil (Ruiz-López et al., 2000). During the Roman Empire, the yellow legume seeds of lupins, commonly known as lupin beans, were widely cultivated throughout the Roman Empire. Some other usages range from medicinal during the time of Hippocrates of Cos, and as gifts and money in Roman times (Cowling et al., 1998).

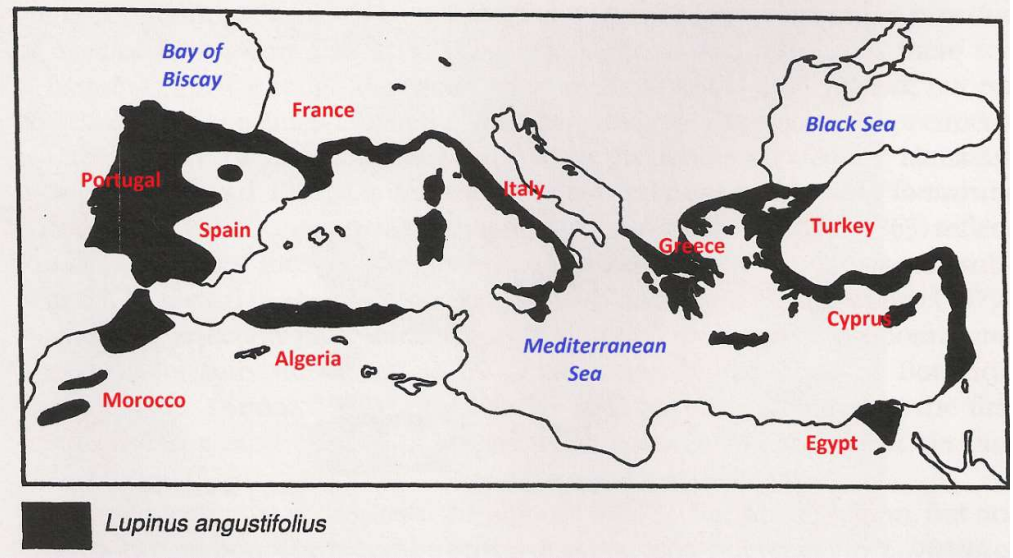

Figure 1 : Distribution of *Lupinus angustifolius* L. in the Mediterranean region.
Source: Gladstones (1998)

Development of lupin as a human food crop was limited due to the alkaloids found in the legume which are toxic, and had to be removed before consumption. For centuries, farmers in the Andes used traditional techniques to debitter (remove alkaloids) from lupins. The seeds were soaked for 5-8 days by placing bags of lupin in flowing river water before being consumed (Cowling et al., 1998). But German plant breeders in the

1920's produced the first selections of low alkaloid or "sweet" lupin, which can be directly consumed by humans or livestock (Putnam, Oplinger, Hardman, & Doll, 1997). Between the 1930s and the 1940s, sweet lupin was slowly introduced into the modern food industry. However, lupin is better known as feed for monogastric and ruminant animals (Cowling et al., 1998).

2.1.1.2 Australian Sweet Lupin

Australian Sweet Lupin (ASL) refers to the legume crop *Lupinus angustifolius* (narrow-leaved Lupin) cultivated in Australia. It is the largest grain legume crop grown in Western Australia, producing between 500,000 and 1,000,000 tonnes annually, which is considerably more than any other state in Australia, or any other country in the world. Figure 2 shows the distribution of lupin production areas in Australia.

Figure 2 : Distribution of lupin production area in Australia
Source: Anonymous (2009)

The reason for lupin being successfully grown in Western Australia, as compared to other parts of the world, is due to the fact that the grain legume is highly adaptive to the acidic sandy soils and the Mediterranean climate of south-western Australia (French & Buirchell, 2005). ASL is the major species found in Australia, having proved to be the most adaptable to Australian conditions and has been the subject of intensive breeding effort based in Western Australia (Landers, 1991). During the past 20 years, Australia has exported more than 15 million tonnes of Australian sweet lupin to countries all over the world, including Spain, The Netherlands, Indonesia, Japan, South Korea, Thailand, and Taiwan (Sipsas, 2008a).

ASL is mostly used in a crop rotation as they can add nitrogen into the soil, reduce cereal diseases, improve soil structure and add diversity to farm production (Landers, 1991). By adding nitrogen and controlling cereal diseases, crop rotation has a higher probability of improving cereal yield or grain protein content in the following crop (Perry et al., 1998). Having a high protein content, ASL is favoured as an on-farm feed for most farm animals. However, with the increasing awareness of the health benefits of ASL, lupins are slowly being incorporated into food products for human consumption (Jayasena & Quail, 2004). However, the inclusion in food is somewhat restricted due to the undesirable beany flavour that lowers consumer acceptability.

ASL is readily distinguished from other cultivated lupins by its foliage. The narrow leaflets are only 1.5-4 mm wide in wild types (up to 6 mm in cultivated types), as compared to the leaflets of other varieties ranging from 8-20 mm wide. Full domestication of ASL did not occur until low alkaloid varieties were selected in Germany in the late 1920s and the sweet gene iucundis was recombined with non-shattering pods and white flowers and seeds by Dr. Gladstones in the 1960s (Cowling et al., 1998). The work that Dr. Gladstones did, together with the University of Western Australia and the Department of Agriculture and Food (DAFWA), was considered a major breakthrough, and the domestication of lupin to ASL marked the beginning of grain lupin cropping in Australia (P. White, French, McLarty, & Grains Research and Development Corporation, 2008). Figure 3 shows a close up picture of an ASL plant. For practical purposes, in this thesis, the word lupin is referred to *Lupinus angustifolius* (Australian Sweet Lupin) unless otherwise stated.

Figure 3: Australian Sweet Lupin

Picture taken by author during the lupin society workshop at Wongan Hills on the 14th September 2011.

2.1.1.3 Lupin Processing

To utilise lupin for human consumption, lupin seeds must first go through a series of processes to produce lupin flour. According to Food Standards Code for Cereals and Cereal Products (Standard 2.1.1), flour is defined as "products of grinding or milling of cereals, legumes or other seeds". From this definition, it is understood that lupin flour is the end product of milling lupin seed.

To produce lupin flour, seeds are sorted and graded to remove any foreign objects by using a vibrating screen. The cleaned whole seeds are then dehulled using a dehuller and then separated with an aspirator. The clean kernels are then milled using a range of mills depending on the intended usage or customer preference. The most popular mill used in Western Australia is the rotor mill which was used in this present study.

2.1.2 Composition of Lupin

Lupin is a type of grain legume that belongs to the family of Leguminosae, which includes peas, various beans, soy, chickpeas, and peanuts. Lupin is also known as a

non-endospermic, dicotyledons plant due to the cotyledons being developed as the plant storage tissues of the seed. Hence, a grain legume stores nitrogen (protein) and carbon as its reserves, while grains high in oil content such as soy and peanuts store oil as their reserve substances. However, lupin is different from the rest, as it stores a high amount of non-cellulose, non-polysaccharide (NSP) in its thickened walls of the cotyledon (Pfoertner & Fischer, 2008).

The polysaccharide content of lupin cotyledon is mainly galactan, a form of polymerised galactose, and the outer layer coating of the seed, known as the hull, is mainly made of cellulose or hemicelluloses, and accounts for 25% of the seed weight (Mohamed & Rayas - Duarte, 1995; Petterson, 1998b).

The inner seed is called cotyledons (kernel) where most of the seed's energy is stored within the kernels, in the form of oil, oligosaccharides, and non starch polysaccharides (Petterson, 1998b). Unlike other common crops such as rice, wheat, and other legumes such as field peas and lentils, lupin contains very little starch (Sipsas, 2008a). Figure 4 shows a diagram of the lupin seed.

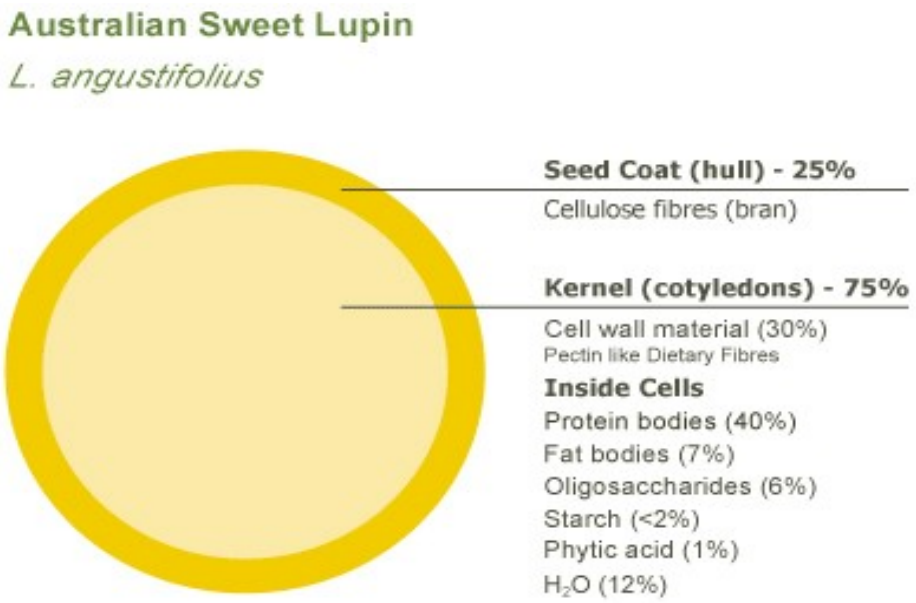

Figure 4: The lupin seed

Source: Sipsas (2008a)

Lupin flour is the product of the finely milled, dehulled lupin kernels. It is pale yellow in colour and produces a golden colour when incorporated into baked goods. The addition of lupin flour to wheat flour-based products has the potential to increase dietary fiber content and improve protein content and quality (Hall & Johnson, 2004). Unlike highly processed legume ingredients such as purified fibre and protein isolates, lupin flour also provides a wide range of phytochemicals, including antioxidants and phytosterols, with health benefits (Jayasena, Leung, & Nasar - Abbas, 2008). The chemical composition of lupin flour is shown in Table 2.

Table 2: The chemical composition of lupin and soybean flour

Nutrient	lupin flour[a] (%)	soybean flour[b] (%)
Protein	41	35 - 40
Fat	7	15 - 20
Polysaccharides (non- starch)	29	30
Oligosaccharides	6	-
Lignin	1	-
Moisture	12	10 - 13
Ash	3	5

Source: a – Lee (2007); b – Riaz (2006)

The composition of lupin is deemed far superior to soy in terms of having a significantly lower fat content and a higher dietary fibre content than soy. This gives lupin an edge in terms of having a better nutritional value, and by being more economical than soy; lupin has major potential in replacing soybean in food products.

2.1.2.1 Carbohydrate

The composition of the lupin seed coat (hull) is very similar to that of a soy hull. Lupin and soy hulls contain predominantly structural polysaccharides; cellulose, hemicelluloses, and pectins, along with low amounts of lignin, protein, and lipid (Petterson, 1998b; Riaz, 2006). One hundred grams of lupin hull contains 95 grams of

fibre. Xylose, the main building block of hemicelluloses, is present in large quantities, followed by uronic acid (Sipsas, 2008a). These insoluble carbohydrates components (dietary fibres) give valuable contribution to the overall benefit of lupin consumption because consuming adequate amount of dietary fibres has been linked to a reduction in the risk of heart disease and cancer, as well as the improvement of bowel function (Riaz, 2006).

As for the kernel, the main carbohydrates are non-starch polysaccharides (NSP), which consist mainly of galactose, arabinose, and uronic acid. This makes lupin distinct from cereals and other legumes, in which starch is the main storage carbohydrate (C. L. White, Staines, & Staines, 2007). About 20% of NSP are water-soluble, and are considered to have bioactive and textural properties because of their viscous property and the effect on intestinal transit time (Sipsas, 2008a). Cotyledon NSP is also able to hold a large amount of water and maintains normal gut motility (Sipsas, 2008a). A comparison of carbohydrate content in lupin hulls and cotyledons is shown in Table 3. It is shown that hull contains three times more dietary fibre than cotyledons, meanwhile, a cotyledon has a significantly higher amount of oligosaccharides compared to the hull and is also higher in sucrose. In terms of comparison of total dietary fibre (TDF) in legumes, lupin was found to have a significantly higher total dietary fibre content (34%) as compared to peas (14%) and soy (19%) (Bähr, Fechner, Hasenkopf, Mittermaier, & Jahreis, 2014) which adds to the appeal of lupin as a potentially sought after food ingredient.

Table 3: The carbohydrate content in lupin hull and cotyledon (variety: Danja)

Polysaccharides	Hull	Cotyledons
Dietary fibre	91	31*
- Cellulose	51	1.3
- Lignin	1.3	0.9
Oligosaccharide	0.4	7.4
Sucrose	1.4	3.5
Starch	0.4	0.7

(Petterson, 1998b)

*Mainly Non Starch Polysaccharide (NSP)

Another polysaccharide component found in lupin is the oligosaccharide. The lupin oligosaccharide-fraction (5-12%) contains raffinose, stachyose, verbascose, and ajugose. Raffinose has one galactose moiety linked to sucrose through an α 1,4 bond, while stachyose has two, verbascose three, and ajugose four molecules (Sipsas, 2008a). It is a well known fact that oligosaccharide is a rich source of food for the beneficial bifidobacteria found in the lower intestine. Bifidobacteria has been known to prevent or reduce infectious gut diseases with symptoms such as diarrhoea, as it is highly resistant to pathogens' colonisation in the intestines. Some bifidobacteria strains have even been studied, to help protect the host from carcinogenic activities in the intestines, by decreasing the production and/or activity of potential carcinogens such as ammonia. These, in turn, help to reduce the incidence of colon cancer and other intestinal disorders (Picard et al., 2005).

2.1.2.2 Protein

Legumes have always been the main sources of protein for vegetarian diets. Lupin has a very high amount of protein (45%) comparable to soy (49%) and is significantly higher than pea (24%) (Bähr, Fechner, Hasenkopf, et al., 2014). Most of the proteins consist of globulin type storage proteins called conglutins, making up about 85% of the total protein (7s and 11s storage protein), while the remaining 15% of proteins are albumins, which are soluble at pH 5 and vary in size (Lee, 2007; Sipsas, 2008a).

In a study by Mariotti, Pueyo, Tomé, and Mahé (2002), it was determined that the Real Ileal Digestibility (RID) of lupin flour is similar to soy protein, which is 91%. As the availability of amino acids is primarily determined by their digestibility measured at the end of the small intestine (which is the ileum), and no absorption at the large intestine, the term RID is established to measure the bioavailability of amino acids, which, in this study, is on healthy human men. The amino acid score of lupin is 0.87, which is slightly lower than soy protein. Apart from lupin flour having a significantly higher amount of Arginine than soy protein; other amino acids do not have any significant difference. Like most legumes, lupin has a low amount of sulphur-containing amino acids (cysteine and methionine) which is normally found in abundance in meat and certain grains. Hence, it is necessary to combine lupin with cereal protein to get a balanced amino acid profile in the diet. It is also interesting to

note that the digestibility and bioavailability of the ASL protein is similar to soy protein.

Table 4 shows the essential amino acid profile of lupin flour protein and soybean flour protein in g/16 g N. Normally amino acids are expressed as a percentage of protein or g per 16 g N. From the table, it can be seen that the amino acid content is comparable; however, certain amino acids, such as Arginine and Tyrosene, are slightly higher in lupin flour than in soybean flour. This shows that lupin can be a viable option to replace soybean when consuming protein due to the similarities in amino acid content.

Table 4: Amino acid analysis of lupin and soybean flour (g/16 g N)

Amino acid	Lupin flour [a]	Soybean flour[b]
Arginine	12.0	7.3
Histidine	2.41	2.6
Isoleucine	3.97	4.6
Leucine	6.61	7.8
Lysine	4.66	6.4
Methionine	0.72	1.1
Phenylalanine	3.65	5.0
Threonine	3.13	3.9
Tryptophan	0.97	1.4
Valine	3.64	4.6
Cysteine	1.48	1.4
Tyrosine	5.09	3.8

Source: Source: a – Petterson (1998a) b - Vaidehi and Kadam (1989)

2.1.2.3 Fat

The oil content in lupin is approximately 7%. This amount is higher than most plant legumes but is lower compared to soybean, which has around 20% oil. The main fatty

acid component of lupin are the polyunsaturated fatty acid (PUFA) oleic and linoleic acid. Table 5 shows the comparison of the fatty acid profile of lupin and soybean.

Lupin contains 81% unsaturated fat and 19% saturated fat. The lipid content of Australian Sweet Lupin is comprised of triglycerides (71%), phospholipids (15%), free sterols (5%), glycolipids (3.5%), sterol and wax esters (0.5%), free alcohols (0.4%), and unidentified waxy material (4%). The sterols present in the non-saponifiable fraction of the oil are sitosterol and campesterol as the main components, and stigmasterol and avenasterol as secondary (Lee, 2007).

Table 5: The fatty acid profile of lupin and soybean (%)

Fatty acid	Average lupin [a]	Average soybean [b]
Lauric acid (12:0)	-	0.1
Myristic acid (14:0)	0.2	0.2
Palmitic acid (16:0)	10.3	10.7
Palmitoleic acid (16:1)	0.1	0.3
Stearic acid (18:0)	4.8	3.9
Oleic acid (18:1)	34.0	22.8
Linoleic acid (18:2)	37.0	50.8
Linolenic acid (18:3)	6.2	6.8
Arachidic acid (20:0)	0.7	0.2
Gadoleic acid (20:1)	0.3	-
Eicosadienoic acid (20:2)	0.4	-
Eicosatrienoic acid (20:3)	0.2	-
Arachidonic acid (20:4)	<0.01	-
Behenic acid (22:0)	1.3	<0.5
Erucic acid (22:1)	<0.01	-
Lignoceric acid (24:0)	0.1	-

Source: a - Petterson, Sipsas, and McIntosh (1997); C. L. White et al. (2007)
b – Pryde (1980); X. Wang, Wang, and Johnson (2002)

Apart from a higher content of linoleic acid in soya and oleic in lupin, there is no significant difference in the fatty acid profile of both legumes. Both also contain a

slight amount of linolenic acid, which is the most susceptible to auto-oxidation compared to oleic and linoleic (Pryde, 1980). As both legumes have a high linoleic acid content, they can be unstable during processing and storage due to oxidation, thus producing the beany or off-flavour which will create a negative effect on the overall taste of these legumes.

2.1.2.4 Minerals and Trace Elements

Lupin can be a valuable source of most of the minerals, especially Calcium (Ca), Phosporus (P), Potassium (K), and Sulphur (S). According to Sipsas (2008a), the calcium and phosphate contents in lupin are higher than in cereals. The mineral (ash) content between different lupin varieties varies between 3.2 and 4.6 g/100 g dry matter (Sipsas, 2008a). Among the possible reasons, it could be due to the different mineral content found in the soil used for cultivation and the genotype influence (Petterson, 1998b). Table 6 shows the mineral contents of lupin and soybean respectively.

Table 6: Mineral contents of lupin (g/kg) and soybean

Mineral	ASL[a]	Soybean[b]
Calcium	2.4	2.0[b]
Magnesium	1.8	2.2[b]
Phosphorus	3.3	4.9[c]
Potassium	8.8	25[c]
Sodium	0.4	0.1[c]
Sulphur	2.5	n.a.
Iron	0.07	0.07[b]
Zinc	0.04	0.04[b]

Source: a- Petterson et al. (1997); C. L. White et al. (2007), b – Sandberg (2002), c - U.S. Department of Agriculture and U.S. Department of Heallth and Human Services (2010)

Apart from the high amount of Potassium in soy, and low amount of Natrium, the mineral content of lupin and soy are comparable, with each having sufficient amounts

of minerals. This serves as an advantage for food products that have lupin incorporated into it.

2.1.2.5 Antioxidant

Antioxidants have long been used in the food industry to prolong the shelf life of food products and retarding the production of off-flavour by inhibiting the oxidative process of unsaturated fatty acids (Tsaliki, Lagouri, & Doxastakis, 1999). However, of late, there has been an increasing interest on natural antioxidants in food, given the growing knowledge and awareness of the general public that antioxidants are also able to combat free radicals and prevent different types of diseases caused by free radicals. Major health problems mediated by free radicals include cancer, atherosclerosis, rheumatoid arthritis, inflammatory bowel diseases, immune system decline, brain dysfunction, cataracts, and malaria. These can be prevented or delayed by natural antioxidants (Gordon, 2003).

Natural antioxidants mainly consists of plant-based phenolic compounds that can be found in almost all parts of the plants (Gordon, 2003). There are different types of natural antioxidants found in fruits, vegetables, and seeds of plants, such as phenolic compounds, nitrogen compounds, vitamins, and terpenoids (Cai, Luo, Sun, & Corke, 2004). However, the major components found in lupin are polyphenols which are mainly tannins and flavonoids (Martínez-Villaluenga et al., 2009; Oomah, Tiger, Olson, & Balasubramanian, 2006). Lupin has also been reported to have a small amount of tocopherol, which is in inverse proportion with the fat content found in lupin (Lampart-Szczapa, Korczak, Nogala-Kalucka, & Zawirska-Wojtasiak, 2003) and isoflavones, which is a phytoestrogen that belongs to the flavonoid group (Ranilla, Genovese, & Lajolo, 2009; Von Baer et al., 2006). It has been suggested that the vitamin E content in lupin is comparable to that of soybean but is lower than sunflower and rapeseed oil (Lampart-Szczapa et al., 2003).

A study by Tsaliki et al. (1999) on *Lupinus albus* ssp Graecus showed that there was a correlation between the antioxidant activity (evaluated by the β-carotene bleaching method) and the presence of total phenolic compounds in the lupin seed flour at 13.6% (extracted with cold methanol) and 20.7% (extracted with hot methanol), which were

higher than soybean. Lupin protein isolate had a higher antioxidant level than soy protein isolate, and hence can be recommended as a good ingredient for functional food formulations.

In contrast, studies conducted on different species of lupin, including ASL, show that the antioxidant activities were independent of the total phenolic content (Lampart-Szczapa et al., 2003; Oomah et al., 2006; Ranilla et al., 2009) and tocopherol (Lampart-Szczapa et al., 2003). The phenolic content of *L. angustifolius* var. Zapaton have been divided into four different compound families, namely flavones (76% of identified phenolic), hydroxybenzoics (18%), isoflavones (4%), hydroxycinnamics (1.2%), and dihydroflavonols (0.4%), respectively (Dueñas, Hernández, Estrella, & Fernández, 2009).

Sirtori et al. (2004) found that there is a negligible amount of isoflavones (0.56 mg/100 g FW) in white lupin (*L. albus*) seeds. This finding is contradicted by Ranilla et al. (2009) who showed that only the species *Lupinus murtabilis* has genistein and agenistein derivatives, and none were found in *Lupinus albus* and *Lupinus angustifolius*. Results in the studies were expressed as mg genistein by 100 g of sample in fresh weight (FW). Furthermore, the *L. murtabilis* cv. H-6 was noteworthy because of its high total isoflavone content in seed coats (87 mg/100 g FW), cotyledon (30.8 mg/100 g FW), and hypocotyls (6.1 mg/100 g FW) ($p < 0.05$), showing that isoflavones are indeed present in certain lupin species. However, even the promising amount of isoflavones found in this study is incomparable to the higher amount of isoflavones found in soybean in the USA, which is reported to be ranging from 116 to 274 mg of isoflavones/100 g FW (C. Wang, Sherrard, Pagadala, Wixon, & Scott, 2000).

There are a number of factors that might affect the antioxidant activities in lupin. Their content depends on the species, variety, growing location, and maturity of the legumes (Dueñas et al., 2009). However, heating (at a temperature of 105°C for an hour) did not significantly change the effectiveness of antioxidant activities in the 3 lupin species studied, which includes ASL (Lampart-Szczapa et al., 2003).

Recently, the germination of lupin seed has started to attract interest and has been studied by a number of researchers as a way to increase antioxidant activities. It has

been proven to increase the antioxidant activities in legumes, including lupin (Dueñas et al., 2009; Fernandez-Orozco et al., 2006). According to a recent study, the concentration of total phenolic compounds in methanolic extracts of germinated ASL flours were increased by about 700% as compared to ASL flour of ungerminated seeds (Rumiyati, 2010; Rumiyati, James, & Jayasena, 2012). The germinated ASL flour was also successfully incorporated up to 8% in muffins which resulted in higher total phenolic compounds and phytosterols content (Rumiyati, James, & Jayasena, 2015). Hence, germination can be used to increase the antioxidant level of lupin before flour manufacturing for food applications.

2.1.3 Health Benefits of Lupin

Presently, there is increasing scientific evidence on the important health benefits of Australian sweet lupin. Researchers around the world are increasingly studying the potential of lupins contribution to combat obesity and its associated health problems to diabetes and heart diseases.

2.1.3.1 Reducing risk of Type-2 Diabetes

Lupin has the lowest Glycaemic index (GI) of all commonly consumed grains (Sipsas, 2008a). GI is used to measure the carbohydrates' impact on the blood sugar levels. By having a slower carbohydrate breakdown, the glucose is released more slowly into the body, and this is considered low GI. A low GI food is better for people with diabetes as it improves blood glucose control and also blood lipids.

A study was conducted by Hall, Thomas, and Johnson (2005) on eleven healthy human subjects in Victoria, Australia, where each subject consumed an ASL flour bread breakfast once, and a standard white bread breakfast twice, albeit on different mornings, at least 7 days apart, after an overnight fast of 10-12 hours. Subjects were also asked to only consume high GI food given on a list, to avoid the "second meal effect", and to abstain from alcohol and excessive exercise. On the test day, blood was collected every 15 minutes up till 120 minutes after breakfast began. Glucose and insulin levels were measured, and perception of satiety and sensory evaluation were also done on the test day. The inclusion of 7.7 g of ASL flour to the breakfast resulted

in a glycaemic index that was comparable to that with high fibre enriched bread. The reduced GI of the ASL bread might have been due to the bread's higher lupin content which resulted in a higher insulin response. This proves that incorporating lupin flour into white bread significantly reduces the blood glucose and insulin responses in human subjects, as compared to consuming normal bread. However, there was no clear evidence on the effect ASL flour has on satiety, which contrasts with another previous study done on the same subject matter. This is probably due to the small number of subjects, which might not have supplied adequate statistical power in identifying differences in satiety responses.

In 2004, the interaction of lupin seed protein and insulin was studied extensively (Magni et al., 2004). Firstly the interaction was done in-vitro, which positively showed that the lupin seed protein component, namely conglutin γ is capable to bind with insulin. The study was carried to an in vivo assay, using 100 male rats, and the result convincingly showed that the lupin protein component indeed reduced the plasma glucose level of the rats, and the conglutin γ given at the highest dose was comparable to that obtained with half a dose of metmorfin, a well known glucose-lowering drug. This study has certainly pin-pointed the component which is most likely the reason of the reduction of plasma glucose level and the lowering of GI, but further studies should still be done to verify the concurrence of these results.

A study on lupin seed extract on mice was done by Knecht, Nguyen, Auker, and Kinder (2006), whereby the male mice were given seed extracts orally, or intraperitoneally (IP). The seed extracts used were whole seed and dehulled lupin kernel. The study found that the dehulled lupin kernel extract given orally, significantly reduced the blood glucose level, while the one administered intraperitoneally did not show a beneficial decrease of plasma glucose level. The active component of lupin would appear to be concentrated in the interior of the lupin seed. But what is most interesting to note is that lupin has the ability to reduce blood glucose when given orally, and not otherwise. This proves favourable for lupin to be incorporated into food.

On the other hand, three previous studies conducted in 1995 and 2003 incorporating lupin fibre in food have shown no positive effect on the lowering of blood glucose

level in human subjects. The study by Feldman et al. (1995) was done on 14 non-insulin dependent diabetes mellitus (NIDDM) subjects, whereby the lupin fibre taken from the whole seed was incorporated into an Israeli traditional food (melawach) and was consumed by the subjects. No significant difference on consuming fibre containing food and non-fibre containing food, was noted in the study. It is interesting to note the fibre for this study was taken from the whole seed, which consists of lupin hull fibre, whereby the hull fibre has been reported in previous studies to provide no beneficial glycaemic effects in NIDDM subjects (Diaz et al., 1990; Knecht et al., 2006).

A study on lupin fibre was done by Johnson, McQuillan, Sin, and Ball (2003) on 21 healthy male and female subjects, where they were given two breakfasts of control white bread and bread enriched with 9 g of lupin kernel fibre, on different mornings, at least 2 days apart, after an overnight fasting. Results showed that there was no significant blood glucose reduction in the healthy human subjects but there was a significant reduction on the plasma insulin level of lupin fibre-enriched bread compared to the control bread.

Hall, Johnson, Baxter, and Ball (2005), used 38 healthy male subjects using two semi-controlled diets of similar nutritional profile with all but 7 experimental foods incorporated with lupin kernel fibre. Similar to the two previous studies, there was no significant reduction in the blood glucose level and unlike the study by Johnson et al. (2003), there was also no significant lowering of the plasma insulin level of the subjects. This finding further proves that lupin fibre does not reduce blood glucose level in human subjects, both healthy and NIDDM.

2.1.3.2 Reducing Obesity

Satiety is the inhibition of hunger as a result of having eaten. Dietary fibre, when added into food, has been reported to increase satiety (Burley, Paul, & Blundell, 1993; Cani, Joly, Horsmans, & Delzenne, 2005; Slavin & Green, 2007). This is possibly due to their ability to increase bulk and viscosity which lengthen the intestinal phase of nutrient digestion and absorption (Slavin & Green, 2007). There is also increasing evidence that a high protein diet, as compared to a high carbohydrate diet, is more

satiating, resulting in a reduced energy intake (Hodgson & Lee, 2008). As such, studies have been done on the influence of satiety with regards to lupin kernel flour and its purified component of lupin kernel fibre and protein component.

In a study by Archer, Johnson, Devereux, and Baxter (2004) on 33 healthy male subjects, lupin kernel fibre was incorporated to a sausage patty as a fat replacer, the fat content was reduced by one third. Inulin was also used as another product besides lupin kernel fibre and control product (full fat patty), as a comparison. Subjects consuming the lupin fortified sausage patties showed a significantly higher perception of satiety at 285 minutes after meal consumption, as compared to consuming the full fat control sausage or the inulin-replaced sausage. This was observed and measured through the incremental satiety score. There were no differences in the incremental satiety scores for the inulin sausages and full fat sausages respectively. Interestingly, the lupin fibre-fortified sausage patties produced greater effects on perception of satiety in all three sausages despite providing less energy to the normal full fat patties and similar energy to the inulin-replaced sausages.

In a recent study done by Lee et al. (2006) on healthy and non-smoking human subjects, 40% of lupin flour was incorporated into bread. A sensory acceptability test was not done in this study to see the acceptability of 40% lupin flour. Results showed that lupin fortified bread can acutely reduce the appetite. Lupin-enrichment of bread was significantly reduced within meal food intake by approximately 30%, food intake at a subsequent meal by approximately 15%, and self-reported hunger and fullness for 3 hours following a fixed energy meal (Hodgson & Lee, 2008). The lupin flour increases the protein and fibre contents of the consumed bread, thus resulting in an increased feeling of fullness while lowering the energy intake (Lee, 2007; Lee et al., 2006). This shows that lupin flour has the ability to suppress appetite in humans.

However, a study by Hall, Thomas, et al. (2005) incorporating 10% ASL flour in breads did not have a significant effect on satiety and energy intake. This could be due to the small number of subjects in that study, which was 11, providing insufficient statistical power to detect the differences in satiety response. In another study of 38 healthy male subjects, consuming food incorporated with lupin kernel fibre also

showed that lupin kernel fibre did not have a significant effect on satiety and energy intake (Hall, Johnson, et al., 2005). The result still remains inconclusive, although the majority of the studies support the beneficial effect of lupin on satiety. Hence, the effect of lupin flour or fibre on satiety remains worthy of a further investigation.

2.1.3.3 Lowering Cholesterol Level

There has been increasing evidence (Bähr, Fechner, Kiehntopf, & Jahreis, 2015; Bähr, Fechner, Krämer, Kiehntopf, & Jahreis, 2013) suggesting that a food product supplemented with lupin has the ability to lower the blood cholesterol level. An abnormally high level of cholesterol consisting of a high amount of Low Density Lipoprotein (LDL) and a low amount of functional High Density Lipoprotein (HDL), has been linked to be the main cause of cardiovascular-related diseases.

Numerous studies have been conducted to study hypocholesterolemic activity when consuming lupin products. The cholesterol-lowering property of lupin has been found as early as 1998. In a study by Chango et al. (1998), 30 male rats were fed with lupin protein extract. The result showed that lupin has cholesterolemic effect on the rats. Another study done by Rubio, Brenes, and Centeno (2003) on growing broiler chicken, fed on whole lupin and dehulled lupin diet, also showed that the plasma cholesterol levels were lower than the animals fed on control diet.

In another study by Sirtori et al. (2004), conglutin γ, a lupin protein extract was fed to 20 rats who were on a hypercholesterolemic diet for 2 weeks. Results showed that LDL level reduced by 30%, triglycerides level were lower by 17% while the beneficial cholesterol, HDL, level was raised up to 20%. Furthermore, the rats did not experience any known side effects and their body weight was significantly higher than the control rats. The authors also found that lupin protein increases the activity of the LDL receptor in HepG2 cells, which may perhaps be the cause for the hypocholesterolaemic effect observed in vivo. This finding, however, is not applicable to proteins that undergo digestion since cultured cells of HepG2 were used.

A study by Martins et al. (2005) on pigs fed with hypercholesterolemic diet found that feeding the pigs with whole blue lupin seeds for three weeks had exerted a marked

hypocholesterolemic effect. The result was based on the marked decrease in the intestinal absorption of cholesterol, likely to be modulated by bile acid reabsorption and phytosterols. The bile acid metabolism, according to the author, was mainly due to the stimulation done by the ingestion of lupin seeds.

Viveros, Centeno, Arija, and Brenes (2007), randomly selected nine chickens which were fed with lupin seeds. The overall results suggest that lupin seeds' intake had successfully reduced the cholesterol absorption as well as the serum glucose level in chickens and that this seed has the potential to be used as a cholesterol- alleviating agent.

Another study on rats was reported by Bettzieche et al. (2008), to further prove lupin has plasma cholesterol lowering effect. Lupin protein, when compared with casein has significantly reduced the concentration of triacylglycerols (TAG) in the rat liver and LDL in rats. The result suggests possible health promoting properties of lupin protein, which requires further study on its component.

As for human studies, a research was carried out by Hall, Johnson, et al. (2005) on 38 healthy male subjects aged between 24 to 64 years old. Subjects were given Lupin Kernel Fibre (LKF) and a control diet for 1 month. Both diets given have the same background menus plus some seven additional experimental foods. Results were positive, in which LKF diet reduced the total cholesterol and LDL significantly as compared to the control diet, while maintaining the level of HDL. This result echoes numerous other studies mentioned earlier that were done on animal subjects, thus proving that lupin has, in fact, cholesterolemic activity, and should be further studied.

In a more recent study on human subjects by Bähr et al. (2013), 33 hypercholesteromic subjects were given a daily diet of protein drink incorporated with 25 g Lupin Protein Isolate (LPI) and 25 g Milk Protein Isolate (MPI) which was consumed for 8 weeks. LDL cholesterol significantly reduced by 4 weeks, and blood pressure reading reduced at 8 weeks with LPI. It can be concluded that lupin protein could definitely affect cardiovascular risk factors predominantly in individuals with higher hypercholesterolemia.

In another similar recent study by Bähr et al. (2015), 72 hypercholesterolemic subjects were given a daily diet of complex food products incorporated with 25 g Lupin Protein (LP), Milk Protein (MP) and Milk Protein with arginine (MPA) for 28 days randomly. Results showed that LDL cholesterol was significantly lower in LP incorporated diet than MP, and the results were significantly greater in subjects with severe hypercholesterolemia (>6.6 mmol/L) than those with moderate hypercholesterolemia (5.2 mmol/L). It can be concluded that incorporation of 25 g of LP daily into various complex food products produced a lower total and LDL cholesterol, triacylglycerols, homocysteine, and uric acid in hyperchoelsterolemic subjects.

In summary, addition of lupin seed or its component to the diet proves favourable in plasma lipid. This shows that lupin has a great potential in becoming a novel ingredient that has the ability to lower blood cholesterol level and reduce the risk of coronary heart diseases (CHD) in humans.

2.1.3.4 Lowering Blood Pressure

Apart from hypercholesterolemia being heavily linked to CHD, another vital sign of CHD is hypertension. Hypertension is the increase of blood pressure, while blood pressure is the measure of the pressure of the blood flow when it is circulated upon the walls of the blood vessel.

In a study done by Pilvi (2006) to compare the effects of lupin and soy protein diets on blood pressure and vascular function, 34 salt-loaded male rats were used. Rats were divided into 3 groups, each with food content of high NaCl (6%). The first group was given a normal rat chow; the second was given 200 g/kg lupin protein isolate mixed with the standard chow while the third group was similar to the second group, with the exception of substituting the lupin protein with soy protein. Both protein treatments significantly reduced the hypertension of the rats, which was accelerated by the sodium loading. However, only the lupin treatment improved the severely diminished endothelium-dependant vascular relaxation. According to the author, the attenuation

of the hypertension can be explained by the improved vascular function observed in the lupin and soy groups. The lupin group was observed to have a greater effect on blood pressure than the soy group, and also the improvement of the impaired vasolidilation was more marked than in the soy group. This can probably be explained by the relatively high content of its arginine which is 99.3 mg/g in lupin as compared to 57.8 mg/g in soy, hence the ability to improve the vascular function of the rats. According to the author, arginine is a physiological substrate for endothelial nitric oxide synthase (eNOS) and supplementation with L-arginine in previous studies had been shown to improve vascular function.

In two studies done by Lee (2007), 74 overweight and obese male and female human subjects were used in a 16-week study. Participants were randomly assigned to replace 15–20% of their usual diet with white bread (control) or 40% lupin flour substituted bread. Ambulatory blood pressure was taken for 24 hours on the first day prior to the beginning of the diet and 16 weeks later. The result showed a reduction of systolic blood pressure and pulse pressure. The observed differences in the systolic blood pressure is associated with a 10% difference in the prevalence of hypertension, a 4% difference in the risk of coronary artery disease and a 10% difference in the risk of stroke, should this data look at a population level. This study confirms the potential of lupin as a novel ingredient in reducing blood pressure and the risk of coronary heart disease.

2.1.3.5 Improving Bowel Health

It is now common knowledge that consumption of high fibre diets is beneficial to the human bowel health. In a study done by Rubio, Spencer, Grant, and Pusztai (1995), male rats were fed with lupin (360 g lupin/1kg feed) and a control substance of lactalbumin and potato starch. Results showed that the number of *Esterichia coli (E.coli*) in the caecum, colon, and small intestine of the rats fed with lupin diet is significantly lower than those given the control diet. The authors have suggested this finding was due to the fibre component of the lupin seed. Having a low microbiota count of bad bacteria such as *E.coli* in the colon proves favourable in improving bowel health in animal and human subjects.

Rahman, Hossain, and Moslehuddin (1996), showed that subjects consuming lupin in their diet have significantly increased faecal bulk, as compared to consuming the control diet of lactalbulmin. This stimulates intestinal peristalsis, making the faeces passed quicker throughout the large intestine, allowing less time for the reabsorption of water in the colon, and thus benefits the overall bowel health.

Turnbull, Baxter, and Johnson (2005), studied the water binding capacity (WBC), and viscosity of lupin kernel fibre, as compared to other fibre enriched ingredients found in commercial baked products using an in-vitro protocol to simulate the human upper gastrointestinal conditions. Results showed that the WBC and viscosity of the lupin kernel fibre was significantly higher than soy kernel fibre, pea hull, cellulose, and wheat fibre. According to the authors, this might be due to the higher amount of soluble fibre found in lupin as compared to other types of fibres. These findings show that by consuming lupin kernel fibre, it can beneficially modify physiological events such as gastric emptying and improve the rate of nutrient digestion and absorption. However, this study needs to be repeated on human subjects, in order to identify whether this product is truly beneficial to the consumer.

Studies done on humans have found that lupin kernel fibre has the ability to improve bowel health. A study was done by Smith et al. (2006) to determine the effect of lupin kernel fibre on human intestinal microbiota using quantitative fluorescent in situ hybridisation (FISH) analysis whereby 18 healthy male human subjects were used. They were given a control diet and a diet containing 17-30 g lupin per day for 28 days. Fecal samples were collected for 3 days prior to the end of the 28-day period and the microbial population were then analysed using the FISH method. Results showed that the amount of Bifidobacterium spp. were significantly higher in the lupin diet compared to the control diet, while Clostridia spp. were reported to be significantly lower. According to the authors, this result proves that lupin kernel fibre acts as a bifidobacterium factor, which is similar to fructooligosaccharides (FOS) and galactooligosaccharides (GOS) which are well known as prebiotics. This suggests that lupin kernel fibre has beneficial effects in bowel function, in terms of improving faecal output and shortening transit time and overall improving the colon health in humans.

Another study on healthy men gives further evidence that consumption of lupin kernel fibre can beneficially affect the bowel function and lower the risk of colon cancer (Johnson, 2006). In the study, 38 healthy men were given a diet of lupin kernel fibre (17–30 g per day), and a control diet, for a month. Bowel function self-perception, frequency of defecation, transit time, faecal output, pH and moisture, faecal levels of SCFA and ammonia, and faecal bacterial b-glucuronidase activity were then assessed. The lupin kernel fibre diet resulted in an increased frequency of defecation, increased faecal output by 21%, and faecal moisture content while reducing transit time by 17% beneficially reduced the faecal pH. These findings have proven that lupin fibre has certainly improved the markers that are important in maintaining a healthy bowel health; thus lupin fibre plays a significant role in lowering the risk of colon cancer.

2.1.4 Lupin in Animal Feed

Lupins represent an important potential source of protein for animal and human consumption. The plant shows advantages such as tolerance to poor soils, adaptability to temperate climates, and high seed protein content (30–40%) (Jiménez-Martínez, Hernández-Sánchez, & Dávila-Ortiz, 2003). Lupin is regarded as a good quality feed ingredient due to the consistent quality and low content of anti-nutritional factors (Petterson & Mackintosh, 1994) .

2.1.4.1 Livestock Feed

Currently lupin is widely used as an animal feed for ruminants because they are high in protein, low in anti-nutritional factor, and have advantages in terms of handling, storing, and feeding. Supplementation of ruminant diets with lupins has been shown to have many positive effects in terms of growth and reproductive efficiency, equivalent with supplement of cereal grain (Van Barneveld, 1999). Extruded lupin seed fed to cows has been reported to increase milk production and slightly reduce milk fat percentage (Bayourthe, Moncoulon, & Enjalbert, 1998). This finding is supported by a study by C. L. White et al. (2007) on *lupinus albus,* which reported that feeding lactating cows with lupin had resulted in an increased concentration of C18 :

1 in milk, and a reduction of C12 : 0 – C16 : 0, consequently improving the fatty acid profile of milk and complying to the Australian national dietary guidelines for enhanced cardiovascular health in human population.

Low level of starch and high levels of fermentable carbohydrate in lupin is advantageous for ruminants, as it causes low incidents of acidosis and makes it an excellent microbial fermentation substrate (Edwards & Van Barneveld, 1998). However, this is not the case in monogastrics because the complex carbohydrate profile is the major limitation for it to be used as feed, as it influences the net energy yield and has an effect on the utilisation of other nutrients in the diet (Edwards & Van Barneveld, 1998).

Another monogastric feed issue is the amino acid profile of lupin which is low in methionine content as compared to other grain legumes. Since these amino acids are rather important for pigs and poultry, it would mean that their diets will need to be supplemented with other types of feed or amino acids (Edwards & Van Barneveld, 1998). This finding is in agreement with a study by Gdala, Jansman, van Leeuwen, Huisman, and Verstegen (1996), which shows that lupin feed is unsuitable as a sole protein supplement for young pigs due to its amino acid profile; however, there were no growth performance retardations recorded.

The effects of using seed and dehulled lupin on the effects of feeding broiler chicken with lupin meal were studied. The 4 groups of chicken were given 4 different diets accordingly, which were control (60% wheat, 30% soy), lupin/soy (40% lupin, 34% wheat, and 13% soy), lupin/casein (40% lupin, 43% wheat, and 6% soy), and dehulled lupin/soy (32% lupin, 50% wheat, and 7% soy). All samples contained adequate amount of methionine and cysteine to rule out any issues pertaining to lack of these two essential amino acids. The results showed that the chickens on the control and dehulled lupin diet had a significantly higher weight increase than the chickens on the lupin seed diet. The degradation of lupin fibre in birds' small intestine is very restricted. Interestingly, lupin seed diet also had a significantly low feed intake as compared to other samples. Lupin seed meal used in the study contained 360 g/kg NSP (Non-Starch Polysaccharide), hence this might have caused the accumulation of

undigestible lupin fibre in the chicken's intestine. However, in the subsequent experiment, the addition of a protease enzyme extracted from a *Bacillus sp.* bacteria in whole lupin diets improved feed intake and growth compared with birds fed on the unsupplemented diets (Rubio et al., 2003).

2.1.4.2 Aquaculture Feeds

Soy protein is the principal non-animal protein source used in fish diets, and is known to provide excellent high protein feedstuff for fish. However, soy is expensive, as more are being used in human food. Scientists have been trying to find other alternatives of non-animal protein sources which have similar composition as soy and are less costly (Hughes, 1991). Hence, the suitability of lupin as an aquaculture feed is due to its similar amino acid profile, high digestibility, and economic pricing.

Numerous studies have proven that lupins are suitable as aquafeed and results have shown that the replacement of lupin from soy resulted in weight gain and efficiency of feed utilisation equal to that of soy, at a fraction of the cost (Burel et al., 1998; Burel, Boujard, Tulli, & Kaushik, 2000; Glencross et al., 2006; Hughes, 1991). In a study by Farhangi (2001), up to 40% inclusion level of dehulled lupin was shown to be sufficient, without having any other extra supplementation. However, Glencross (2003) suggested that to improve lupin meals, oligosaccharide fraction should be removed, as it reduces the protein digestibility in fish. By doing so through processing, it will result in a significant improvement in the overall nutritional value of a lupin meal.

2.1.5 Application of Lupin in Food

Although lupin seeds at present are mainly used as feed, they are acquiring increasing importance as a source of healthy foods high in protein, high in dietary fibre, low in fat, and high in bioactive compounds.

The utilisation of lupins in western food formulations has been limited, mainly due to the unpleasant beany flavour associated with the grain legume. Nevertheless, lupin could be expanded into new and convenient products that will, in turn, diversify and enhance its market potential (Joray, Rayas-Duarte, Mohamed, & Santen, 2007). The lupin seeds have to be processed before they are suitable for use as a food ingredient (Sipsas, 2008b). In the course of processing lupin for food applications, lupin grains are first dehulled and the cotyledon is ground to produce lupin flour. The flour can be used for different food formulations or can be further fractioned into protein and dietary fibre components (Jayasena & Coorey, 2006). Hence, if the unpleasant beany flavour is removed or significantly reduced to ensure the overall acceptability of food products, then the application of lupin as a food ingredient can be enhanced. To achieve such a reduction of its off-flavour, lupin needs to be processed prior to usage. One such possible application is the applying of heat during the processing stage either prior to dehulling or milling of lupin seed.

2.1.5.1 Baked Products

Lupin baked products are mostly made out of lupin flour. It has a slightly nutty flavour and slightly oily feel (Sipsas, 2008b). According to Petterson (1998b), up to about 10% of lupin flour can be included into wheat or wholemeal flour to produce a more nutritious bread and a more complete food due to its high protein content and improvement of the amino acid profile. In a recent study done by Villarino, Jayasena, Coorey, Chakrabarti-Bell, Foley, et al. (2015), 20% of lupin flour of different lupin varieties were incorporated into bread production and results showed that there were significant improvements in the nutritional, phytochemical, and bioactive compounds of the ASL – wheat bread.

However, the inclusion of more than 5% will result in a lower loaf volume and beyond 10% substitution rate, producing denser pore structure and firmer crumb, most likely from the low elasticity of lupin proteins and the high water-binding capacity of its dietary fibre; resulting in the interruption of the development of the desired wheat gluten network (Villarino et al., 2016). This is agreed by studies done by Pollard, Stoddard, Popineau, Wrigley, and MacRitchie (2002), Ballester, Castro, Cerda, Garcia, and Yanez (1988), Doxastakis, Zafiriadis, Irakli, Marlani, and Tananaki

(2002), and Mubarak (2001), which show that the maximum inclusion of 5% of lupin flour did not affect the loaf volume and crumb structure of the bread. The inclusion of 6% lupin flour has decreased the overall acceptability of the product, with 17% of the panellists stating an aftertaste or bitter taste when asked for the reason of the product being unappealing (Hall & Johnson, 2004).

To obtain the benefit of lupin fibre without affecting the loaf size, structure, texture, and taste of the bread, 4.6% of lupin kernel fibre was incorporated into white bread. It was shown that there was no significant difference in the overall acceptability of lupin fibre enriched bread and control bread (Clark & Johnson, 2002). However, without the inclusion of the lupin flour or the protein nutritional content of the product in terms of amino acid, its profile cannot be improved, making it not as nutritious as the one substituted with lupin flour.

In a study done by James, Jayawardena, and Jayasena (2007), and Jayawardena (2006), on muffins, up to 20% of wheat flour was replaced by lupin flour into muffins without affecting its overall acceptability. However, Clark and Johnson (2002) found that 6% of lupin kernel fibre resulted in a significant reduction in the overall acceptability of the muffin. In another related study, 17% of lupin flour was used in producing muffins, which resulted in 26% of the panellists citing an aftertaste and bitter taste as the reason of the muffin being highly unpalatable to them (Hall & Johnson, 2004).

There have also been studies carried out on incorporation of lupin ingredient in other baked products. For biscuits and chocolate chip cookies, the inclusion range from 10–20% of lupin did not affect the overall acceptability of the product, but also increased the acceptability in terms of appearance (Hall & Johnson, 2004; Hegazy & Faheid, 1990; Jayasena & Nasar-Abbas, 2011; Wittig de Penna, CarreñO, Urrutia, Lopez, & Ballester, 1987). However, there were still 7% comments from panellists in a study done by Hall and Johnson (2004), that cited an aftertaste in the product, proving that lupin indeed carries a beany or bitter flavour, which affects the overall acceptability of most of the products that has lupin included.

Other products such as lupin-incorporated breakfast bar (20% inclusion) have also proved to be successful in terms of overall acceptability. However, the breakfast bar was rated significantly lower in terms of texture, which was deemed hard, chewy, and dry (Hall & Johnson, 2004).

2.1.5.2 Pasta and Noodles

There have been studies on the incorporation of lupin flour into pastas and noodles. As always, the incorporation of lupin helps to increase the nutritional value of food products. However, lupin flour can be incorporated to up to only 20% in instant noodles (Jayasena et al., 2008), and pastas (Jayasena, Nassar-Abbas, Yang, & Senaratna, 2009), without affecting the sensory acceptability of the products. The addition of 20% lupin, however, has significantly improved the protein content by 42% and dietary fibre by 200%.

2.1.5.3 Tofu

Up to 40% of lupin can be incorporated into tofu without affecting the sensory qualities, while improving the nutritional content and lowering the fat content of tofu (Fudiyansyah, Petterson, Bell, & Fairbrother, 1995). However, the colour acceptability of raw tofu reduced significantly at 40% of lupin inclusion, reason being that the conventional tofu was white in colour (Jayasena, Khu, & Nasar-Abbas, 2010). But given the nutritious benefits of lupin incorporation and the low cost of lupin as compared to soy, lupin has huge potential in substituting soy in tofu manufacturing.

2.1.5.4 Meat Products

In a study done by Archer et al. (2004), a small amount (3%) of lupin kernel fibre was incorporated into sausage patties as a fat replacer and was compared to normal patties and inulin included patties. There were no significant differences in the overall acceptability of the sausage patties; however, the normal sausage patties had a significantly higher acceptability in terms of flavour as compared to the sausage patties with the lupin kernel fibre. This is normal as the reduction of fat in products is known to decrease flavour. However, the inclusion of lupin kernel fibre has significantly

improved the perception of satiety of panellists consuming the patties, as compared to those who consumed the normal sausage patties. Thus, lupin kernel fibre appears to have potential as fat replacers in meat products, and for reducing the consumption of fat and energy intake in the population (Archer et al., 2004).

In another study done on sausages, up to 2% of lupin protein isolate produced from *Lupinus albus* was incorporated into the sausage with improved yield and without any detrimental effects on overall sensory acceptability of products which includes texture, colour, and taste. However, by incorporation of 3%, the lupin protein isolate resulted in a significant increase in processing yield and reduction in purge accumulation, and a negative overall acceptability as a result of bitterness and unpleasant taste. This shows that lupin has potential in improving yield and most likely, the nutritional value; however, the taste needs to be improved (Alamanou, Bloukas, Paneras, & Doxastakis, 1996).

2.1.5.5 Traditional Fermented Food Products

Asian countries are well known for producing and consuming traditional fermented foods such as tempe, miso, and soy sauce, which are usually soy-based. Given the similarities of soybean and lupin in certain aspects, soybean has been substituted by lupin in studies on producing tempe, which is a fermented food product. Studies have shown that lupin can be added at up to 60% in the manufacture of tempe without affecting the sensory characteristics (Fudiyansyah et al., 1995; Jayasena, Kardono, Quail, & Coorey, 2007). Following the successful sensory acceptability of the product with a high inclusion rate, the lupin tempe was patented in 2009 under the Australian Patent No. 2009212099 (Jayasena, Quail, & Kardono, 2009) . Rhizopus Oligosporus strain was used as the starter for the fermented product. In producing tempe, the method involves dehulled lupin being washed in cold tap water, boiled for 15 minutes, soaked in acetic acid for 6 hours, cleaned and steamed for 15 minutes, and then cooled to 37°C for surface drying purposes. The prepared lupin kernel was then inoculated at 30°C for 24 hours and transferred to a cooler temperature of 25°C for 72-hour fermentation. The product produced a tempe quality which is very similar to the original Indonesian delicacy, and was highly accepted when tested with Indonesian

panellists (Jayasena, Quail, et al., 2009). The high inclusion of lupin into tempe without affecting the sensory acceptability is believed to be largely due to the method of production of tempe, which involves a series of heat treatments like soaking and boiling followed by fermenting the lupin beans, which would likely have an effect in the reduction of the "beany" flavour associated in lupin.

2.1.5.6 Snacks/Crisps

Lupin-based crisps has been one of the most successful products in term of high lupin inclusion of up to 70% without significant difference in sensory acceptability. Furthermore, compared with normal potato chips, lupin-based crisps had 4 times more proteins and 10 times more dietary fibre, which makes it significantly more nutritious than normal chips (Jayasena, Nassar-Abbas, et al., 2009). Interestingly, the high inclusion of lupin into crisps as compared to other products can be attributed to the preparation of the crisps, which involves heat treatment during its preparation. This would have likely reduced the "beany" flavour associated with lupin.

2.1.5.7 Food Ingredient

Lupin protein isolate has been found to have superior functional characteristics than soy protein isolate and costs significantly less as compared to soy protein isolate (Jayasena, Kartawinata, et al., 2006). The unique functional properties of lupin protein isolate includes emulsifying activity, emulsion stability, foaming capacity, and foam stability. Lupin protein isolate also has a better water holding capacity than soy protein isolates. These functional properties make lupin protein isolate highly suitable as a substitute, for soy protein isolate to be included in various food formulations (Jayasena & Coorey, 2006).

2.1.6 Disadvantages of Lupin

2.1.6.1 "Beany" flavour or off-flavour

There has been a pronounced interest in the utilisation of lupin as food for human consumption instead of animal feed (El-Adawy, Rahma, El-Bedawey, & Gafar, 2001).

The potential of lupin as a food ingredient with numerous health benefits is clearly evident in research findings. (Arnoldi et al., 2007; Petterson et al., 1997; Torres, Frias, Granito, Guerra, & Vidal-Valverde, 2007). Lupin incorporation in food products not only improves nutritional values, especially protein and fibre tremendously, it also improves the colour and texture of a product (Dervas, Doxastakis, Hadjisavva-Zinoviadi, & Triantafillakos, 1999; Doxastakis et al., 2002). However, based on numerous researches conducted in the past 20 years (Alamanou et al., 1996; Clark & Johnson, 2002; Fudiyansyah et al., 1995; Hall, Baxter, Fryirs, & Johnson, 2010; Hall & Johnson, 2004; James et al., 2007; Jayasena, Khu, et al., 2010; Jayasena et al., 2008; Jayasena, Nassar-Abbas, et al., 2009; Jayawardena, 2006), an inclusion of a higher percentage of lupin—either as lupin flour, fibre, or protein isolate—significantly lowers the consumer acceptability of the product. This, therefore, raises concern that the undesirable "beany" flavour arising from the use of lupin ingredients should be greatly minimised. Based on all the studies conducted, incorporating novel legume ingredients such as lupin flour into foods, proves to be a challenge in terms of maintaining sensory appeal, as it can cause an unpleasant and bitter taste.

Cortes Sánchez et al. (2005), contend that the presence of alkaloid is a limiting factor for lupin consumption as elevated concentrations of alkaloid produces bitter taste. Consequently, lupin alkaloid has been eliminated by technological treatment, and bitter seed varieties are slowly being replaced by sweet varieties with low alkaloid content. However, there is still a beany and bitter taste found in lupin-based products even with a low amount of alkaloid.

There have been limited studies on identifying the cause of the "beany" flavour in lupin. However, many studies have been conducted on the beany flavour found in soy, which indicates lipoxygenase (LOX) enzyme as being the cause (Mohamed & Rangappa, 1992; Sheu & Chen, 1991; Vijayvaragiya & Pai, 1991; R. Wang, Zhou, & Chen, 2008; Z. H. Wang, Dou, Macura, Durance, & Nakai, 1998). LOX has also been identified as the enzyme responsible for the development of off-flavours in many other legumes (Yoshie-Stark & Wäsche, 2004). Ha et al. (1992), showing that the development of soy off-flavour compound is due largely to LOX and heat processing has been widely used to inactivate LOX enzymes. Meanwhile, Dahuja and Madaan

(2004) research proves that LOX is the main cause of the beany flavour in soybean and the off-flavour in soybean is largely generated by the action of LOX on polyunsaturated fatty acids (PUFAs) and the non-enzymatic oxidation of these fatty acids seems to be far less a factor in off-flavour development.

The overall odour of lupin flour was examined for the first time by Bader, Czerny, Eisner, and Buettner (2009), and according to the study, the most potent odour-active compound to be characterised in lupin flour was said to be derived from either lipoxygenase activity, secondary metabolism, or activity of microorganisms. Among the large number of odour-active compounds identified in this study are saturated and unsaturated aldehydes, ketones, carboxylic acids, alkyl-methoxypyrazines and terpenes. It is a well-known fact that lipoxygenase activity is a process whereby the lipoxygenase enzyme catalyses the oxidation of linoleic and linolenic acids, esters, and triglycerides, leading to formation of compounds such as as aldehydes, ketones, and alcohol (Eskin, Grossman, Pinsky, & Whitaker, 1977). Hence, this shows that lipoxygenase enzyme is most likely the cause of the beany flavour, as the odour-active compound identified in the research is said to be the same as the hydroperoxide formed during lipoxygenase activity.

To this day, there have been limited studies on the effect of heat treatment on LOX in fruits and vegetables. Most of these findings revealed that the respective heat treatment method is effective, as LOX was inactivated in most results achieving it at 60°C (Anthon & Barrett, 2002; Baysal & Demirdöven, 2007; Borhan & Snyder, 1979; Buranasompob et al., 2007), with the exception of LOX in tomato, which gave negative result to blanching – another form of heat treatment (Anese & Sovrano, 2006). However, LOX in lupin is believed to have a different thermal stability as compared to the other products. According to Yoshie-Stark and Wäsche (2004), LOX in *Lupinus angustifolius* seems to have a higher thermal stability of up to 80°C, even higher than *Lupinus albus* which has a similar thermal stability to LOX in other grains which is 60°C. Therefore, this study is imperative to ascertain if heat treatment is able to eliminate or decrease the beany flavour associated with lupin, and thus, making the highly nutritious lupin food product well received by the consumers.

2.1.6.2 Allergens

In the past few years, there has been a gradual rise in usage of lupin as a food ingredient; hence, there have been an increasing number of research studies on the severity of lupin allergen. There are two ways for lupin to cause allergic reactions, which is through inhalation (Campbell, Jackson, Johnson, Thomas, & Yates, 2007; Novembre et al., 1999), or through ingestion.

Studies have linked α-conglutin and β-conglutin to be the lupin protein candidate that causes allergenicity in lupin (Dooper, Holden, Fæste, Thompson, & Egaas, 2007; Goggin, Mir, Smith, Stuckey, & Smith, 2008; Guillamón et al., 2010; Holden, Sletten, Lindvik, Fæste, & Dooper, 2008). However, no clear identification has been reported.

According to a study conducted by Lindvik, Holden, Løvik, Cvancarova, and Halvorsen (2008), children with sensitisation to lupin are not prone to have a clinical lupin allergy. Avoiding lupin consumption on the basis of lupin sensitisation or peanut allergy would lead to unnecessarily strict diets. Fiocchi et al. (2009), stated that lupin consumption is tolerable among the general population and only affects a minority. He also suggested that products containing lupin ingredient should be clearly labelled, similar to peanut products, given the similar cross-reactive peanut allergens.

The severity of allergic reaction on lupin is linked to the severity of peanut allergy, which can cause anaphylaxis in the allergic sufferer (Fæste, Løvik, Wiker, & Egaas, 2004; Moneret-Vautrin et al., 1999). It has been estimated that 44% of peanut-allergic individuals can also react to lupin. According to Peeters et al. (2007), lupin allergy not only equals peanut allergy in severity, but an allergic reaction to it is not confined to peanut allergy sufferers. However, the severity of reaction in non-peanut allergy sufferers is yet to be determined.

In another, more recent study, done by Bähr, Fechner, Kaatz, and Jahreis (2014) on allergenic reaction in lupin, a comparison of lupin to pea, peanut, and soybean were done using a skin prick test on 163 subjects. It was concluded that lupin allergenicity was comparable or even lower than other legumes tested and was deemed uncommon.

However, subjects with existing allergy with other legumes were at a greater risk of lupin allergy due to cross- reactivity.

Heat treatment is considered in reducing the effects of allergen in lupin due to the known aspect of thermal instability in protein component. According to Alvarez-Alvarez et al. (2005) auto-claving at 138°C for 20 minutes significantly reduces the overall allergenicity of lupin, indicating that prolonged autoclaving might significantly reduce the allergenicity. Food processing techniques, such as pressure cooking and extrusion, might also be able to reduce overall allergenicity of lupin, in addition to the possibility of reducing any beany off-flavours due to LOX.

2.1.6.3 Anti - Nutritional Factor (ANF)

Apart from nutrients, there are also other compounds that can be found in most legumes. Some of these compounds are known as anti-nutritional factor (ANF) or anti-nutrient, and can cause negative effects when consumed. ANF found in food lowers the nutritional value of food by reducing the bioavailability or digestibility of nutrients. For example, ANFs like Phytate are known to inhibit the absorption of dietary minerals such as Zinc (Zn) and Iron (Fe) which are essential to human health (Sandberg, 2002). Domesticated varieties of Australian sweet lupin have a very low content of anti-nutritional factors (see Table 7). These types of ANFs require heat treatment to destroy the lectins and protease inhibitors which can reduce protein digestion and availability (Petterson et al., 1997; C. L. White et al., 2007). The low amount of ANF found in ASL renders them safe for human and animal consumption.

Table 7: Anti-nutritional factors in Australian sweet lupins

Factor	Units	Lupin Average (between species)	Soy
Alkaloids	%	0.02[a]	-
Oligosaccharides*	%	4.07[a]	-
Phytate	%	0.50[a]	0.21[b]
Saponins	mg/kg	573[a]	-

Factor	Units	Lupin Average (between species)	Soy
Tannins (Total)	%	0.29[a]	0.29[b]
Trypsin Inhibitor Activity (TIA)	U/g	0.12[a]	0.28[b]
Chymotrypsin inhibitor Activity (CTIA)	mg/g	0.08[a]	-
Lectin	dilut	n.d[a]	-

*Sum of raffinose, stachyose and verbascose

Source: a- Petterson et al. (1997); b - El-Shemy et al. (2000)

The main anti-nutritional substances found in lupin seeds are various alkaloids of the quinolizidine group. These bitter compounds also make the seed unpalatable (Sujak, Kotlarz, & Strobel, 2006). A pronounced bitter taste was still detected in lupin-enriched white bread even though the alkaloid level was low (0.0029 mg/g). This low level suggests that other factors are responsible for the overwhelming bitterness detected, since the threshold for detecting alkaloid bitterness is in the range of 0.02 mg/g for lupanine (Dupont, Muzquiz, Estrella, Fenwick, & Price, 1994). In Australia, lupin is traded on the basis that it contains less than 0.02% total alkaloid as measured by gas chromatography. Alkaloid content of ASL is 200 folds lower than its bitter wild lupin species. According to a study done by Petterson (1994), the typical alkaloid profile for ASL is 42–59% lupanine, 24–45% 13-hydroxylupanine, 7–15% angustifoline, and 1–15% α-isolupanine. It is a widely known fact that heat treatment does not affect alkaloid content in lupin, hence alkaloid testing is deemed unnecessary when heat is applied to inactivate enzymes or denature proteins.

Saponins are plant glycosides in which the non-sugar moiety is a steroid or a triterpenoid compound. Its content in Australian sweet lupin ranges from 55–730 mg/kg and is considerably lower than most legume species (Sipsas, 2008a). Previously, the anti-nutritional factors (ANFs), namely saponin, tannin, and alkaloid were considered to be the culprits of the decline in sensory acceptability of lupin products. However, saponin is confirmed to have low concentration in lupin compared to other legumes, thus having little impact on the taste (Dupont et al., 1994). Tannin,

meanwhile, is relatively low in lupin, especially in *Lupinus angustifolius* (Lampart-Szczapa et al., 2003), and researchers found no correlation between tannin and bitterness (Dupont et al., 1994).

2.2 Lipoxygenase Enzyme (LOX)

Lipoxygenase enzyme (LOX) is a type of oxidative enzyme that is prevalent in both plants and animals (Liavonchanka & Feussner, 2006; Loiseau, Ly Vu, Macherel, & Deunff, 2001; Siedow, 1991). Table 8 shows some plants and animal sources of lipoxygenase. Legume seeds contain particularly high amount of lipoxygenase compared to others plant sources (Siedow, 1991). The amount of lipoxygenase found in plant and animal tissues differ widely, depending on its cultivars and strains of different organisms, age, type of tissues, and environmental factors (Hsieh, 1994). LOX of soybean is widely studied, mainly due to its association with the grassy-beany and rancid off-flavours (Rackis, Sessa, & Honig, 1979).

Table 8 : Some plant and animal sources of Lipoxygenase

		PLANTS		
Apple	Alfalfa	Banana	Barley	Beans
Broccoli	Cauliflower	Cucumber	Egg Plant	Grape
Maize	Mango	Melon	Mushroom	Oat
Peas	Pear	Potato	Rice	Rye
Soybean	Tobacco	Tomato	Wheat	Watermelon
		MAMMALS		
Platelets		Lungs		Leukocytes
Skins		Reticulocytes		
		FISHES		
Drum	Perch	Emerald Shiner	Bass	Bullhead
Sheephead	Trout	Catfish	Salmon	Blue Gill

Source: Hsieh (1994)

Since LOX reaction results in products that are aromatic, the occurrence of lipoxygenase activity in many produce have been known to affect certain food properties, particularly during storage, both, in positive and negative ways (Siedow, 1991). Among some unwanted effects of lipoxygenase reactions are the destruction of essential fatty acids, colour changes, and most importantly, off-flavour production and rancidity (Ludikhuyze, Indrawati, Van den Broeck, Weemaes, & Hendrickx, 1998).

2.2.1 Structure and Mechanism

Lipoxygenase (linoleate:oxygen oxidoreductase, EC 1.3.11.12) is a class of non-haem iron-containing dioxygenase that catalyses the oxygenation of polyunsaturated fatty acids (PUFA) containing a cis, cis-1, 4-pentadiene structure to form conjugated diene hydroperoxide (Siedow, 1991), as shown in Figure 5:

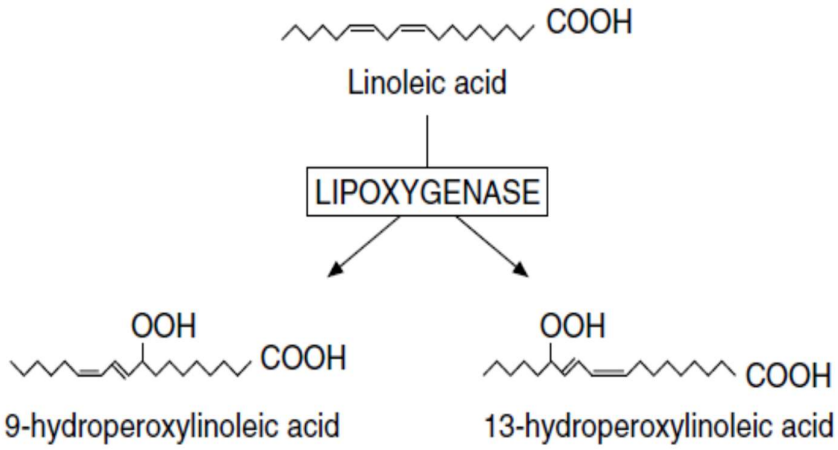

Figure 5: The formation of 9- and 13-hydroperoxides from linoleic acid by lipoxygenase

Source: Loiseau et al. (2001)

According to Hsieh (1994), there are four major steps to lipoxygenase initiated oxidation of polyunsaturated fatty acids.

1. The activation of enzyme from a ferrous state to a ferric state by the presence of trace amounts of hydroperoxide.

2. The process of hydrogen subtraction from the substrate unsaturated fatty acid.
3. Stereospecific attachment of an oxygen molecule.
4. The formation of a fatty hydroperoxy anion and regeneration of ferric enzyme for the next cycle.

The hydroperoxide produced from the LOX reaction on the PUFA can be broken down by other enzymes to form undesirable aroma compounds. According to Van Den, Raymundo, and Mendoza (1982), this hydroperoxide degradation to odorous carbonyl compound is most likely the cause of the beany and off-flavour in high LOX legumes such as soybean and lupin; however, there have been limited studies ever since to support this theory. Lipoxygenase activity is also reported to be accelerated with the addition of water into cereal products (Barnes & Galliard, 1991), which is a requirement in food processing.

2.2.2 Effects of LOX in Food

It is a known fact that the enzyme LOX has an undesirable effect on the acceptability of food products. LOX has been identified as the enzyme responsible for the beany/off-flavour of several food such as soybeans (Z. H. Wang et al., 1998), green beans, green peas and corn (Barrett & Theerakulkait, 1995; Yoshie-Stark & Wäsche, 2004). Besides off-flavour, LOX has also been known to cause off-odour (Ramezanzadeh, Rao, Windhauser, Prinyawiwatkul, Tulley, et al., 1999). Malekian et al. (2000) suggested the linking of LOX to rancidity in legume storage.

According to a study done by Z. H. Wang et al. (1998) on beany flavour in soymilk, the typical beany flavour causing compounds were found to be pentanol, hexanol, heptanol, hexanal, and ethyl vinyl. Studies have shown that the activity of the lipoxygenase enzyme, which causes the alteration of polyunsaturated fatty acids to hydroperoxides and secondary compounds, are one of the main causes of being off-flavour, as previously mentioned (Bader et al., 2009). To prevent the development of such beany off-flavours, one option would be to inactivate LOX before any such flavour develops.

LOX enzyme, purified from different sources, presents similar characteristics, non-haem iron, similar molecular weight, specificity for a cis-cis-pentadiene substrate, etc. (Olias & Valle, 1988). However, enzymes from different sources have slight differences in terms of pH, heat stability, different inhibitor response, etc. Hence, even though many studies have been conducted on soy LOX, it is imperative to study lupin LOX.

2.2.3 LOX Lupin

There have been very few studies on lupin LOX. The results of LOX activity in those literatures also seem to contradict one another, especially in terms of heat stability, which is the focus of this study. This may be due to different lupin variety used in these studies.

One of the first few studies done on lupin LOX was by Mahmoud, Refat, Rasmy, and Mohamed (1986), with the comparison of heat stability of LOX in 6 major legumes found in Egypt. The 6 legumes studied were soybeans (*Glycine max*), faba bean (*Vicia faba*), lentils (*Lens esculenta*), chick peas (*Cicer aerietinum*), lupin (*Lupinus termis*), and peas (*Pisum sativum*). As most other studies, the author agreed the study was pertinent due to the fact that LOX causes off-flavour, hence the interest in studying LOX in 6 different legumes. Samples were prepared by milling into powdered form and extracting with sodium phosphate buffer. The aliquot of crude extract was then subjected to heat treatment, which involves heating in water bath for 2 minutes at temperatures ranging between 30 and 100°C. Results showed that LOX activity of LOX samples started to decrease significantly at 60°C, while lupin LOX significantly decreased at 80°C. This shows that lupin LOX has the highest degree of heat stability as compared to the rest of the legumes studied. This could be the reason lupin has been consistently associated with off-flavour as compared to other legumes, due to its LOX's resistance to high temperature.

In another study done by Najid, Beneytout, Leblanc, Tixier, and Rigaud (1988), LOX from lupin was extracted and purified before being subjected to a series of studies on its characteristics, including heat stability. *Lupinus albus* seeds were dried, grounded, and extracted using phosphate buffer, before being centrifuged and purified. Heat

stability was measured at different stages during the purification process, which were crude extract, DEAE- Trisacryl eluate, and electric focusing column eluate, at different temperatures. It is worth mentioning that the heat treatment used in this study was not mentioned anywhere in the paper and the LOX assay uses a different type of substrate, which is arachidonic acid, unlike the conventional linoleic acid used in most LOX assays. Results showed that LOX activity significantly decreased up to 80% at 70°C and denatured completely at 80°C. The results are slightly different from the earlier study by Mahmoud et al. (1986) in terms of temperature, however, this is probably due to the different varieties used between two experiments, different substrates, and the lengths of heat treatment of samples.

In a more recent study by Yoshie-Stark and Wäsche (2004), *Lupinus albus* (Chile) and *Lupinus Angustifolius* (Australia) were used. Each was milled, extracted, and partially purified using ammonium sulphate precipitation. Results showed that LOX from *L. albus* was thermally stable until 60°C, before it significantly decreased and denatured at 100°C. *L.angustifolius* was stable until 80°C. This shows that ASL is thermally stable, as compared to *L.albus.* However, study on *L. albus* shows complete denaturation at 80°C and not as low as 60°C like in the previous study (Najid et al., 1988). Both studies also did not point out the type of heat treatment used.

It is worth noting that in all of the studies done on heat stability in LOX, all of the LOX were isolated and partially/fully purified before being studied. As a result, information may not reflect the effect on LOX in lupin flour. From a food processing point of view, it is important to study the LOX reaction and behaviour in its food complex, lupin seed, or lupin flour forms.

2.2.4 Eliminating LOX

LOX has been known to be the most probable cause of beany flavour in most food products (Dahuja & Madaan, 2004). LOX can be thermally inactivated above 60°C which will also result in a longer shelf life (Baysal & Demirdöven, 2007). However, since different LOX from different plant varieties have a different behaviour, this might mean that lupin LOX might not be inactivated at 60°C, or will have an even higher temperature stability as compared to soy LOX.

No studies have been conducted so far on heat treatment of LOX, prior to milling. It is imperative to investigate the LOX activity of samples heat treated, prior to dehulling and milling. LOX interacts with PUFA and creates by-products which causes an off-flavour. Hence, in theory, if the LOX was deactivated or reduced prior to milling, there will be minimal off-flavour due to minimum interaction between low amount of LOX and PUFA when the product is in flour form.

By reducing/eliminating LOX through heat treatment, it is anticipated that the food product will have an improved taste. However, there is still a slight possibility that there are other factors which also could contribute to the off-flavour in lupin. Hence, in this study, a food product will be prepared by using the heat treated samples, and analysed using sensory acceptability.

2.3 Heat Treatment Technology

2.3.1 Evidence of Usage in Food/Grains

Heat treatment is a well-established food processing technique and has long been used, mainly to improve shelf life and flavour. For instance, heat treatment in milk helps in improving shelf stability, palatability, texture, and flavour (Scanlan, Lindsay, Libbey, & Day, 1968), although a study by Contarini, Povolo, Leardi, and Toppino (1997) suggests that extreme temperature could be responsible for the development of off-flavour. Though researches focusing on the effect of heat treatment of grains are very limited, most of their findings indicate that processing of grains using heat treatment improves shelf life, making it convenient and creating favourable taste for consumer consumption (Ha et al., 1992; Slavin, Jacobs, & Marquart, 2001). For grains like wattle seed, they are heat-treated by roasting them at a high temperature before being incorporated into beverages, baked products, and dairy foods, mainly as a flavouring agent (Ee, Rehman, Agboola, & Zhao, 2009). Meanwhile research done by Carrão-Panizzi, Beléia, Prudêncio-Ferreira, Oliveira, and Kitamura (1999) also suggests that pre-heating of grain promotes better flavour in soybean product. Heat treatment of oat flour resulted in the reduction of the level of unwanted flavours like bitterness and

astringency, and improved the overall flavour of the flour (Molteberg, Solheim, Dimberg, & Frølich, 1996).

2.3.2 Effects on LOX

There have been limited studies on the effects of heat treatment on LOX activity, specifically in addressing the issue of LOX' probable link to the beany flavour. S. H. Wang and Toledo (1987) were among the first few who had used microwave heat treatment to on soybean lipoxygenase in soaked soybean, resulting in inactivity at 2450 MHz and 240 seconds.

In a different study done by Ramezanzadeh, Rao, Windhauser, Prinyawiwatkul, Tulley, et al. (1999), microwave heating was used to heat rice bran to 107°C (850 watt at 3 minutes). The rice bran was mixed with water prior to microwaving to increase moisture from 7.4 to 21%. The reason for the added moisture was because a moisture level of less than 21% has resulted in the rice bran being too dry with some burns. The samples were kept in a –80°C freezer for 2 days before packing them in different packaging for storage study. They found that microwave treatment (with high moisture content) increases LOX rather than decrease. The outcome of the study, however, showed that the amount of time and temperature combination was not sufficient to fully inactivate LOX activity. However, it produced a very low amount of FFA (Free Fatty Acids), indicating a reduction in the substrate (polyunsaturated fatty acid – PUFA) present for LOX activity.

2.4 Summary

Australia is the world's largest producer of Australian Sweet Lupin. Lupin is widely available at a cheaper price compared to soybean. Historically, lupin has been used as a traditional food for thousands of years in the Mediterranean region and the Andean highlands, providing good track record of safety. Lupin has also been safely used as animal feed and has been proven beneficial to ruminants and fishes.

Lupin has an impressive nutritional composition, having high fibre and protein contents, and fat and starch contents, valuable mineral contents, and phytochemicals such as antioxidants that are beneficial to health.

Numerous studies have demonstrated the health benefits of lupin. Studies have shown that lupin flour has the ability to reduce blood glucose level, decreasing the risk of diabetes mellitus (type 2) and has a beneficial effect in increasing satiety. Lupin also has the ability to lower blood cholesterol and reduce blood pressure, thereby decreasing the risk of coronary heart diseases (CHD). Additionally, lupin kernel fibres has beneficial effects in bowel function, in terms of improving faecal output, shortening transit time, and acts as a prebiotic to Bifidobacterium, thereby improving the overall colon health.

Lupin has great potential to be successfully incorporated, at a low percentage, into numerous food products such as baked products, pasta and noodles, tofu, meat products, traditional fermented foods, and snacks and crisps. However, a higher inclusion rate results in poor consumer acceptability. One of the main reasons for the poor consumer acceptability is the beany flavour associated with LOX enzyme that needs to be addressed in the hope of increasing the consumer acceptability. For these reasons, it is important to understand the correlation between the LOX enzyme and the beany flavour. Effects of food processing treatments in terms of heat treatment, time, and milling combinations need to be taken into consideration to inactivate the LOX, so as to improve the overall acceptability of lupin incorporated foods.

3.0 Materials and Methods

The effects of different types of heat treatments and time temperature combinations on the quality of lupins were studied. The first step of the research was to determine an assay to measure lipoxygenase (LOX) activity. The next step was to screen the 25 lupin varieties to select 3 varieties for further studies. The final stage was to select one variety which would be incorporated into the product development for sensory evaluation. The effects of the different heat treatments on the LOX activity, and its subsequent impact on the sensorial acceptability, texture, and colour were determined and that correlated with the quality of heated lupin.

3.1 Materials

3.1.1 Lupin samples

The Australian Sweet Lupin (ASL) seeds (*Lupinus angustifolius*) used in this study was grown in Wongan Hills Research Station, Department of Agriculture and Food, Western Australia (DAFWA) were harvested in 2009, 2010, and 2011, and these were provided. All seed samples were kept in a cool room (4°C) until further use. Table 9 shows the lupin varieties used in this study.

Table 9: List of lupin varieties used in the study

Lupin varieties On the label:	Number assigned to the variety	Plot	Harvest number
Uniwhite	1	1001	1
Uniharvest	2	1002	2
Unicrop	3	1003	3
Marri	4	1005	5
Illyarrie	5	1006	6
Yandee	6	1008	8
Chittick	7	1010	10
Danja	8	1012	12
Geebung	9	1013	13
Gungurru	10	1015	15
Yorrel	11	1016	16
Warrah	12	1018	18
Merrit	13	1020	20
Myallie	14	1022	22
Kalya	15	1024	24
Wonga	16	1026	26
Belara	17	1028	28
Tallerack	18	1029	29
Tanjil	19	1032	32
Moonah	20	1024	24
Quilinock	21	1037	37
Jindalee	22	1039	39
Mandelup	23	1040	40
Coromup	24	1042	42
Jenabillup	25	1044	44

3.1.2 Chemicals

Analytical grade chemicals were used in this study, unless otherwise stated. The details of the laboratory chemicals used together with the respective suppliers are listed in Table 10.

Table 10 : Details of chemicals used in LOX

Supplier	Chemicals
Sigma – Aldrich, Sydney, Australia	Sodium tetraborate decahydrate ACS reagent (≥99.5%) Sigma B9876, Boric acid ACS reagent (≥99.5%) B0394, Disodium hydrogen phosphate anhydrous/Sodium phosphate dibasic (Sigma S7907), Tween 20 (Sigma P1379), Linoleic acid (cis-9, cis-12-octadecadienoic acid) (Sigma L1376)
Acros Organics, Geel, Belgium	Potassium phosphate monobasic, extra pure (ACR205925000), Acetic acid ACS reagent (ACR423225000)
Merck Millipore, Bayswater, Victoria, Australia	Sodium acetate anhydrous (Merck 106268), Sodium chloride (Merck 106404)

Note: Description of chemicals presented as product name and product number

3.2 Methods

3.2.1 Lupin milling

Lupin samples were dehulled and milled prior to usage. Samples in seed form was dehulled using a dehuller (Figure 6), manufactured by S.K. Engineering and Allied Works (Bahraich, Uttar Pradesh, India), and widely known as the "SK System". There are 5 components in the dehuller used for processing pulses, depending on the nature of the pulse processed. After dehulled at 7 mm setting, the kernels and hulls were air separated, using an aspirator (built in-house by the Department of Agriculture and Food, Western Australia, DAFWA, Kensington, Western Australia). The dehuller was

cleaned using a vacuum cleaner before milling each sample to ensure there was no cross - contamination. After removing the hulls and separation, the dehulled samples were manually separated to ensure that they were free from hulls and whole seeds. Samples were packed in GLAD plastic Snap Lock bags (sandwich size -15 cm x 17 cm) and were kept in a freezer (-18°C) until needed.

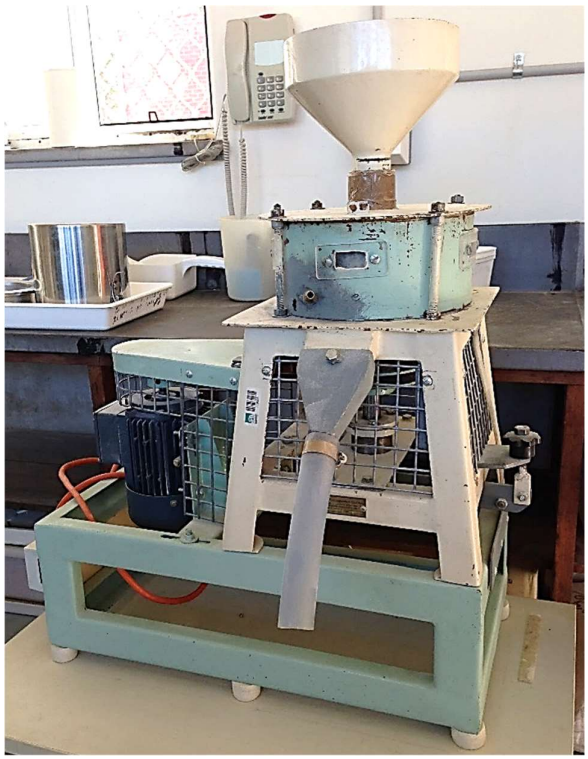

Figure 6 : Lupin dehuller

Dehulled lupin kernel samples were milled using a Retsch mill ZM 200 (Retsch GmbH, Hans, Germany). The screen size used throughout the study was 500 µ. The kernels were poured into the feeder at an amplitude of 40-50 rpm. A low feed rate was used to minimize heat generation. The temperature of milled flour was monitored using a thermocouple, in order to ensure that the heat generated from milling was low. Milled lupin flour samples were kept in a fresh Glad Snap Lock plastic bag and stored in a freezer (-18°C) until further use. Lupin flour yielded from seed to flour was also recorded, by weighing each variety of seed samples prior to their dehulling and milling, and reweighing the final product, which is the lupin kernel flour. Figure 7 shows the lupin miller used in the experiment.

Figure 7 : Lupin miller (Retsch mill ZM 200)

3.2.2 LOX assay

The following LOX assay was conducted through the modifications of methods used by Mohammed & Rangappa (1992) and Cato et al. (2006).

3.2.2.1 Defattening of samples

Lupin flour (10 g) was placed in a beaker. 30 mL of hexane was carefully added, and then carefully stirred for a minute with a glass rod to allow homogeny. The solution was then covered with aluminium foil, and was left overnight at 4°C. Excess solvent was decanted the next day, and samples were left to air - dry for 6 hours under a fume cupboard.

3.2.2.2 Preparation of substrate solution (2.5% linoleic acid)

Substrate solution was prepared fresh daily on ice to prevent degradation. A 0.05 M borate buffer (pH 9) solution (5 mL) was filled into a 10 mL brown bottle with screwcap top. Tween20 (0.25 mL) was carefully dissolved into the borate buffer, avoiding any vigorous mixing to prevent air bubbles. Linoleic acid (0.25 mL) was added into the solution and 1 N NaOH (0.65 mL) was added, and the solution was then completed with the addition of distilled water (3.85 mL), making the solution's volume 10 mL.

3.2.2.3 Enzyme extraction from lupin flour

Milled lupin flour (1 g) was weighed and transferred to 100 mL volumetric flask. Then 50 mL of acetate buffer (pH 5.0), containing 0.1 M - NaCl was added. The volumetric flask was filled with the sample and buffer was placed in a container filled with ice for 15 minutes, shaken at every 5 minutes. After 15 minutes, 1.5 mL of the sample was transferred to 1.5 mL Eppendorf tube, and was centrifuged at 10,000 rpm for 5 minutes. The supernatant produced from the centrifugation process was transferred to a new Eppendorf tube. Eppendorf used is Eppendorf 5810R (Eppendorf, Hamburg, Germany)

3.2.2.4 Enzyme assay

The UV spectrophotometer (Varian Cary 50 Probe UV-Visible spectrophotometer by Varian Inc, (Agilent Technologies, Santa Clara, California) was pre – warmed, and the temperature of the cell holder was equilibrated to 25°C by water circulation. The spectrophotometer was then set to 234 nm for LOX analysis. Phosphate buffer (0.1 M, pH 6.8), which was equilibrated 25°C in a water bath (Kottermann Serial no. 3044, Köttermann GmbH & Co KG, Uetze/Hänigsen, Germany) earlier was used. In the test tube, phosphate buffer (2850 µL, 0.1 M, pH 6.8, 25°C), substrate solution (50 µL) and enzyme extract (100 µL) was added, and then was vortexed for 10 seconds before placing into the cell holder circulated with 25°C water from the water bath before

measuring the absorbance of the solution, using the probe connected to the spectrophotometer. The absorbance at 234 nm was measured. Before starting, the spectrophotometer absorbance was calibrated to zero, using deionized water at 25°C before placing the blank. The absorbance was re-calibrated to zero using a blank solution containing 2.95 mL phosphate buffer and 50 µL substrate solution (without any enzyme extract addition). Initially the absorbance was recorded from 0 -5 minutes at one minute intervals to confirm that the absorbance at 234 nm was increasingly leaner in their manner. After the initial test to ensure the linearity of the absorbance line, every other sample's absorbance was only recorded at 1 minute and 4 minutes respectively.

3.2.2.5 Calculation and expression of results

One unit of LOX activity was represented as an increase in 0.001 unit of absorbance at 234 nm per minute, per gram of lupin on dry basis, as calculated by using the following formula (Mohamed & Rangappa, 1992).

$$\text{LOX activity} = \frac{\text{Abs (4 min)} - \text{Abs (1 min)}}{3} \times \frac{1}{1 \times (100\text{-}M)/100} \times \frac{50{,}000}{B}$$

Abs (4 min): Absorbance at 234nm at 4 min of reaction
Abs (1 min): Absorbance at 234nm at 1 min of reaction
M: Moisture content of lupin sample
B: Volume (µL) of enzyme extract of lupin used (It is usually 100)

3.2.3 Moisture content

The moisture content was measured as per the AOAC method (-925.10-) (AOAC, 2000). Dried and empty dishes were pre-weighed and suitably labelled according to the samples used (W_0). The total weight of sample on the pre-weighed dish was recorded as W1. The samples in the pre-weight dishes were then dried in an oven set at 105ºC until constant weight, which was approximately 24 hours. The dried samples in the pre-weight dishes were then cooled down to room temperature in a desiccator and weighed as W_2. Moisture content in the samples was calculated as shown in Equation 1:

$$\text{Moisture content (\%)} = \frac{(W_1 - W_2)}{(W_1 - W_0)} \times 100 \quad (1)$$

Where:

W_0 = weight of empty dish (g)

W_1= weight of dish and sample (g)

W_2= weight of dried dish and sample (g)

3.2.4 Ash content

The ash content of the samples was determined by using a method by Osborne (1978). The required amount of silica dishes and lids was placed in a muffle furnace (Thermolyne furnace 48000, Thermo Fisher Scientific Inc. Iowa, U.S.A.) that was set at 550

°C

for 15 minutes. The dishes and lids were then removed and cooled in a desiccator for one hour. After the dishes were cooled, each dish and lid was weighed to the nearest milligram (W1). Approximately 5.0 g of sample were then added into each dish and was weighed with the lid on (W2). The dishes which were filled with samples were then placed on a hot plate under a fume-hood and temperature was slowly increased until smoking ceased and the samples become thoroughly charred. The dishes were then transferred in to the muffle furnace at 550 °C and ashed until constant weights, approximately overnight. The dishes were then removed from the furnace and placed in a desiccator for one hour to cool down to room temperature. Once cooled to room temperature, the dishes were re-weighed to the nearest mg (W3) and the weight of the ash were calculated by their difference as per equation 2.

$$\%\ \text{Ash} = \frac{(W3 - W1)}{(W2 - W1)} \times 100 \quad (2)$$

Where:

W_1 = weight of empty dish and lid (g)

W_2= weight of dish, lid and sample (g)

W_3= weight of dried dish, lid and sample (g)

3.2.5 Protein content

Protein content of the samples was determined by the Kjeldahl method AOAC method number 920.87 (AOAC, 2000). The analysis was carried out using a Tecator™ Digestion systems (FOSS, Hilleroed, Denmark) and a Kjeltec 1030 auto analyser (FOSS, Hilleroed, Denmark).

Lupin flour (0.5 g) was weighed onto a pre-weighed N free filter paper. The weighed sample was slowly poured into the bottom of a digestion tube and appropriately labelled. Kjeltab (a catalyst to increase the speed of reaction which constituents consists of potassium sulphate, copper and selenium) was added into each individual test tube filled with the sample, glass bead (to absorb heat and minimize bumping), 8 mL of digestion acid (95% concentrated sulphuric acid, 5% concentrated phosphoric acid) and 4 mL of hydrogen peroxide. For the blank 0.5 g of sucrose was used instead of the samples. The tubes were then placed into a preheated digester set at 420°C. Samples were then digested until the solutions became clear or pale yellow. The digested samples were then cool down to room temperature before 75 mL of distilled water were slowly added. A receiver flask containing 25 mL of boric acid and indicator (bromocresol green : 20 mL in 20 L and methyl red : 14 mL in 2 L)was placed under the condenser outlet of the Kjeltec machine. Approximately 50 mL of 40% sodium hydroxide (NaOH) was poured into the digestion tube and steam - distillated. The distillation was continued until approximately 125 mL of distillate solution was collected into the receiving flask. The receiving flask was then dropped down and steam was turned off. The ammonia content trapped in the boric acid was titrated against 1 M hydrochloric acid until the grey endpoint was reached. The number of equivalents of acid used in titration is the same as the number of equivalents of ammonia present in the sample (% N). The protein percentage was calculated by the multiplication of % N with a conversion factor (*f*) of 6.25 as per equation 3.

$$\% \text{ protein} = \frac{(\text{sample titre mL} - \text{blank titre mL}) \times \text{M HCl} \times 14.1 \times f \times 100}{\text{mg sample}} \quad (3)$$

Where : F = conversion factor = 6.25

3.2.6 Fat content

Fat content was determined using the AOAC (2000) method number (-963.15-) using a Buchi E-816 SOX extraction unit (Buchi Labortechnik, Flawil, Switzerland) . Dried lupin flour samples were used in the experiment. Approximately 1.5g of the sample was placed into a pre-weighed empty thimble and the weight was recorded up to four decimal places (Ws). The extraction cup (a glass beaker where the fat will be collected later in the experiment) containing two glass beads was weighed up to four decimal places (We). A white plastic holder was attached to the thimble and the thimble was then placed into the extraction chamber of the Buchi fat extractor. Subsequently the weighed extraction cup was placed on the bottom hot plate. The unit was then lowered to seal the extraction cups. Setting was then made according to the Buchi manual (*Extraction Unit E-812/816 Hot Extraction*) and cooling water flow was turned on - this step is crucial to ensure that any petroleum ether vapours are condensed and returned to the sample. Once sealed, 100 mL of petroleum ether was added to the top of the unit (above the distilling coils) using the funnel. The machine was then started and the extraction cycle lasted for about 90 minutes. Following the extraction, the solvent remaining in the extraction cup was evaporated and the cups containing the extracted oil were then dried in an oven at 105°C for 24 hours. The dried extraction cups were weighed and the weight was recorded as Wc. The oil content was calculated as per the equation below:

$$\% \text{ Fat content} = \frac{Wc - We}{Ws} \times 100 \quad (4)$$

Where:

W_s = weight of sample (g)

W_e = weight of empty cup (g)

W_c = weight of dried cup and oil after extraction (g)

3.2.7 Colour measurement

Colour values of lupin flour samples were measured using a Spectrophotometer Minolta CM-508i (Hunterlab Ultrascan Sphere Spectrophotometer-Hunter Associates Laboratory Inc., Reston, VA) that uses the CIELAB colour system (L^* a^* b^*) and the

average readings at three predetermined points on the sample was recorded. The instrument was calibrated against a standard white colour plate. The *L** represents black to white colours with a range value between 0 (pure white) to 100 (pure black). For axis a* and b*, the values run from positive to negative and have no specific numeral limits. On the a*, positive values indicate the amount of red while negative values indicate the amount of green. For b*, positive values indicate yellowness and negative values indicate blue. Meanwhile value 0 is neutral grey. Figure 8 shows the Minolta spectrophotometer used in the study.

Figure 8 : Minolta Spectrophotometer

3.2.8 Heat treatment

Three of the lupin varieties were selected based on their LOX levels (which were high and medium) as well as commercial values. The three selected varieties were subjected to heat treatment at seed, dehulled seeds and flour. The 3 types of heat treatment used were conventional oven, microwave oven and electric pressure cooker at variable time and temperature combinations. Heat treatment types were chosen based on their ease of availability and time - saving capacity in the food industry.. Microwave and pressure cooker were chosen based on the needs of the Ready to Eat / café industry and the

catering industry, where these techniques are common. The oven heating technique was used considering the food manufacturing industry All of the heated samples were subjected to LOX assay.

Approximately 10 g of each sample were weighed, placed into a soda glass petri dish (Schott) with the diameter of 60 x 15 mm ensuring the height of each sample was uniform (15 mm). Figure 9 shows the lupin flour sample in the petri dishes.

Figure 9 : Lupin flour sample in soda petri dishes prior to heat treatment

For convention oven (Contherm Thermotec 2000 Oven by Contherm Scientific, Lower Hutt, Wellington, New Zealand), the temperature range used were 50°C, 60°C, 70°C, 80°C and 90°C while the heating times were 5, 10 and 15 minutes. The details of the time and temperature and lupin stage combinations are as per Table 11.

Table 11: Time-temperature combination of LOX heat treatment

No	Types of heat treatment	LOX activity; mean ± SD		
		Seed	Kernel	Flour
0	0°C temperature 0 time	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
1	50°C temperature 5 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup

No	Types of heat treatment	LOX activity; mean ± SD		
		Seed	Kernel	Flour
2	50°C temperature 10 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
3	50°C temperature 15 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
4	60°C temperature 5 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
5	60°C temperature 10 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
6	60°C temperature 15 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
7	70°C temperature 5 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
8	70°C temperature 10 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
9	70°C temperature 15 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
10	80°C temperature 5 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
11	80°C temperature 10 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
12	80°C temperature 15 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
13	90°C temperature 5 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
14	90°C temperature 10 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
15	90°C temperature 15 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
16	900 watts 30 seconds	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
17	900 watts 60 seconds	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
18	900 watts 90 seconds	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
19	900 watts 120 seconds	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
20	900 watts 150 seconds	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup

No	Types of heat treatment	LOX activity; mean ± SD		
		Seed	Kernel	Flour
21	70kPa 1 minute	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
22	70kPa 2 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
23	70 KPa 3 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
24	70 kPa 4 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup
25	70 kPa 5 minutes	Belara, Danja, Mandelup	Belara, Danja, Mandelup	Belara, Danja, Mandelup

For microwave oven 900 watt was used (Panasonic model NN – 6455A (serial no. AW 601202045) Panasonic Corporation (formerly known as Matsushita Electric Co Ltd., Osaka, Japan). High watt was used and the times were 30, 60, 90, 120 and 150 seconds. The details of the time and temperature and lupin stages combination are as per Table 11.

For electric pressure cooking (the pressure used was 70kPa) the Tefal minut pressure cooker CY4000 (Tefal, Subsidiary of Groupe SEB, Haute- Savoie, France) was used and the time combinations were 1, 2, 3, 4 and 5 minutes. The details of the time and temperature and lupin stages combination are as per Table 11.

3.2.9 Development of lupin chapatti

The sample with the best result from the heat treatments (time and temperature combination) was selected and incorporated to chapatti.

The raw materials used to produce chapatti were Atta flour (Pilsbury Chakki Atta – Stone milled whole wheat flour purchased from Aus Bangla Enterprise, Cannington, Western Australia, Australia), lupin flour, water and table salt (Saxa table salt). The chapatti formulations used are shown in Table 12. The dry materials were first mixed in a Hobart Mixer N-50G Serial number 14025605 (Hobart Corporation, Troy, Ohio USA) for 2 minutes using dough hook and speed no. 1 (low speed) to encourage homogeneity. Figure 10 shows the mixer used in this study. Water was then added

slowly while kneading. Dough was continued to be kneaded for 5 minutes. The dough was then covered with a damp towel to prevent drying and left to be rested for 30 minutes inside the mixing bowl. The dough was then separated into round balls weighing 50 g each. A chapatti Cllws ‘roti maker’ (Maharaja Whiteline, New Delhi, India) was used to flatten the dough and to produce uniformed thickness. This was to ensure that the amount of heat used to cook each chapatti sample remained constant. Figure 11 shows the chapatti maker used in this study. After the dough was flattened, the dough was then transferred into a hot plate (model number H0909BA, serial number 9404181, Wenesco, Chicago, USA) at a temperature of 320°C for one minute on each side, repeating once. Figure 12 shows the hot plate used in this study. Although traditionally chapatti is cooked on a stove or on direct heat or fire, the amount of heat applied will vary from chapatti to chapatti when using this traditional method. A hot plate was used in the study to ensure uniform heat application for each sample.

Figure 10: Hobart Mixer

Figure 11 :Maharaja chapatti maker

Figure 12 : Hot plate used for chapatti heating

To produce chapatti with heat treated lupin flour, the chapattis were incorporated with different percentages (based on the % of lupin per 100g of flour) of heat treated lupin flour as shown in Table 12. The heat treated flour was produced using the oven heat treatment at 80°C for 5 minutes.

Table 12 : Chapatti formulation

Ingredients	Control	20% lupin	30% lupin	40% lupin
Atta flour	1000 g	800 g	700 g	600 g
Heat treated lupin flour	-	200 g	300 g	400 g
Table salt	10 g	10 g	10 g	10 g
Water	700 mL	700 mL	700 mL	700 mL

3.2.10 Texture analysis

The texture of the chapatti sample were measured as it is a key quality trait of chapatti which includes how it tears and folds, how it feels in the hands and in the mouth (Fenton, Solah, Williams, Gujral, & Diepeveen, 2011). The texture was assessed using an extensibility test on the Texture Analyser (TA.XT2, Stable Microsystems, Surrey, United Kingdom) using a method by Fenton et al. (2011) to stretch and tear a strip of chapatti. Three chapatti strips were cut from one chapatti and the distance between the tension grips was set at 30 mm and 10 mm and each was clamped by tension grip. The chapatti strips were stretched at a speed of 1.0 mm/sec for a distance of 15 mm. The parameters of peak force to rupture, distance to peak force and area under the curve values were measured.

3.2.11 Sensory evaluation

The sensory acceptability of the chapatti samples was measured by using the 9 Point Hedonic Scale Test developed by Jones, Peryam, and Thurstone (1955), which is a commonly used scale for measuring food acceptability, modified by Jayasena, Khu, et al. (2010). The samples were evaluated for appearance, colour, flavour, texture and

their overall acceptance (Appendix A). Treatment combinations are listed in the Table 13.

Table 13 : Chapatti samples used for sensory evaluation

Chapatti samples	Atta flour (%)	Lupin flour - untreated (%)	Lupin flour – heat treated (%)
1	100	-	-
2	80	20	-
3	70	30	-
4	60	40	-
5	80	-	20
6	70	-	30
7	60	-	40

Sixty panelists (as per the requirement of the standard ISO 8586:2012 (ISO, 2012)) were recruited to participate in the sensory evaluation. Participants were informed of the task and asked to complete a consent form (Appendix B).

The chapatti samples were served in quarters and they were accompanied with a small bowl of vegetarian dal makhani curry produced by Delhi 6 Authentic Indian Restaurant, 271, Amherst road, Canning Vale, WA consists of urad dal, kidney beans, onion, green chillies, ginger garlic paste, tomatoes, cumin seeds, cloves, green cardamoms, black cardamoms, cinnamon, bay leaf, red chilli powder, nutmeg powder, low fat cream, dry fenugreek leaves and butter. A glass of water and water crackers were provided as well to cleanse the palate between tastings.

3.3 Statistical analysis

Data were analysed using SPSS for Windows version 23. To identify whether the data was normally distributed, Shapiro Wilk's test was conducted to measure for normal distribution followed by boxplots to identify the outliers were used. For parametric data one-way ANOVA (Tukey's post hoc test) was applied to determine the

differences between samples. Independent t-test was used for comparing between 2 groups of samples. For samples that have unequal variances, result was interpreted with unequal variance t test or Welch t test. Other test used was Levene‘s homogeneity test of variance. Non - parametric data were analysed by non-parametric Kruskal-Wallis test followed by Mann-Whitney U test. A 0.5% level of significance was applied in the statistical tests, where p-value of less than 0.05 indicated the presence of significant differences.

4.0 Results and Discussions

4.1 Chemical and physical properties

The Australian Sweet Lupin used in this study was grown in the Wongan Hills, region of Western Australia. The study evaluated the Lipoxygenase (LOX) activities in 25 varieties over a three year harvesting periods. The significance differences between LOX activities between years and varieties and the proximate composition (moisture, ash, protein, fat, and colour) were determined.

Lipoxygenase activity is associated with the beany flavour in soy (R. Wang et al., 2008) and other legumes (Yoshie-Stark & Wäsche, 2004). Dahuja & Madaan (2004) found that LOX is the main cause of beany flavour in soybean and the off-flavour in soybean is largely generated by the action of LOX on polyunsaturated fatty acids (PUFAs). Currently there has been limited studies on LOX in lupin in terms of off flavour or beany flavour (Stephany, Bader-Mittermaier, Schweiggert-Weisz, & Carle, 2015). When applied to food products, this beany flavour makes the product unacceptable. It is believed that reducing LOX activities is a prerequisite for the manufacture of acceptable lupin – incorporated food products as this flavour affects consumer acceptability negatively.

The aim of this study was to gather knowledge on the LOX activities of the 25 varieties of lupin grown in Western Australia to understand the LOX variations in different varieties and the factors that may be involved in affecting the LOX activity. To date, there have been no published studies on the LOX activities of Australian Sweet lupin varieties. Hence it is important to gather data on the LOX activity of different varieties so that low LOX varieties can be grown for food applications thereby minimizing the negative impact on foods containing lupin.

4.1.1 LOX activity

The 25 lupin varieties used in the initial phase of this study were harvested from 2009 to 2011, in three harvesting seasons. However, the harvesting year 2010, was considered a drought which was the driest year on record, with annual rainfall 40-50% lower than normal , based on report from the Australian Bureau of Meteorology (2011). Such a drought has impacted the harvest and only 18 varieties were available for testing instead of 25 varieties.

4.1.1.1 LOX activity in 25 varieties

The LOX activities of lupin varieties are presented in Table 14. As expected some of the varieties have different LOX activity.

Table 14 : LOX activities of 25 lupin varieties grown in 2009, 2010 and 2011.

Lupin variety	2009 (mean ± SD)	2010 (mean ± SD)	2011 (mean ± SD)
Belara	95.95± 9.97^{gh}	92.66± 2.76^{defg}	101.14± 6.43^{hij}
Chittick	99.91± 3.44^{ghi}	105.24± 2.05^{hi}	101.06± 14.77^{hij}
Coromup	153.40± 6.33^{l}	188.53± 7.92^{k}	152.96± 0.32^{m}
Danja	99.25± 9.00^{ghi}	128.20± 11.81^{j}	101.60± 12.66^{hij}
Geebung	61.91± 6.90^{bc}	90.90± 9.66^{def}	71.60± 4.47^{bcd}
Gungurru	76.35± 11.07^{d}	96.42± 4.85^{fgh}	79.60± 6.17^{def}
Illyarrie	108.34± 6.24^{ij}	N/A	109.20± 5.31^{ijk}
Jenabillup	118.45± 11.02^{j}	83.75± 2.40^{cde}	118.11± 5.14^{k}
Jindalee	82.58± 6.30^{def}	N/A	79.13± 11.20^{def}
Kalya	72.41± 6.05^{cd}	115.83± 14.48^{i}	65.91± 7.90^{bc}
Mandelup	155.82± 10.65^{l}	92.81± 2.45^{efg}	158.98± 10.37^{m}
Marri	105.47± 11.05^{hi}	N/A	110.99± 5.23^{jk}
Merrit	76.07± 6.39^{d}	71.19± 1.47^{ab}	76.34± 4.08^{cde}
Moonah	147.79± 12.81^{l}	112.25± 7.64^{i}	151.61± 11.60^{m}
Myallie	88.84± 5.05^{efg}	97.59± 5.47^{fgh}	96.10± 7.30^{gh}

Lupin variety	2009 (mean ± SD)	2010 (mean ± SD)	2011 (mean ± SD)
Quilinock	75.02± 11.71^{d}	N/A	69.30± 2.87^{bcd}
Tallerack	91.96± 3.34^{fg}	80.18± 7.53^{bc}	88.33± 5.11^{fg}
Tanjil	79.43± 15.30^{def}	68.20± 6.34^{a}	82.99± 3.39^{ef}
Unicrop	90.16± 10.79^{fg}	81.42± 1.44^{cd}	89.00± 10.63^{fg}
Uniharvest	77.83± 4.73^{de}	N/A	79.27± 6.04^{def}
Uniwhite	54.75± 3.64^{ab}	N/A	62.85± 9.21^{b}
Warrah	46.03± 6.97^{a}	N/A	51.07± 4.51^{a}
Wonga	59.08± 5.24^{b}	86.34± 6.27^{cde}	69.05± 10.92^{bcd}
Yandee	133.49± 2.92^{k}	111.40± 1.10^{i}	136.95± 8.91^{l}
Yorrel	97.63± 5.66^{ghi}	104.26± 4.81^{ghi}	98.22± 5.42^{ghi}

*Different alphabets vertically indicates significant differences of LOX activity between varieties ($P<0.05$).

**(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

*** Missing data (labelled as N/A) in 2010 due to unavailability of varieties

From the data in Table 14 for the year 2009, the top 3 varieties that had the highest amount of LOX activity were Mandelup (155.82 ± 10.65), Coromup (153.40 ± 6.33) and Moonah (147.79 ± 12.81). Meanwhile among the varieties with the lowest LOX activity for 2009 are Warrah (46.03 ± 6.97) and Uniwhite (54.75 ± 3.64) (Figure 13).

Only 18 varieties were available from the 2010 harvest. From the data in Table 14 for the year 2010, Coromup (188.53 ± 7.92) had the highest amount of LOX activity, followed by Danja (128.20 ± 11.81) and Kalya (115.83 ± 14.48) respectively. Meanwhile among the varieties with the lowest LOX for 2010 are Tanjil (68.20 ± 6.34) and Merrit (71.19 ± 1.47) (Figure 14). The difference in the lowest LOX varieties for 2010 compared to the 2009 and 2011 could be due to the drought and the lupin plants' response to this. This finding will be further discussed in 4.1.1.3 LOX activity between years.

The top 3 varieties that have the highest amount of LOX activity for the year 2011 as shown in Table 14 are Mandelup (158.98 ± 10.37), Coromup (152.96 ± 0.32) and Moonah (151.61 ± 11.60). Meanwhile the varieties with the lowest LOX for 2009 is Warrah (52.07 ± 4.51) (Figure 15). The varieties with the highest and lowest LOX activity were more or less similar over the testing period, which is an indication that some low LOX varieties such as Warrah may be better for food applications than others due to LOX having an impact on flavour that makes products unacceptable.

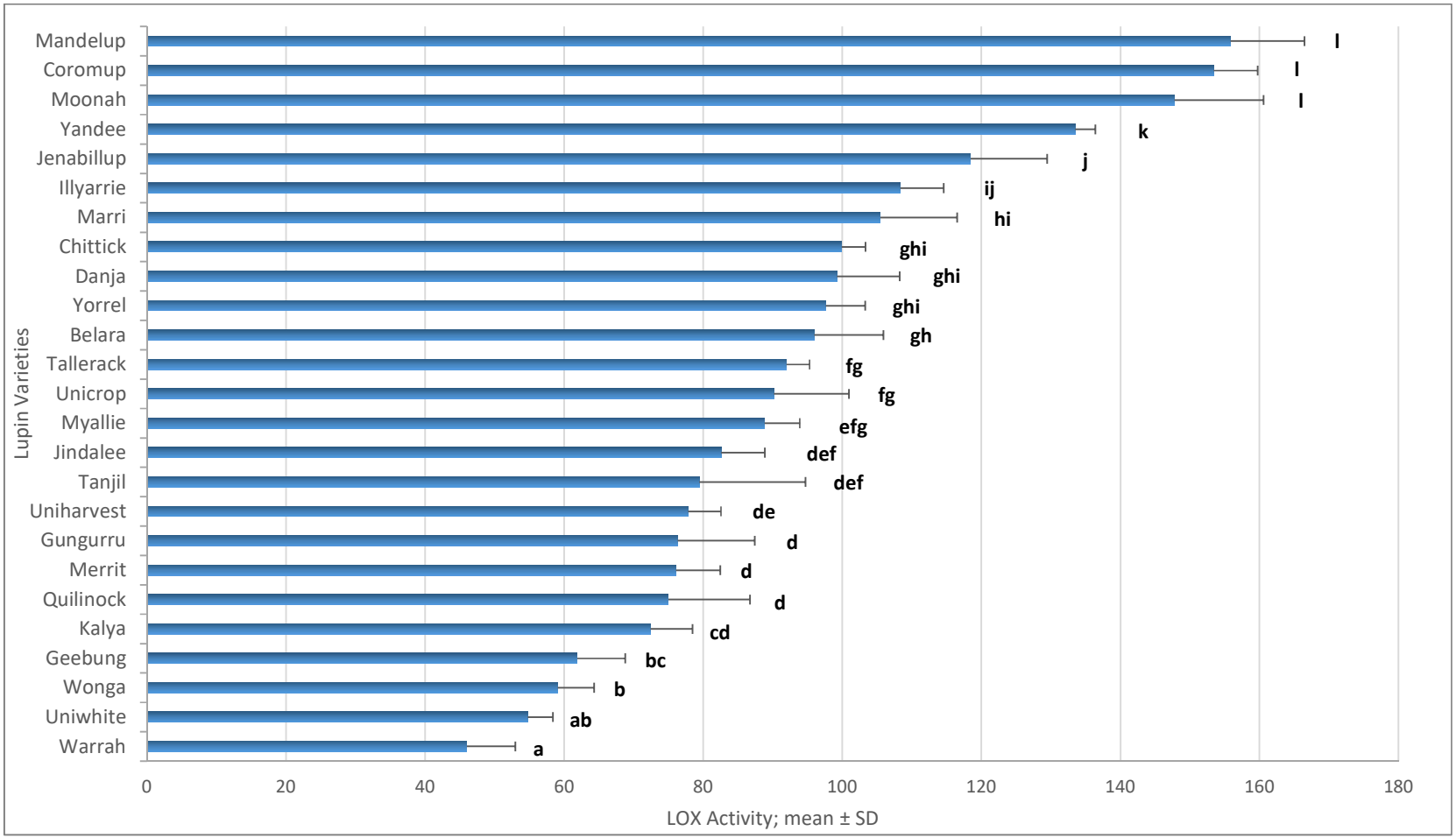

Figure 13: LOX activity of lupin varieties harvested in 2009

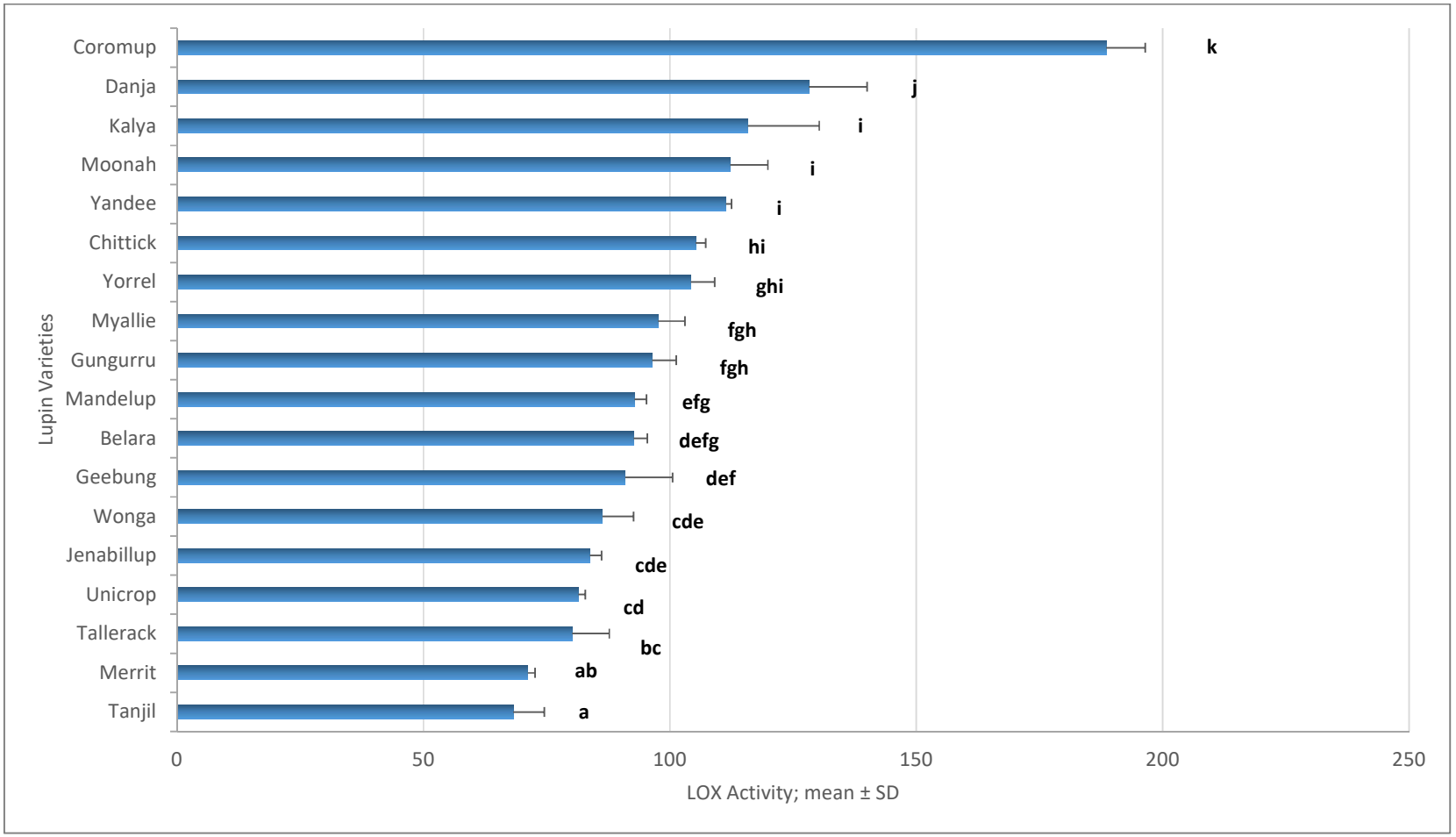

Figure 14 : LOX activity of lupin varieties harvested in 2010

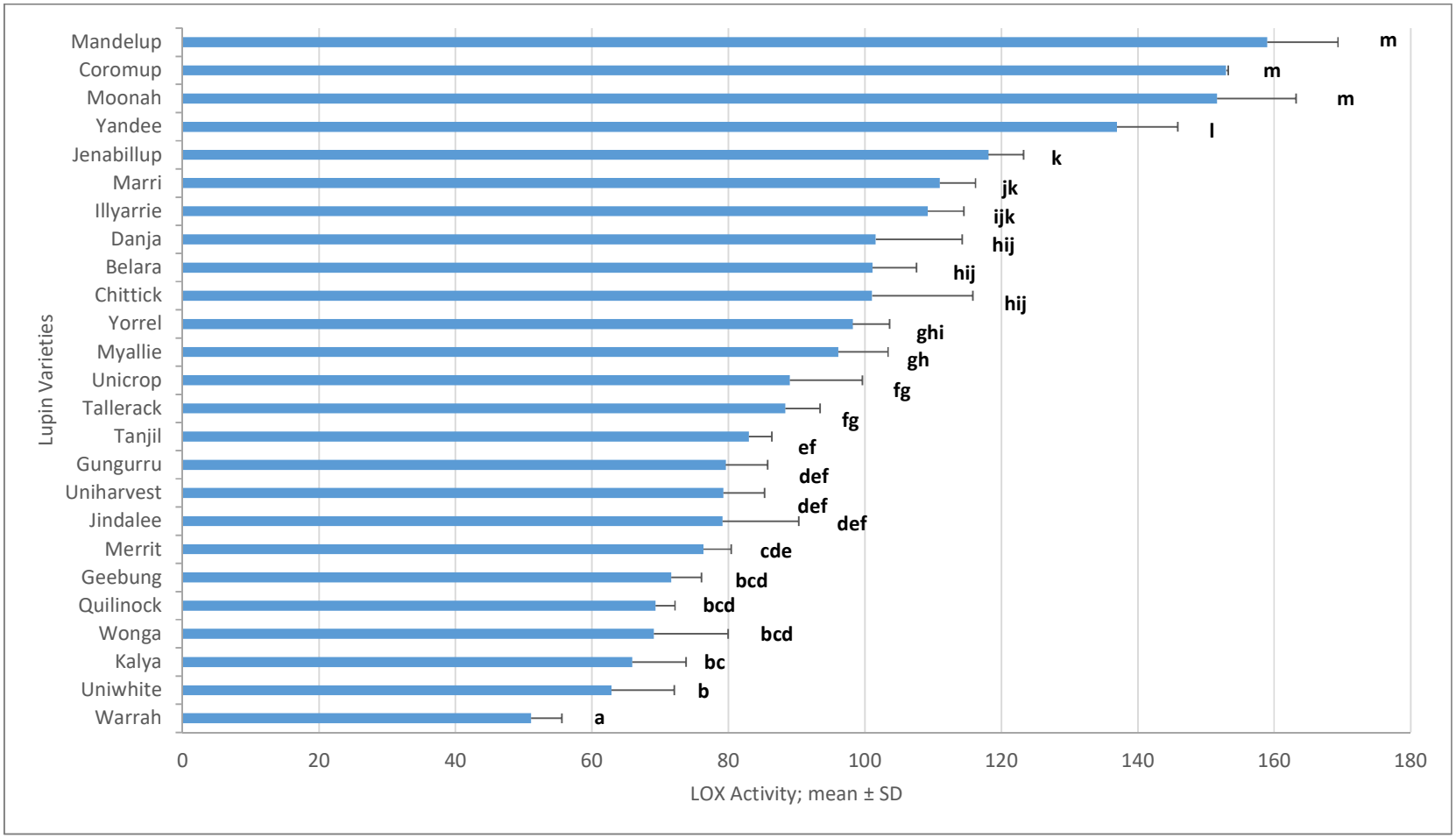

Figure 15: LOX activity of lupin varieties harvested in 2011

4.1.1.2 LOX Average

The LOX activity of the commercially grown varieties of lupin that were determined in the 3 harvesting years were calculated to display the average activity allowing for easier ranking purposes. Result is presented in Table 15. Data for 2010 were not considered in the average calculation as some samples of the varieties were not available.

In terms of differences between varieties, there is a significant difference between varieties ($P<0.05$) as shown in Table 15. The ranking from high to low can be seen in Figure 16. Mandelup, Coromup and Moonah had the highest amount of LOX activity while Warrah had the lowest LOX activity. The study shows that there are significant differences in the LOX activity between varieties in all 3 years and also in the average data.

Table 15: Average LOX activity of 25 lupin varieties

Lupin varieties	LOX activity average; mean ± SD
Belara	98.54± 8.25^{kl}
Chittick	100.48± 9.95^{lm}
Coromup	153.21± 4.49^{q}
Danja	100.42± 10.24^{lm}
Geebung	66.76± 7.47^{bcd}
Gungurru	77.98± 8.48^{fg}
Illyarrie	108.77± 5.39^{n}
Jenabillup	118.28± 7.96^{o}
Jindalee	80.85± 8.59^{g}
Kalya	69.16± 7.39^{cde}
Mandelup	157.40± 9.88^{q}
Marri	108.27± 8.53^{mn}
Merrit	76.20± 4.96^{efg}
Moonah	149.70± 11.50^{q}
Myallie	91.95± 6.75^{ijk}
Quilinock	72.16± 8.47^{def}
Tallerack	90.14± 4.45^{ij}
Tanjil	81.46± 9.35^{gh}
Unicrop	89.58± 9.93^{hi}
Uniharvest	78.55± 5.08^{fg}
Uniwhite	59.38± 8.10^{b}
Warrah	48.55± 6.07^{a}
Wonga	64.06± 9.56^{bc}
Yandee	135.22± 6.22^{p}
Yorrel	97.93± 5.14^{jkl}

*Different alphabets vertically indicates significant differences of LOX activities between varieties ($P<0.05$).

**(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

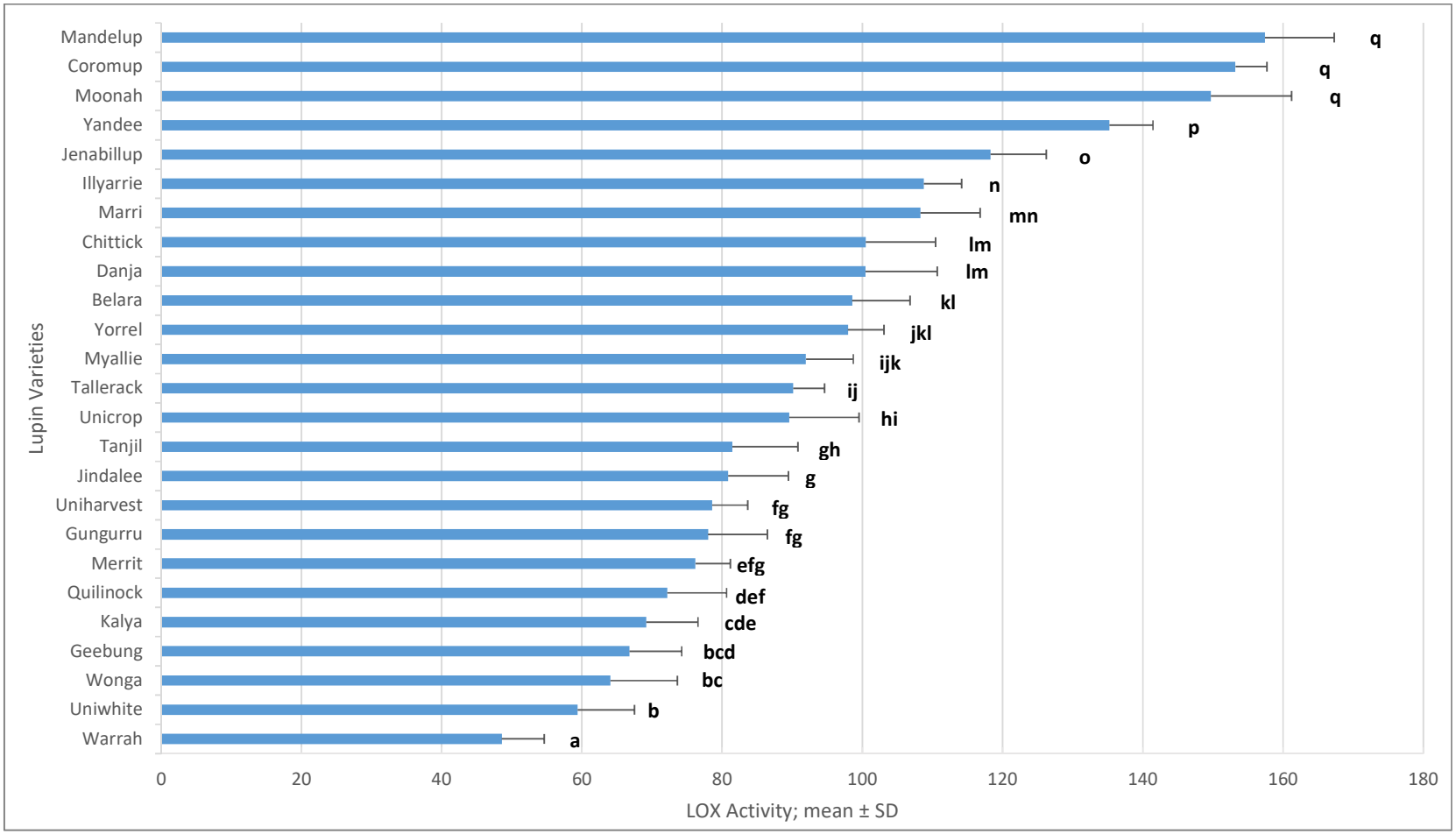

Figure 16: Average LOX activity of 25 varieties

As per observation in Figure 16, it is indicated that Mandelup (157.40± 9.88), Coromup (153.21± 4.49) and Moonah (149.70± 11.50) ranked high compared to the rest of the 25 varieties. It is interesting to note that Coromup and Mandelup are the most commonly grown lupin varieties in Western Australia (P. White et al., 2008). It is also interesting to highlight that both varieties have high protein contents, similar to Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015) finding that Coromup has the highest protein content in her study. These findings will be further discussed in section 4.1.2.5.

Prior to 2015, there have been no published studies on the LOX activity of Australian sweet lupin. Most of the previous studies targeted at LOX extraction, purification and characterization from lupin. Stephany et al. (2015) studied the LOX activity of luteus, albus and angustifolius; including 9 varieties of angustifolius tested. None of the varieties tested were Australian sweet lupin varieties. The specific LOX activity in the 2015 study was expressed in units of enzyme activity per milligram protein with one unit of enzyme causes an absorption increment of 0.001 per min. However, in this current study the unit of enzyme activity is expressed in unit of enzyme activity per gram of protein with one unit of enzyme causes an absorption increment of 1 per minute. Hence, to compare the results between the previous study with the current study, the findings from the previous study were converted to get the same unit.

Stephany et al. (2015) study reported a higher amount of LOX activity in the samples, ranging from 100 – 1000 units per gram protein compared to the lower amount of LOX activity found in this current study, ranging from 49 to 162 unit per gram protein. There are many factors that could contribute to these differences. Firstly, this could be due to the different types and varieties used in the study. Local ASL lupin are specially – breed species that were domesticated from low alkaloid varieties selected from Germany (Cowling et al., 1998) and were also found to have low anti nutritional factors (ANF) (Petterson et al., 1997) hence it is possible that ASL variety contain low LOX compared to its wild bitter varieties. The lupin that were used, which were harvested in 2010 from Steinach, Germany, were also grown in different conditions compared to the ASL used in this study. Other factors that could have contributed to the high level of LOX differences could be the different method of milling, extraction,

and measuring LOX. However, it is interesting to note that the results in the Stephany et al. (2015) study showed that there was significant difference between LOX activity among different species whereby *luteus* species was found to have the lowest LOX activity and *albus* species has LOX activity that were comparable to half of the 9 varieties used for *angustifolius* species. Meanwhile between the 9 *Lupinus angustifolius* varieties, there were significant differences between them as well, which supports the results of the current study.

4.1.1.3 LOX activity between years

There were no significant differences in the LOX activity of lupin varieties harvested in year 2009 and 2011. However, there were significant differences in 12 out of 18 varieties in 2010 harvest compared to varieties harvested in 2009 and 2011 (Table 16).

Table 16: Comparison of LOX activity between the year 2009, 2010 and 2011

Lupin variety	2009 (mean ± SD)	2010 (mean ± SD)	2011 (mean ± SD)
Belara	95.95± 9.97[a]	92.66± 2.76[a]	101.14± 6.43[a]
Chittick	99.91± 3.44[a]	105.24± 2.05[a]	101.06± 14.77[a]
Coromup	153.40± 6.33[a]	188.53± 7.92[b]	152.96± 0.32[a]
Danja	99.25± 9.00[a]	128.20± 11.81[b]	101.60± 12.66[a]
Geebung	61.91± 6.90[a]	90.90± 9.66[b]	71.60± 4.47[a]
Gungurru	76.35± 11.07[a]	96.42± 4.85[b]	79.60± 6.17[a]
Illyarrie	108.34± 6.2[a]	N/A	109.20± 5.31[a]
Jenabillup	118.45± 11.02[a]	83.75± 2.40[b]	118.11± 5.14[a]
Jindalee	82.58± 6.30[a]	N/A	79.13± 11.20[a]
Kalya	72.41± 6.05[a]	115.83± 14.48[b]	65.91± 7.90[a]
Mandelup	155.82± 10.65[a]	92.81± 2.45[b]	158.98± 10.37[a]
Marri	105.47± 11.05[a]	N/A	110.99± 5.23[a]
Merrit	76.07± 6.39[a]	71.19± 1.47[a]	76.34± 4.08[a]
Moonah	147.79± 12.81[a]	112.25± 7.64[b]	151.61± 11.60[a]
Myallie	88.84± 5.05[a]	97.59± 5.47[a]	96.10± 7.30[a]
Quilinock	75.02± 11.71[a]	N/A	69.30± 2.87[a]
Tallerack	91.96± 3.34[a]	80.18± 7.53[b]	88.33± 5.11[ab]

Lupin variety	2009 (mean ± SD)	2010 (mean ± SD)	2011 (mean ± SD)
Tanjil	79.43± 15.30[ab]	68.20± 6.34[a]	82.99± 3.39[b]
Unicrop	90.16± 10.79[a]	81.42± 1.44[a]	89.00± 10.63[a]
Uniharvest	77.83± 4.73[a]	N/A	79.27± 6.04[a]
Uniwhite	54.75± 3.64[a]	N/A	62.85± 9.21[a]
Warrah	46.03± 6.97[a]	N/A	51.07± 4.51[a]
Wonga	59.08± 5.24[a]	86.34± 6.27[b]	69.05± 10.92[a]
Yandee	133.49± 2.92[a]	111.40± 1.10[b]	136.95± 8.91[a]
Yorrel	97.63± 5.66[a]	104.26± 4.81[a]	98.22± 5.42[a]

*Different alphabets horizontally indicates significant differences of LOX activities between years ($P<0.05$).

**(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

According to the report from Australian Bureau of Meteorology (2011), the harvesting year of 2010 was the driest year on record (annual rainfall occurring between 40-50% lower than normal). The other two years have had an average rainfall, with 2011 having a slightly higher average rainfall. Hence, the difference in LOX in 2010 harvest compared to other years were most likely due to the drought conditions, in which case the drought could have caused water stress (water deficit) which can adversely affect the plant growth and productivity in the plants (Osakabe, Osakabe, Shinozaki, & Tran, 2014). The results also indicate that the normal year to year differences in the environment may not affect LOX as the effect on LOX would only be if there are drastic changes in the growing conditions, such as a substantial drought.

According to Siedow (1991), the role of LOX production in plants were yet to be clearly identified. However, according to Brash (1999), among LOX enzyme's role is to synthesis hydroperoxide from free fatty acid substrates, creating a pathway for producing intermediate products such as jasmonic acid and aldehyde biosynthesis and end products such as 12-HETE in the pathway that acts as biological mediator/signalling molecules which are beneficial to the plants. Signalling molecules are molecules that are responsible for transmitting information between plant cells.

Lipoxygenase are also thought to be involved in inducing structural or metabolic changes in the cell (Brash, 1999).

The findings by Williams and Harwood (1997) on the effect of drought on the lipoxygenase pathway (LOX) in olive oil indicated there was significant difference in the volatile products produced in the LOX pathway of drought-affected plants and irrigated plants. Hence it was concluded by them that drought influences the LOX enzyme activity and pathway, which affect the end products. The findings of Williams and Harwood (1997) was supported in the current study, where the LOX activity in the tested varieties were different for the 2010 harvesting season which was affected by a drought compared to 2009 and 2011.

According to P. White et al. (2008), water shortage is the most important environmental constraint to lupin production in Western Australia. In Western Australia, a method called drought escape is applied. This method involves the matching of a particular crop's growth to the pattern of water supply, so that it's life cycle is complete while water remains accessible. This is done by planting in early autumn and by spring, the grain is fully grown. The harvesting is normally in January the following year and for this study purpose, the sample year is named after the year the samples were harvest. However, in this case, as the drought occurs after the lupin species have been fully grown (which was between November until March), it shows that the full grown lupin plants' LOX activity could still be affected by the drought. This shows that environmental factor such as drought could have a significant effect in the LOX activity of lupin.

Another important factor that should be considered, when comparing LOX differences between years is the storage conditions of the lupin seeds in each year, which includes the storage temperature and length of time the seeds were stored. According to a study done by Kong and Chang (2009) on soybean, factors such as storage temperature and length of storage plays an important role in determining the quality of the seed. There is a natural fluctuation of temperature and humidity occurring during the storage. In the current study, all of the seed samples were initially stored in the warehouse before being kept in cool room (4

°C
). This would mean that seed samples from 2009 and 2010 harvest were in storage longer than 2011, hence the probability of storage conditions affecting the LOX quality of the seeds. However, despite differences in storage length, seed samples from 2009 and 2011 harvest had no significant difference in LOX activity, it can be concluded that storage condition of lupin in seed after harvest does not have an effect in LOX activity.

4.1.2 Compositional analysis of 25 varieties of lupin

In the preliminary study, apart from the LOX activity, lupin flour yield, protein, ash, and fat content were also determined. Due to the limited amount of lupin sample available,it was decided that the analysis would be done on the 2011 samples, as the availability of 2009 and 2010 seed samples were beyond the scope of the study. Table 17 shows the overall percentages of yield, moisture, ash, fat and protein in different varieties.

4.1.2.1 Lupin flour yield

Lupin flour yield from all of the lupin varieties were calculated (in percentages) based on the amount of lupin flour produced from the whole lupin seeds. Figure 17 shows the percentage of lupin yields. Yield calculation is important to identify the amount of flour produced. The higher the yield in a particular variety, the more economical the end product will be, which would be beneficial to the food industry when applied to food manufacture. Referring to yield percentages from Table 17, there is significant difference between the different varieties in terms of lupin yield percentages. The average yield percent for lupin is 52.39 ± 2.93%. Among the factor that affects yield is also the type of dehuller used, as an efficient dehuller is able produce a higher yield (Reichert, Oomah, & Youngs, 1984).

Table 17: Comparison of percentages of lupin flour yield, moisture, ash, protein and fat content of lupin varieties

Lupin varieties	Yield (%)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)
Belara	49.80 ± 4.52^{abc}	7.50± 0.09^{cde}	3.82 ± 0.27^{cdef}	7.64± 0.69^{abcd}	42.49± 1.19^{cde}
Chittick	51.07 ± 0.10^{abcde}	7.77± 0.04^{fgh}	3.85 ± 0.40^{cdef}	7.91± 0.30^{abcd}	42.08± 0.91^{bcd}
Coromup	54.50 ± 0.72^{cdef}	7.92± 0.12^{hij}	3.44 ± 0.09^{bc}	7.86± 0.98^{abcd}	48.82± 0.73^{l}
Danja	51.37 ± 3.42^{abcdef}	7.88± 0.07^{ghi}	3.79 ± 0.45^{bcde}	7.36± 0.71^{ab}	41.89± 0.40^{bcd}
Geebung	52.36 ± 0.39^{abcdef}	7.63± 0.01^{ef}	3.84± 0.24^{cdef}	7.41± 0.54^{ab}	40.84± 1.11^{ab}
Gungurru	53.98 ± 1.94^{cdef}	7.84± 0.06^{ghi}	3.44± 0.17^{bc}	7.86± 0.09^{abcd}	45.57± 0.74^{hij}
Illyarrie	52.93 ± 1.29^{abcdef}	7.40± 0.03^{bc}	3.84± 0.25^{cdef}	7.76± 0.23^{abcd}	43.08± 0.60^{def}
Jenabillup	47.78 ± 1.57^{a}	7.85± 0.08^{ghi}	3.58± 0.28^{bcde}	7.71± 0.92^{abcd}	46.73± 0.89^{jk}
Jindalee	52.90 ± 2.21^{abcdef}	7.61± 0.07^{ef}	3.77± 0.41^{bcde}	7.48± 0.60^{abc}	44.44± 0.86^{fgh}
Kalya	50.09 ± 3.92^{abc}	8.12± 0.01^{kl}	3.66± 0.30^{bcde}	7.82± 0.23^{abcd}	43.67± 2.03^{efg}
Mandelup	52.23 ± 3.16^{abcdef}	7.71± 0.21^{fg}	3.46± 0.08^{bc}	7.81± 0.29^{abcd}	48.66± 0.83^{l}
Marri	52.91 ± 2.31^{abcdef}	7.15± 0.07^{a}	3.37± 0.13^{ab}	8.00± 0.15^{bcd}	40.24± 0.53^{a}
Merrit	49.30 ± 4.96^{ab}	8.06± 0.11^{jkl}	4.21± 0.07^{f}	7.64± 0.20^{abcd}	46.83± 0.99^{jk}
Moonah	56.65 ± 1.99^{f}	7.48± 0.17^{cde}	3.55± 0.22^{bcde}	7.75± 0.98^{abcd}	44.04± 0.78^{fg}
Myallie	55.81 ± 1.52^{def}	7.39± 0.01^{bc}	3.90± 0.52^{def}	7.13± 0.70^{a}	46.80± 0.61^{jk}
Quilinock	56.00 ± 1.42^{ef}	8.21± 0.05^{l}	3.56± 0.45^{bcde}	8.33± 0.51^{cd}	45.90± 1.52^{ijk}

Lupin varieties	Yield (%)	Moisture (%)	Ash (%)	Fat (%)	Protein (%)
Tallerack	51.25 ± 1.77^{abcdef}	7.64± 0.01^{ef}	4.23± 0.16^{f}	8.08± 0.42^{bcd}	44.58± 1.11^{ghi}
Tanjil	50.93 ± 1.31^{abcde}	7.59± 0.09^{def}	3.01± 0.34^{a}	8.14± 0.73^{bcd}	44.81± 0.96^{ghi}
Unicrop	50.80 ± 3.11^{abcde}	7.37± 0.09^{bc}	3.81± 0.30^{cdef}	7.86± 0.06^{abcd}	43.74± 0.87^{efg}
Uniharvest	55.02 ± 1.82^{cdef}	7.35± 0.18^{bc}	3.50± 0.30^{bcd}	7.38± 0.73^{ab}	42.27± 0.81^{cd}
Uniwhite	53.67 ± 2.25^{cdef}	7.89± 0.01^{ghij}	3.92± 0.26^{ef}	7.58± 0.67^{abcd}	41.21± 0.97^{abc}
Warrah	50.71 ± 2.99^{abcd}	7.99± 0.01^{ijk}	3.65± 0.24^{bcde}	7.94± 0.06^{abcd}	46.39± 0.97^{jk}
Wonga	50.23 ± 2.68^{abc}	8.01± 0.15^{ijk}	3.51± 0.26^{bcde}	7.84± 0.31^{abcd}	47.09± 0.87^{k}
Yandee	53.56 ± 4.03^{bcdef}	7.28± 0.10^{ab}	3.76± 0.31^{bcde}	7.85± 0.13^{abcd}	43.16± 0.39^{def}
Yorrel	53.86 ± 3.37^{cdef}	7.40± 0.03^{bcd}	3.68± 0.40^{bcde}	8.41± 0.37^{d}	44.27± 0.80^{fgh}
Average:	52.39 ± 2.93	7.68 ± 0.29	3.69 ± 0.37	7.77 ± 0.55	44.40 ± 2.48

*Values are means ± standard deviations of measurements expressed in percentages (%).

**Different letters in the same column indicates a significant difference (P <0.05).

*** All samples are calculated on dry weight basis.

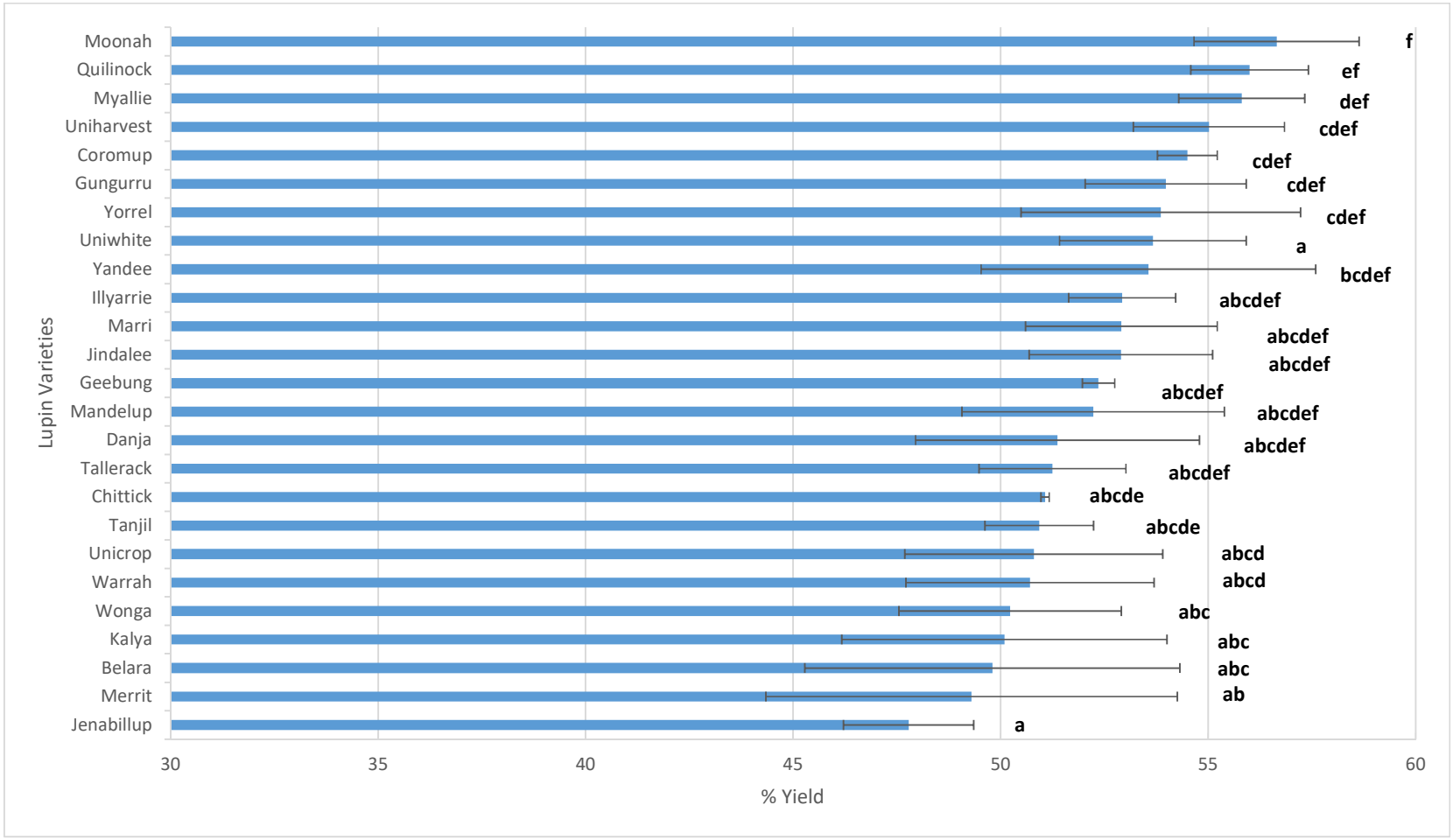

Figure 17: Yield percentages of lupin in 25 varieties

4.1.2.2 Moisture content

Moisture content is important in storage shelf life of seed and flour as the high moisture content result in lower shelf life (Butt, Nasir, Akhtar, & Sharif, 2004). According to Ziegler et al. (2016), grain moisture content and storage temperature was proven to affect grain quality such as bioactive compounds content of soybeans under long term storage. Moisture contents are shown in Figure 18. There were significant differences in moisture content between samples (Table 17). The average moisture content percentage obtained from the study is 7.68 ± 0.29%, which falls within the range of the figures reported in literature, which is between 6.9% (Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson, 2015) to 12% (Lee, 2007). The moisture percentage is also lower than the moisture content of soybean which is 10-13% (Riaz, 2006). In Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015) study, the lupin samples used were grown in Geraldton, Western Australia (except the Mandelup variety) and were from different crop years. Hence there is a possibility that the result could be slightly different due to the different environmental conditions. It is also interesting that Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015) also found that there were significant differences in moisture content between the 6 varieties that were used in that study.

It is also believed that different storage condition could also affect moisture content. In Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015) study, the samples were vacuum packed and stored in the cool room. Hence, it is believed that different storage condition and growing location could be the reason the moisture content varies between the current study and the previous studies.

4.1.2.3 Ash content

Ash content represents the total mineral content (Marshall, 2010). Ash content is shown in Figure 19. The average ash percentage obtained from the study of the 25 varieties is 3.69 ± 0.37%. This result is slightly higher than the literature data which reported ash content ranging between 2.5 -3.5% (Lee, 2007; Villarino, Jayasena,

Coorey, Chakrabarti-Bell, & Johnson, 2015) which is lower than the ash content of soybeans which is 5% (Riaz, 2006). In a study by Trugo, von Baer, and von Baer (2003) on ash content in different lupin species, ASL is reported to be 3.2% which is lower than the finding of the current study. Meanwhile *Lupinus luteus* has the highest ash content of 5.3%. The highest ash contents were found in Tallerack (4.23± 0.16%), Merrit (4.21± 0.07%), Uniwhite (3.92± 0.26%), Myallie (3.90± 0.52%), Chittick (3.85 ± 0.40%), Geebung (3.84± 0.24%), Illyarie (3.84± 0.25%), Belara (3.82 ± 0.27%) and Unicrop (3.81± 0.30%). Tanjil (3.01± 0.34%) and Marri (3.37± 0.13%) were among the lowest ash contents. Interesting to note that Coromup (3.44 ± 0.09%) and Mandelup (3.46± 0.08%) were among the varieties with lower ash contents however were among the highest LOX activity and protein levels. However, even though Marri was among the varieties with the lowest ash content, unlike Coromup and Mandelup, Marri has the lowest protein content. This shows that there is no correlation between ash and protein contents.

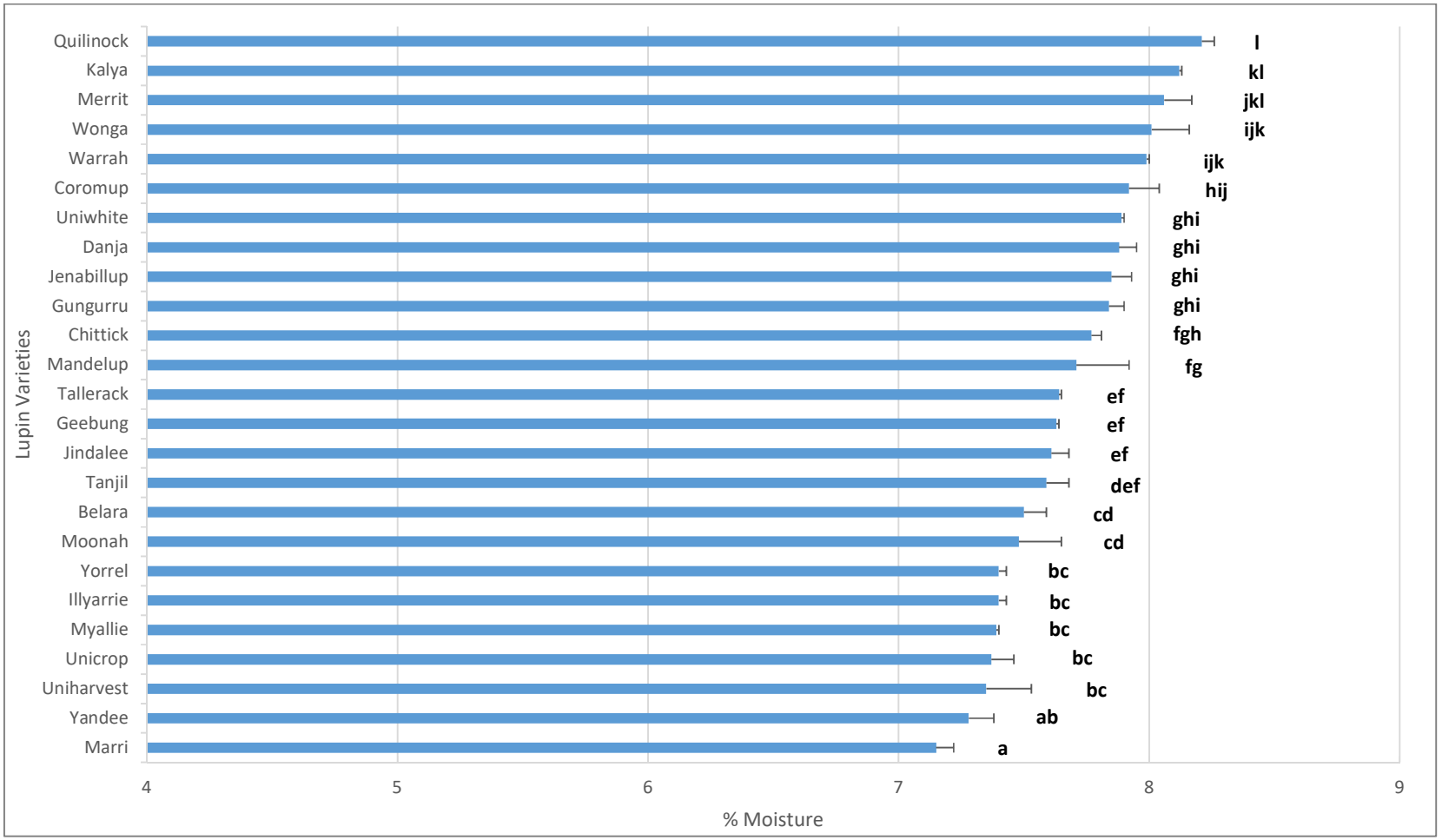

Figure 18 : Moisture content of 25 varieties of lupin

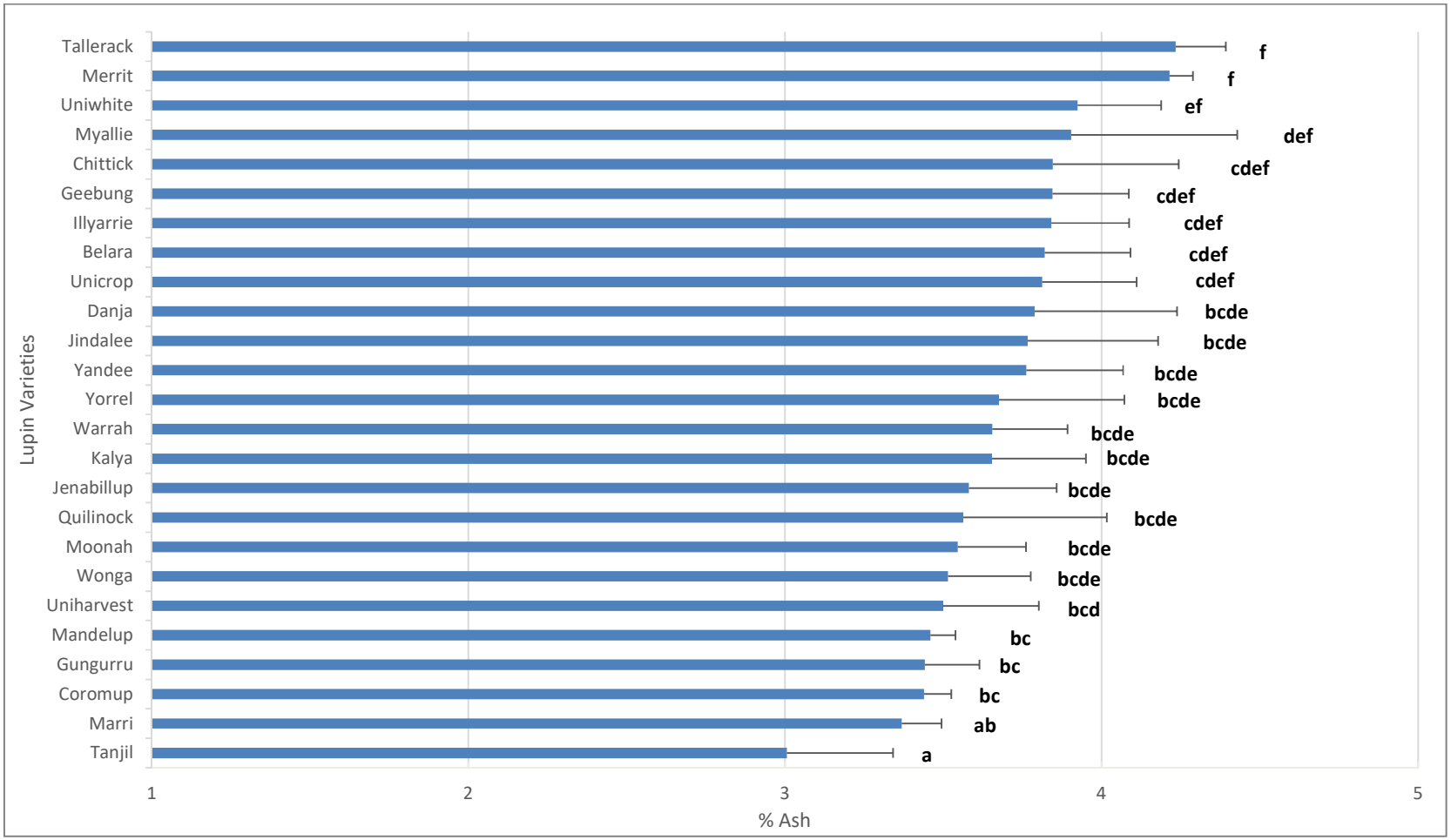

Figure 19 : Ash content of 25 varieties of lupin

4.1.2.4 Fat content

Fat content is shown in Figure 20. The average fat content was 7.77 ± 0.55%. This is comparable to the literature figures on the fat content in lupin which ranges between 6.7 to 8.2% (Lee, 2007; Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson, 2015). Lupin fat, which consists mainly of polyunsaturated fatty acid, is less than half the amount of fat in soy, which is around 15 -20% (Riaz, 2006). In terms of differences among varieties as shown in Table 17, it was found that there are significant differences of fat level between some of the 25 varieties (P>0.05).

In a study done by Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015), the fat content of the 6 varieties had significant differences between them. Interestingly, the fat content for Mandelup from Villarino et al. (2015) study is lower than the result found in this current study which is 6.7%. The Mandelup variety samples for both studies were taken from Wongan Hills however the samples were from different cultivation years. This shows that the fat content of lupin may have changed due to different cultivation and environmental condition variations between years.

4.1.2.5 Protein content

The average protein content was 44.40 ± 2.48%. This is comparable to those reported in the literature which was 41% (Lee, 2007) but slightly higher than reported by Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015) which ranged from 36.8 to 39.5%. Protein in lupin has been known to be higher than the protein in soybean which was 35 -40% (Riaz, 2006). In terms of differences between the 25 varieties, it was found that there were significant differences as shown in Table 17.

Both Coromup (48.82± 0.73%) and Mandelup (48.66± 0.83%) had the significantly highest protein content, while Marri (40.24± 0.53%), Geebung (40.84± 1.11%) and Uniwhite (41.21± 0.97%) were among the lowest protein content (Figure 21). This finding is similar with recent research whereby Coromup had one of the highest protein

content among the lupin varieties tested (Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson, 2015; P. White et al., 2008). When considering applying lupin into food to increase protein, such varieties could be applied, however, the LOX activity of these varieties need to be considered as well due to the impact on flavour.

In terms of correlation between LOX activity and protein content, as has been mentioned earlier, LOX activity in Coromup (153.21± 4.49) and Mandelup (157.40± 9.88) was among the highest LOX activity and also protein content. However, Marri (40.24± 0.53%) was among the lowest protein content has a comparably high LOX (108.27± 8.53) activity while Warrah, which has among the lowest LOX activity (48.55± 6.07), has a comparably high protein content (46.39± 0.97%). This mixed results show that there is a low probability of a correlation between LOX activity and protein content.

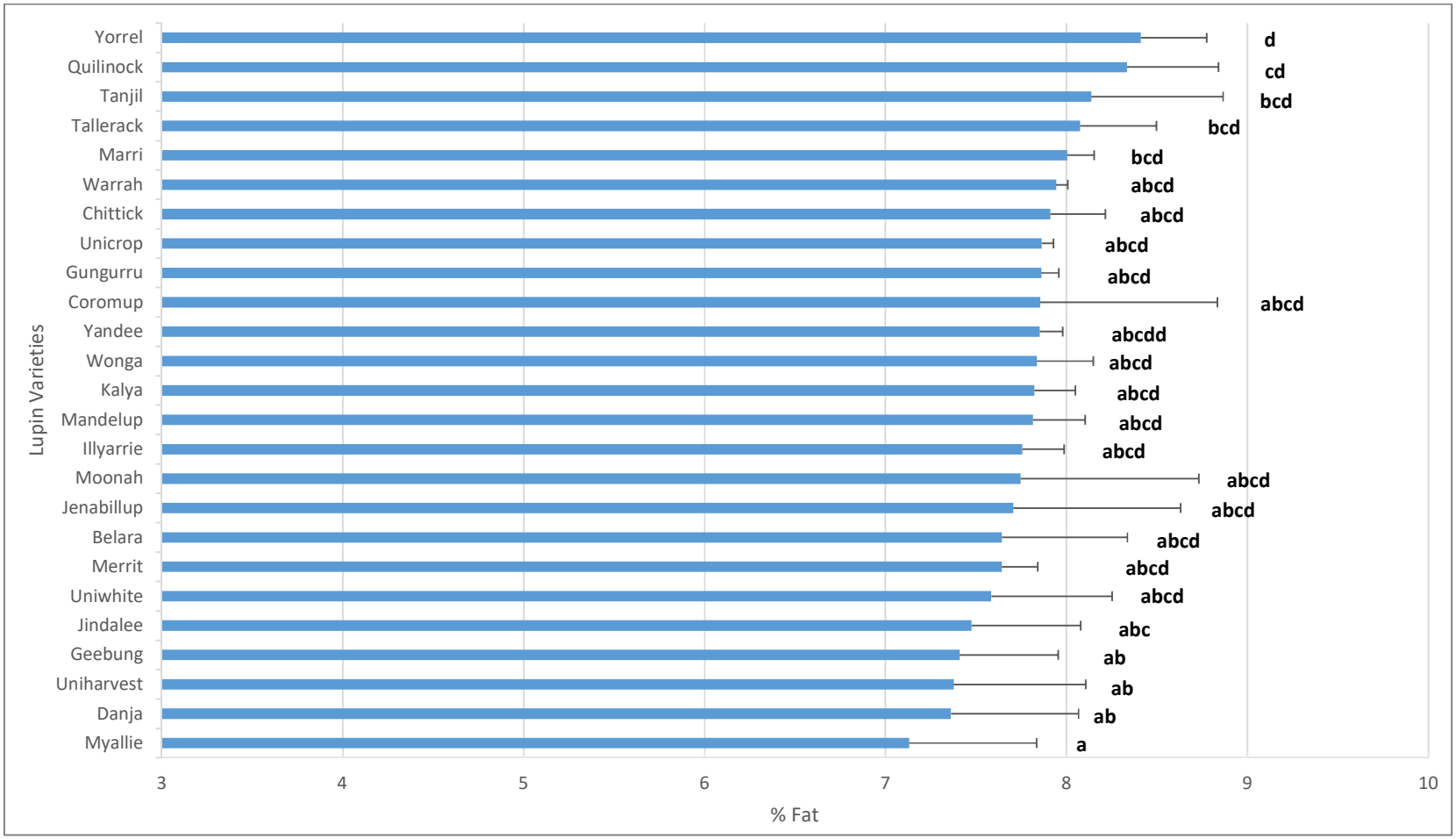

Figure 20 : Fat content in 25 varieties

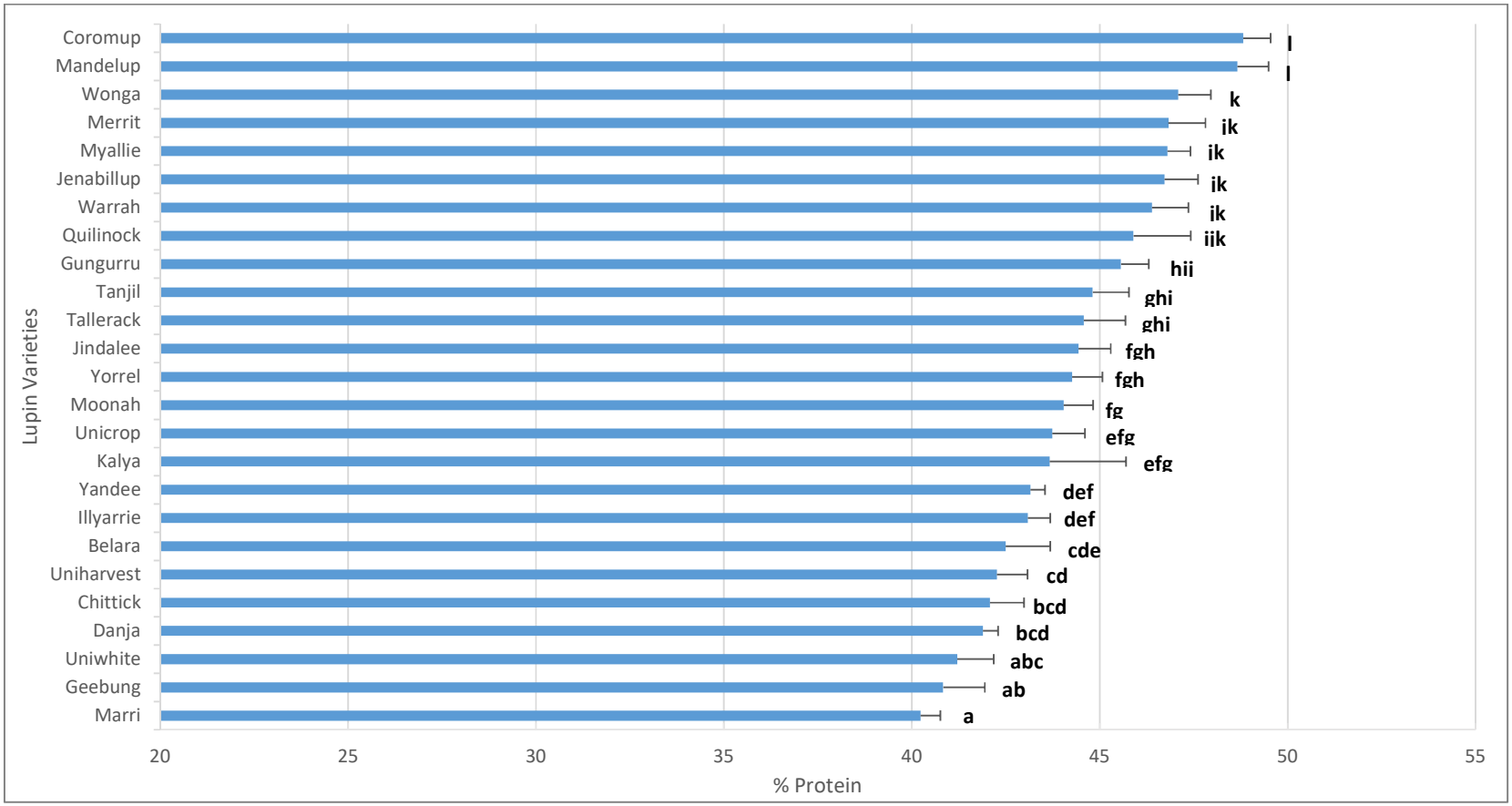

Figure 21 : Protein content in 25 varieties

4.1.3 Colour measurement

Colour measurement of the 25 varieties is shown in Table 18. The colours of the 25 lupin varieties are expressed as L* (lightness, extending from 0 (black) to 100 (white), b* from +128 to -127 (yellowness, positive value is yellow and negative value is blue) and a* from +128 to -127 (redness, positive value is red and negative value is green).

Among the varieties with the highest level of lightness are Unicrop (90.65 ± 0.21), Uniharvest (90.50 ± 0.13) and Merrit (90.38 ± 0.13) (Figure 22), while Belara (88.81 ± 0.29) Kalya (88.89 ± 0.85) had the lowest amount of lightness hence being the darkest, which means if applied to food products there would have a darker tone than when Unicrop or similar was applied. Belara (0.72 ± 0.17) had the highest level of redness, while Uniwhite (-0.58 ± 0.03), Uniharvest (-0.47 ± 0.09), Marri (-0.45 ± 0.16) and Geebung (-0.51 ± 0.11) had the highest level of greenness (Figure 23). Interestingly nearly half of the varieties have positive reading, which indicate half of the samples being redder and the other half to be greener with reading average between +0.7 and -0.6. In food application, it is important to understand the product and the customers' expectations in terms of colour. If the expectation is a redder product then a lupin variety that has a redder hue should be chosen where possible and vis versa for a product that should be greener.

From all three (L*, a*, b*) readings, b* appears to be the most relevant in comparing the colour of lupin samples as b* reading shows the yellowness. Belara (31.77 ± 0.31), Mandelup (31.73 ± 0.69) and Yandee (31.77 ± 0.31) had the highest level of yellowness while Uniwhite (25.59 ± 1.59), Marri (26.75 ± 0.99) and Unicrop (26.85 ± 0.25) were among the least yellow varieties (Figure 24). This information is important especially for food manufacturers as varieties with high yellow reading can be incorporated in their food products such as pasta where yellowness is desirable and appealing.

It is interesting to note that varieties with high level of LOX such as Mandelup (31.73 ± 0.69) and Coromup (30.57 ± 0.26) had high yellow readings, while Uniwhite, Warrah and Geebung which had low LOX level had low yellow readings. It could be

possible that there is a correlation between the yellowness of the lupin flour with the amount of LOX level. When lupin flour is incorporated into food product, the yellow colour gives a considerable appeal to food products (Dervas et al., 1999; Doxastakis et al., 2002). Hence in terms of appearance, varieties with higher yellowness would have a higher appeal when incorporated into food products, especially in products such as egg noodles and pastas. However, this might have a negative impact on the food's flavour, hence the importance of eliminating or reducing LOX in lupin.

Table 18 : Brightness, greenness and yellowness of 25 varieties of lupin varieties

Lupin variety	**Colour**		
	L* (brightness)	**a* (greenness)**	**b* (yellowness)**
Belara	88.81 ± 0.29^{a}	0.72 ± 0.17^{j}	31.77 ± 0.31^{k}
Chittick	90.18 ± 0.14^{efgh}	-0.26 ± 0.09^{b}	27.67 ± 0.43^{cde}
Coromup	89.78 ± 0.13^{cde}	-0.11 ± 0.05^{bcd}	30.57 ± 0.26^{j}
Danja	89.47 ± 0.06^{bc}	0.20 ± 0.26^{h}	29.68 ± 0.80^{hi}
Geebung	89.99 ± 0.75^{defg}	-0.51 ± 0.11^{a}	27.04 ± 0.28^{bc}
Gungurru	90.30 ± 0.22^{ghi}	-0.25 ± 0.10^{b}	27.27 ± 0.35^{bc}
Illyarie	90.06 ± 0.10^{defg}	-0.01 ± 0.05^{cdefg}	27.59 ± 1.00^{cd}
Jenabillup	90.26 ± 0.16^{fgh}	-0.03 ± 0.05^{cdef}	28.54 ± 0.17^{fg}
Jindalee	89.89 ± 0.32^{def}	-0.26 ± 0.06^{b}	28.26 ± 0.50^{ef}
Kalya	88.89 ± 0.85^{a}	0.07 ± 0.02^{efgh}	30.44 ± 0.22^{j}
Mandelup	89.43 ± 0.12^{bc}	0.13 ± 0.04^{fgh}	31.73 ± 0.69^{k}
Marri	90.31 ± 0.37^{ghi}	-0.45 ± 0.16^{a}	28.62 ± 0.57^{fg}
Merrit	90.38 ± 0.13^{ghi}	-0.18 ± 0.13^{bc}	30.22 ± 0.13^{ij}
Moonah	89.37 ± 0.13^{b}	-0.07 ± 0.05^{defg}	30.42 ± 0.23^{j}
Myallie	89.79 ± 0.19^{cde}	0.53 ± 0.13^{i}	29.51 ± 0.30^{h}
Quilinock	90.01 ± 0.45^{defg}	0.51 ± 0.36^{i}	29.18 ± 0.27^{gh}
Tallerack	89.90 ± 0.27^{def}	-0.07 ± 0.12^{cde}	29.71 ± 0.40^{hi}
Tanjil	90.00 ± 0.59^{defg}	0.16 ± 0.23^{gh}	26.85 ± 0.25^{b}
Unicrop	90.65 ± 0.2^{li}	-0.26 ± 0.08^{b}	27.15 ± 0.19^{bc}
Uniharvest	90.50 ± 0.13^{hi}	-0.47 ± 0.09^{a}	28.21 ± 0.13^{def}
Uniwhite	90.34 ± 0.26^{ghi}	-0.58 ± 0.03^{a}	28.48 ± 0.14^{f}

Lupin variety	Colour		
	L* (brightness)	**a* (greenness)**	**b* (yellowness)**
Warrah	90.11 ± 0.18^{efgh}	0.17 ± 0.03^{gh}	27.44 ± 0.17^{bc}
Wonga	90.01 ± 0.39^{defg}	-0.16 ± 0.03^{bcd}	30.85 ± 0.92^{j}
Yandee	90.15 ± 0.06^{efgh}	-0.03 ± 0.21^{cdef}	31.77 ± 0.31^{k}
Yorrel	89.69 ± 0.19^{bcd}	0.21 ± 0.28^{h}	27.67 ± 0.43^{cde}
Average	89.92 ± 0.55	-0.04 ± 0.35	28.80 ± 1.72

*Different letters in the same column indicates a significant difference ($P<0.05$).

**Values are means ± standard deviations of measurements of 3 readings per variety from each of 2 different batches of flour.

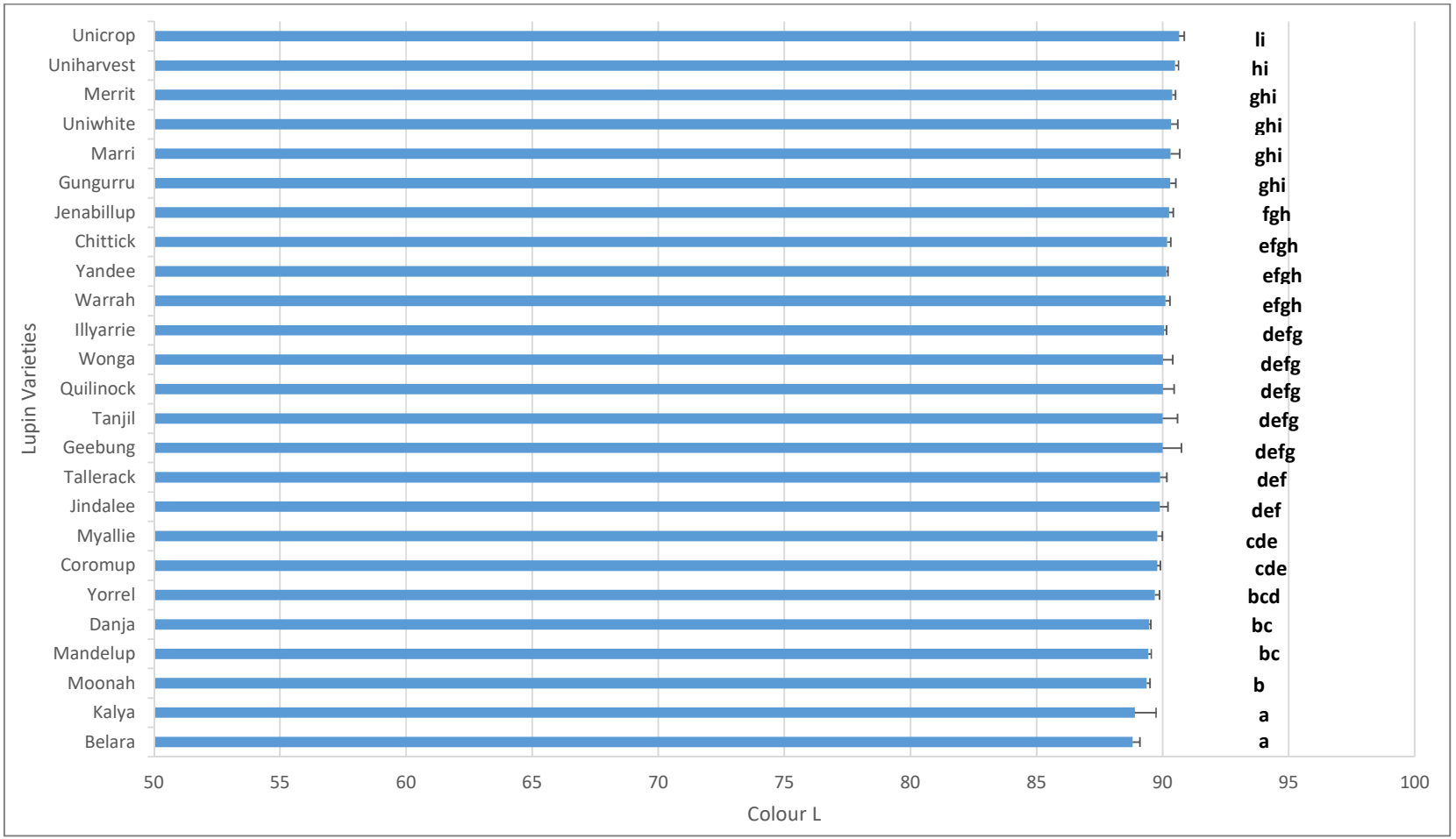

Figure 22 : L* (lightness) reading of lupin in different varieties

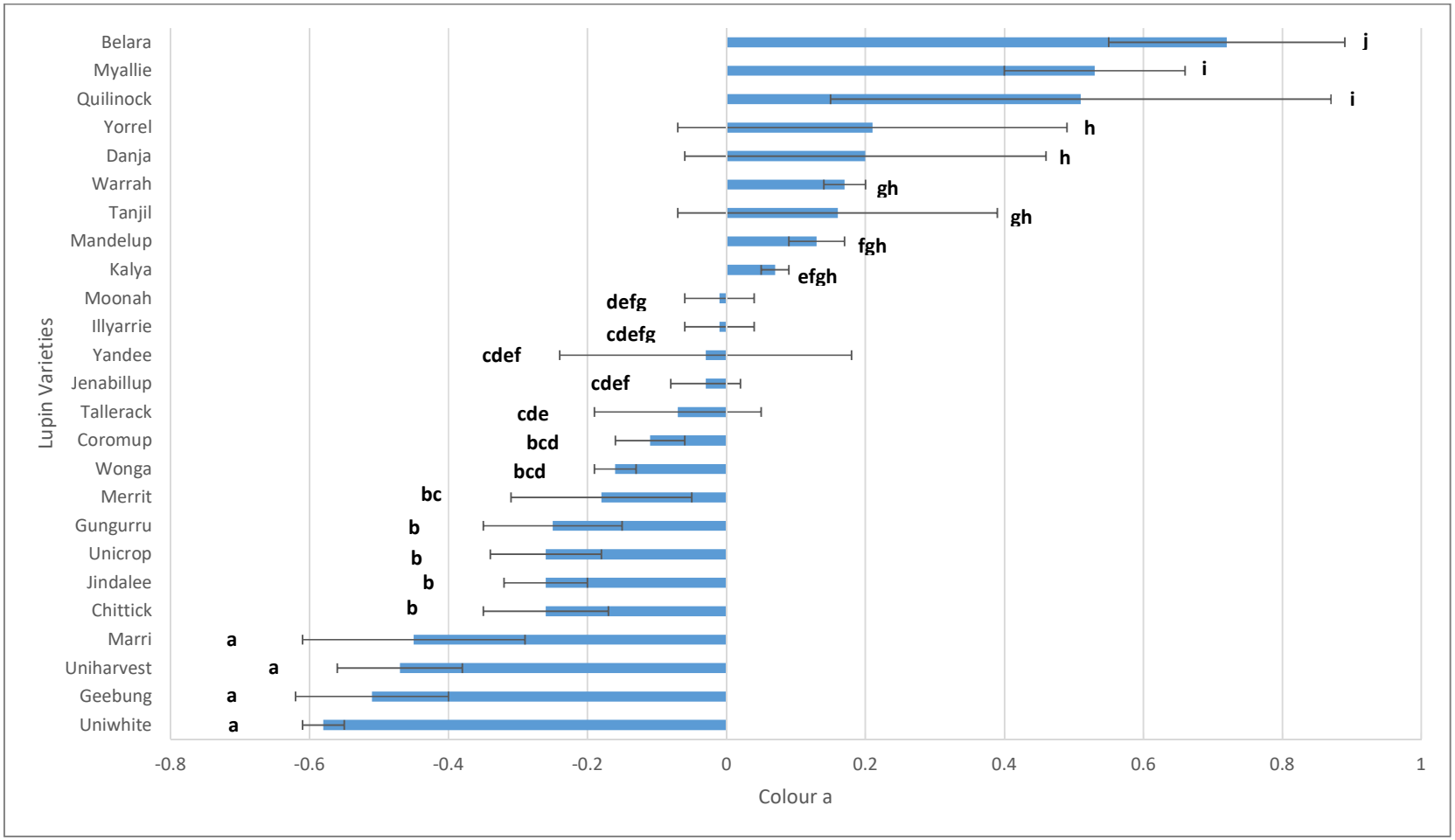

Figure 23 : a* (redness and greenness) reading of lupin in different varieties

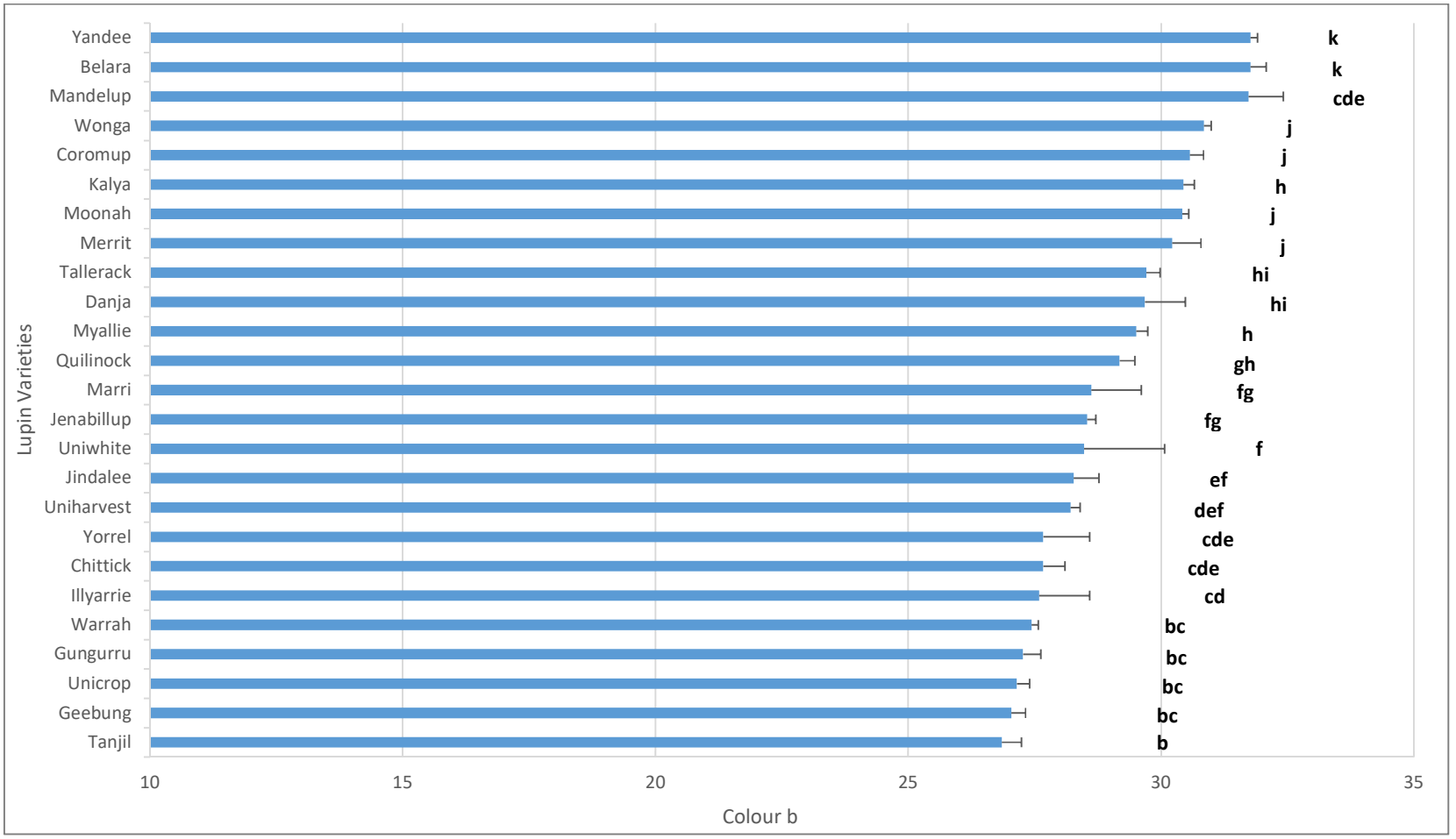

Figure 24 : b* (yellowness) reading of lupin in different varieties

4.2 Effects of heat treatment on the LOX activity of lupin

Based on the LOX activity and the availability of the lupin seeds, three Australian Sweet Lupin varieties, Belara, Danja and Mandelup were selected to determine the effect of heat treatment. Mandelup was among the highest LOX activity level from all varieties and at the time of the current research was the most commonly grown ASL variety in Australia. Both Danja and Belara had medium LOX levels. It is believed that choosing samples with a higher LOX level is beneficial for this study as a greater amount of reduction of LOX in heat treatment and the impact of heat on LOX can clearly be demonstrated. Another important aspect of using high LOX level varieties is that heat treatment that is able to reduce the LOX levels in a higher variety will be able to achieve a greater reduction in lower LOX varieties.

The types of heat treatment used were oven heating, microwaving and pressure cooking. The aim was to identify the time and temperature combinations to reduce the LOX level of lupin by using these three methods of heat treatments. The study also measured the colour of heat treated lupin flour, apart from the LOX level, to determine if there are any correlations between the LOX level and colour of lupin. To date, there have been limited published studies that has been carried out in using heat treatment to reduce LOX activities in lupin.

4.2.1 Effect of different processing stages on LOX

In this study, lupin samples from all three varieties were heat treated in different processing stages (seed, kernel and flour) and tested for LOX level with results averaged between the three varieties. The various stages and the heating regimes are shown in Table 19.

Table 19 : Comparison of LOX level of heat treated lupin between stages in different processing stages (seed, kernel, flour)

No	Heat Treatment			LOX activity; mean ± SD		
	Type	Condition	Duration	Seed	Kernel	Flour
0	OH	0°C	0	119.94 ± 25.37[a]	119.25 ± 25.63[a]	119.57 ± 25.56[a]
1	OH	50°C	5 minutes	104.21 ± 17.61[a]	99.34 ± 15.09[a]	106.42 ± 10.36[a]
2	OH	50°C	10 minutes	103.64 ± 18.79[a]	101.47 ± 12.64[a]	101.68 ± 16.59[a]
3	OH	50°C	15 minutes	106.55 ± 10.29[a]	101.74 ± 16.43[a]	99.22 ± 15.31[a]
4	OH	60°C	5 minutes	91.34 ± 13.64[a]	89.13 ± 10.34[a]	88.61 ± 6.78[a]
5	OH	60°C	10 minutes	89.68 ± 12.27[a]	87.77 ± 10.64[a]	85.43 ± 7.54[a]
6	OH	60°C	15 minutes	88.68 ± 6.93[a]	85.77 ± 7.53[a]	87.24 ± 10.64[a]
7	OH	70°C	5 minutes	68.72 ± 6.26[a]	67.02 ± 7.86[a]	68.36 ± 5.50[a]
8	OH	70°C	10 minutes	71.00 ± 5.30[a]	68.80 ± 5.79[a]	68.26 ± 6.54[a]
9	OH	70°C	15 minutes	68.41 ± 6.48[a]	68.56 ± 5.54[a]	68.38 ± 5.81[a]
10	OH	80°C	5 minutes	37.08 ± 6.30[a]	40.69 ± 3.36[a]	39.31 ± 3.80[a]
11	OH	80°C	10 minutes	37.39 ± 6.96[a]	37.38 ± 4.40[a]	40.96 ± 4.40[a]
12	OH	80°C	15 minutes	39.58 ± 3.75[ab]	41.12 ± 4.46[a]	36.61 ± 6.11[b]
13	OH	90°C	5 minutes	35.98 ± 3.20[a]	35.23 ± 3.99[a]	36.88 ± 5.15[a]
14	OH	90°C	10 minutes	37.31 ± 5.31[a]	33.17 ± 5.22[a]	33.87 ± 4.83[a]
15	OH	90°C	15 minutes	36.73 ± 2.13[a]	34.01 ± 4.88[a]	36.21 ± 2.05[a]
16	MW	900 watts	30 seconds	87.00 ± 8.19[a]	87.48 ± 7.93[a]	89.43 ± 8.50[a]
17	MW	900 watts	60 seconds	79.93 ± 5.50[a]	81.56 ± 4.85[a]	79.74 ± 5.05[a]
18	MW	900 watts	90 seconds	66.45 ± 8.22[a]	69.97 ± 6.43[a]	64.00 ± 10.08[a]
19	MW	900 watts	120 seconds	46.73 ± 4.88[a]	44.93 ± 7.35[a]	43.31 ± 9.59[a]
20	MW	900 watts	150 seconds	44.01 ± 5.13[a]	42.78 ± 5.74[a]	43.87 ± 7.04[a]
21	PC	70 KPa	1 minute	88.40 ± 9.05[a]	87.81 ± 6.06[a]	89.19 ± 6.40[a]
22	PC	70 KPa	2 minutes	83.77 ± 5.03[a]	82.65 ± 6.31[a]	81.48 ± 4.98[a]
23	PC	70 KPa	3 minutes	75.18 ± 4.65[a]	71.15 ± 8.97[a]	71.78 ± 9.03[a]
24	PC	70 KPa	4 minutes	59.70 ± 6.98[a]	57.86 ± 6.47[a]	53.81 ± 9.77[a]
25	PC	70 KPa	5 minutes	44.99 ± 8.39[a]	43.32 ± 7.06[a]	43.97 ± 5.05[a]

* OV – Oven ; MW – Microwave; PC – Pressure Cooker

*No significant differences of LOX activity between different processing stages collected

**Different letters in the same row indicates a significant difference (P <0.05).

***(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

There were no significant differences between heat treatments on LOX of samples between different processing stages. It was believed that there could be a possibility that LOX is stable and inactive at seed stage and is activated when dehulled (kernel)

or milled (flour). Hence,lupin should be heat treated in its seed or dehulled form to reduce LOX activity. This results showed that LOX is activated even at seed form. Torres-Penaranda, Reitmeier, Wilson, Fehr, and Narvel (1998) found that soymilk produced from lipoxygenase free soybean had significantly less beany flavour compared to normal soybeans, which shows that not only is LOX activity related to beany flavour, the beany flavour can be foundeven in its seed form.

According to a study conducted by Yasumatsu and Moritaka (1964) on rice, linoleic acid decreased during storage through gradual oxidization, producing carbonyl that affects flavour. Building on this study, Suzuki et al. (1999) studied the correlation between flavour and lipoxygenase of rice seed during storage using normal rice seed and lipoxygenase free rice seed, and found that that the lipoxygenase free rice seed had significantly better flavour than the normal rice seed. Hence the importance of inactivating LOX activity in lupin prior to food applications.

Table 20 : Comparison of LOX level of heat treated lupin between types of heat treatments in different processing stages (seed, kernel, flour)

No	Heat Treatment			LOX activity; mean ± SD		
	Type	Condition	Duration	Seed	Kernel	Flour
0	Control	0°C	0	119.94 ± 25.37^{i}	119.25 ± 25.63^{h}	119.57 ± 25.56^{i}
1	OH	50°C	5 minutes	104.21 ± 17.61^{h}	99.34 ± 15.09^{g}	106.42 ± 10.36^{h}
2	OH	50°C	10 minutes	103.64 ± 18.79^{h}	101.47 ± 12.64^{g}	101.68 ± 16.59^{h}
3	OH	50°C	15 minutes	106.55 ± 10.29^{h}	101.74 ± 16.43^{g}	99.22 ± 15.31^{h}
4	OH	60°C	5 minutes	91.34 ± 13.64^{g}	89.13 ± 10.34^{f}	88.61 ± 6.78^{fg}
5	OH	60°C	10 minutes	89.68 ± 12.27^{g}	87.77 ± 10.64^{f}	85.43 ± 7.54^{fg}
6	OH	60°C	15 minutes	88.68 ± 6.93^{fg}	85.77 ± 7.53^{f}	87.24 ± 10.64^{fg}
7	OH	70°C	5 minutes	68.72 ± 6.26^{cd}	67.02 ± 7.86^{de}	68.36 ± 5.50^{d}
8	OH	70°C	10 minutes	71.00 ± 5.30^{de}	68.80 ± 5.79^{e}	68.26 ± 6.54^{d}
9	OH	70°C	15 minutes	68.41 ± 6.48^{cd}	68.56 ± 5.54^{e}	68.38 ± 5.81^{d}
10	OH	80°C	5 minutes	37.08 ± 6.30^{a}	40.69 ± 3.36^{abc}	39.31 ± 3.80^{ab}
11	OH	80°C	10 minutes	37.39 ± 6.96^{ab}	37.38 ± 4.40^{abc}	40.96 ± 4.40^{ab}
12	OH	80°C	15 minutes	39.58 ± 3.75^{ab}	41.12 ± 4.46^{abc}	36.61 ± 6.11^{ab}
13	OH	90°C	5 minutes	35.98 ± 3.20^{a}	35.23 ± 3.99^{a}	36.88 ± 5.15^{ab}
14	OH	90°C	10 minutes	37.31 ± 5.31^{ab}	33.17 ± 5.22^{a}	33.87 ± 4.83^{a}
15	OH	90°C	15 minutes	36.73 ± 2.13^{a}	34.01 ± 4.88^{a}	36.21 ± 2.05^{ab}
16	MW	900 watts	30 seconds	87.00 ± 8.19^{fg}	87.48 ± 7.93^{f}	89.43 ± 8.50^{g}

No	Heat Treatment			LOX activity; mean ± SD		
	Type	Condition	Duration	Seed	Kernel	Flour
17	MW	900 watts	60 seconds	79.93 ± 5.50^{ef}	81.56 ± 4.85^{f}	79.74 ± 5.05^{ef}
18	MW	900 watts	90 seconds	66.45 ± 8.22^{cd}	69.97 ± 6.43^{e}	64.00 ± 10.08^{d}
19	MW	900 watts	120 seconds	46.73 ± 4.88^{b}	44.93 ± 7.35^{c}	43.31 ± 9.59^{b}
20	MW	900 watts	150 seconds	44.01 ± 5.13^{ab}	42.78 ± 5.74^{bc}	43.87 ± 7.04^{b}
21	PC	70 KPa	1 minute	88.40 ± 9.05^{fg}	87.81 ± 6.06^{f}	89.19 ± 6.40^{g}
22	PC	70 KPa	2 minutes	83.77 ± 5.03^{efg}	82.65 ± 6.31^{f}	81.48 ± 4.98^{fg}
23	PC	70 KPa	3 minutes	75.18 ± 4.65^{de}	71.15 ± 8.97^{e}	71.78 ± 9.03^{de}
24	PC	70 KPa	4 minutes	59.70 ± 6.98^{c}	57.86 ± 6.47^{d}	53.81 ± 9.77^{c}
25	PC	70 KPa	5 minutes	44.99 ± 8.39^{ab}	43.32 ± 7.06^{bc}	43.97 ± 5.05^{b}

* OH – Oven ; MW – Microwave; PC – Pressure Cooker

**Different letters in the same column indicates a significant difference (P <0.05).

***(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

The type of heat treatment had a significant effect on LOX activity (Table 20). From Table 20, it can be seen that all three methods were able to successfully reduce LOX effectively. The best method is oven heat treatment that produces the greatest reduction at 80°C at 5 minutes which is similar to pressure cooker at 5 minutes and microwave at 150 seconds. Moist heat such as pressure cooker is generally effective in removing biological material such as activating enzymes and microbes. However, it produced flour that seem damp and was deemed unsuitable to be used for future food product development. Microwaves appears to be the most ineffective because it caused the sample to dry and have burnt spots, plus the reduction in LOX was not as effective compared to other methods up till 120 seconds which will then cause burn spots in sample. The effects of microwave will be further discussed in 4.2.3.2.

4.2.2 Effect of time on LOX

When it comes to oven heating, there were no significant differences between the time of 5, 10 and 15 minutes on LOX as shown in Table 21. It was initially anticipated that the longer the heat treatment, the greater the reduction of LOX activities level differ between the time. However, based on result, given that there was no significant

difference, it is possible that the maximum time of 15 minute was too short to produce a significant difference between the three times of 5, 10 and 15. Of course when comparing between temperatures, there were significant difference as per Table 22 which will be discussed further in 4.2.3.1. However, it can also be said that the maximum time might have been 5 minutes for the LOX to be denatured, hence there were no significant difference at 10 and 15 minutes due to a possibly complete denaturation of LOX during the heat treatment. Effect of time on LOX for microwave oven and pressure cooker for 3 different varieties are shown in Table 21. There was significant difference between the time for both microwave oven and pressure cooking heat treatment. The effect of microwave and pressure cooker will be further discussed in 4.2.3.2 and 4.2.3.3 respectively.

Table 21 : Effects of time and type of heat treatment on LOX activity of lupin varieties

No	Heat Treatment			LOX activity; mean ± SD		
	Type	Condition	Duration	Belara	Danja	Mandelup
0	OH	0°C	0	105.74 ± 3.04^{l}	98.59 ± 5.37^{q}	154.43 ± 4.10^{o}
1	OH	50°C	5 minutes	95.88 ± 6.26^{k}	91.86 ± 4.11^{op}	122.24 ± 4.86^{n}
2	OH	50°C	10 minutes	93.03 ± 3.09^{k}	90.24 ± 3.88^{o}	123.51 ± 4.88^{n}
3	OH	50°C	15 minutes	94.58 ± 6.88^{k}	91.95 ± 4.42^{op}	120.98 ± 3.33^{mn}
4	OH	60°C	5 minutes	82.33 ± 2.68^{j}	84.11 ± 2.67^{mn}	102.64 ± 7.29^{l}
5	OH	60°C	10 minutes	81.49 ± 2.20^{j}	80.23 ± 1.52^{kl}	101.15 ± 4.80^{kl}
6	OH	60°C	15 minutes	81.94 ± 2.58^{j}	81.47 ± 3.00^{lm}	98.28 ± 2.64^{j}
7	OH	70°C	5 minutes	63.22 ± 3.07^{g}	64.46 ± 1.21^{h}	76.41 ± 2.53^{f}
8	OH	70°C	10 minutes	65.67 ± 2.26^{g}	67.81 ± 3.79^{i}	74.57 ± 6.51^{f}
9	OH	70°C	15 minutes	64.62 ± 2.45^{g}	64.83 ± 2.56^{hi}	75.90 ± 1.72^{f}
10	OH	80°C	5 minutes	36.70 ± 2.63^{cd}	35.94 ± 3.53^{bcd}	44.45 ± 2.20^{b}
11	OH	80°C	10 minutes	34.35 ± 2.47^{bc}	36.83 ± 4.55^{cd}	44.55 ± 2.81^{b}
12	OH	80°C	15 minutes	35.90 ± 1.93^{bcd}	36.87 ± 4.90^{cd}	44.53 ± 2.52^{b}
13	OH	90°C	5 minutes	33.17 ± 2.02^{ab}	33.60 ± 1.47^{ab}	41.32 ± 1.46^{a}
14	OH	90°C	10 minutes	31.33 ± 2.63^{a}	31.85 ± 2.13^{a}	41.16 ± 3.36^{a}
15	OH	90°C	15 minutes	33.73 ± 2.52^{ab}	34.07 ± 2.62^{abc}	39.14 ± 1.94^{a}
16	MW	900 watts	30 seconds	82.45 ± 2.63^{j}	82.92 ± 2.90^{lmn}	98.54 ± 2.74^{jk}
17	MW	900 watts	60 seconds	78.34 ± 4.36^{i}	77.20 ± 3.49^{jk}	85.69 ± 2.01^{h}
18	MW	900 watts	90 seconds	63.81 ± 3.31^{g}	59.75 ± 6.40^{g}	76.86 ± 1.95^{f}
19	MW	900 watts	120 seconds	38.37 ± 2.72^{de}	43.39 ± 6.18^{e}	53.20 ± 2.56^{d}

No	Heat Treatment			LOX activity; mean ± SD		
	Type	Condition	Duration	Belara	Danja	Mandelup
20	MW	900 watts	150 seconds	40.76 ± 3.09^{e}	38.95 ± 2.36^{d}	50.94 ± 1.89^{c}
21	PC	70 KPa	1 minute	83.50 ± 5.62^{j}	84.94 ± 1.90^{n}	96.96 ± 2.54^{j}
22	PC	70 KPa	2 minutes	82.52 ± 2.10^{j}	76.72 ± 2.19^{j}	88.66 ± 2.43^{i}
23	PC	70 KPa	3 minutes	70.30 ± 4.41^{h}	66.02 ± 4.61^{hi}	81.79 ± 2.84^{g}
24	PC	70 KPa	4 minutes	54.96 ± 3.43^{f}	50.35 ± 6.16^{f}	66.06 ± 3.70^{e}
25	PC	70 KPa	5 minutes	38.03 ± 4.25^{de}	42.13 ± 3.50^{e}	52.12 ± 1.63^{cd}

* OH – Oven; MW – Microwave; PC – Pressure Cooker

**Different letters in the same column indicates a significant difference (P <0.05).

***(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

4.2.3 Effect of heat treatment on lupin LOX

There was no significant difference in LOX activity between seed, kernel or flour or heat treatment times (5, 10 and 15 minutes). In this section, the results have been reported combining all three stages and treatment times. However, as the LOX values between the varieties were significantly different, Mandelup was chosen as it has one of the highest LOX activity considering that if the LOX activity in this variety is reduced, it can be expected to reduce the LOX activity in any variety that has a lower level. As previously mentioned in 4.2, using samples with a higher LOX level will show a greater amount of reduction of LOX in heat treatment hence the impact of heat on LOX can be clearly seen. Also, by using higher LOX level, it demonstrates that heat treatment that is sufficient in reducing high LOX levels should be able to have an even greater reduction in lower LOX level.

4.2.3.1 Effect of temperature in oven heat treatment

Table 22 shows the effect of temperature in oven heat treatment on LOX activity. There is a significant decrease in LOX activities with increasing to oven temperature from 50 - 90°C in all three time (Figure 25). The temperature 50°C was chosen as the

minimum cut off point to start recording because according to literature, lupin LOX activity has been reported to start deactivating at 60°C (Najid et al., 1988) although more recent report suggested at 80°C (Yoshie-Stark & Wäsche, 2004). The results from the current study supports the earlier finding by Yoshie-Stark and Wäsche (2004) that lupin LOX activity reduced to 44.51 ± 2.45 when heated at 80°C.

Table 22 : LOX level of heat treated lupin (variety: Mandelup) in oven

No.	Oven temperature (°C)	LOX activity (mean ± SD)	Reduction compared to the control (%)
1	0	153.67 ± 3.08^{a}	0
2	50	122.24 ± 4.41^{b}	20
3	60	100.69 ± 5.43^{c}	34.5
4	70	75.63 ± 4.11^{d}	50.8
5	80	44.51 ± 2.45^{e}	71
6	90	40.54 ± 2.53^{f}	74

*Different letters in the same column indicates a significant difference ($P < 0.05$).

**(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

4.2.3.2 Effect of time in microwave heat treatment

The effect of microwave heating time on lupin LOX is shown in Table 23. Each 30 seconds increase in heating time resulted in a significant reduction in the LOX activity. This is due to the differing heating pattern of conventional oven heats particles from the outside to inside while microwave oven's electromagnetic waves cause internal heating of particles within the microwave cavity, providing distribution of heat from the inside (Malekian et al., 2000). In the current study, heating lupin in microwave resulted in slightly burnt and dry lupin which could result in unappealing taste and texture and hence it was decided not to incorporate it in the product development phase. Also the LOX activity was not reduced to the levels reported in oven heat treatment by microwaving. Figure 26 also shows the decreasing pattern of lupin LOX with increasing time for all three varieties.

To date, there are no published studies on the effect of microwave heating on LOX activity in lupin however there was a study done in 1999 on microwave heating on LOX activity in rice bran during storage (Ramezanzadeh, Rao, Windhauser, Prinyawiwatkul, & Marshall, 1999). There was no reduction in LOX activity in the initial reading after the microwave heating and the heated samples were packed and stored and LOX activity were measured in a four-week interval. Reduction in LOX activity was shown after 12 weeks in storage and there were no reports of burnt spot and dried sample. This study differs with the current study due to the samples were added water to increase moisture content from 7% to 21%, unlike the samples used in the current study was not added water to. According to Zhou, Puri, Anantheswaran, and Yeh (1995), the microwave power absorption in food samples were largely because of the presence of water molecules. Hence the possible reason as to why the 1999 study added water to increase moisture. However, unlike the current study, the 1999 did not see a reduction in LOX activity after microwave heating until 12 weeks later. This showed that adding water to sample could cause the microwave heating to be ineffective in reducing LOX activity unlike the current study.

Table 23 : LOX level of heat treated lupin (variety: Mandelup) in microwave oven (900 watts)

No.	Time (Seconds)	LOX activity (mean ± SD)	Reduction compared to the control (%)
1	0 (control)	154.03 ± 4.00^{a}	0
2	30	98.54 ± 2.74^{b}	36
3	60	85.69 ± 2.01^{c}	44
4	90	76.86 ± 1.95^{d}	50
5	120	53.20 ± 2.56^{e}	65
6	150	50.94 ± 1.89^{f}	66.9

*Different letters in the same column indicates a significant difference ($P < 0.05$).**(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

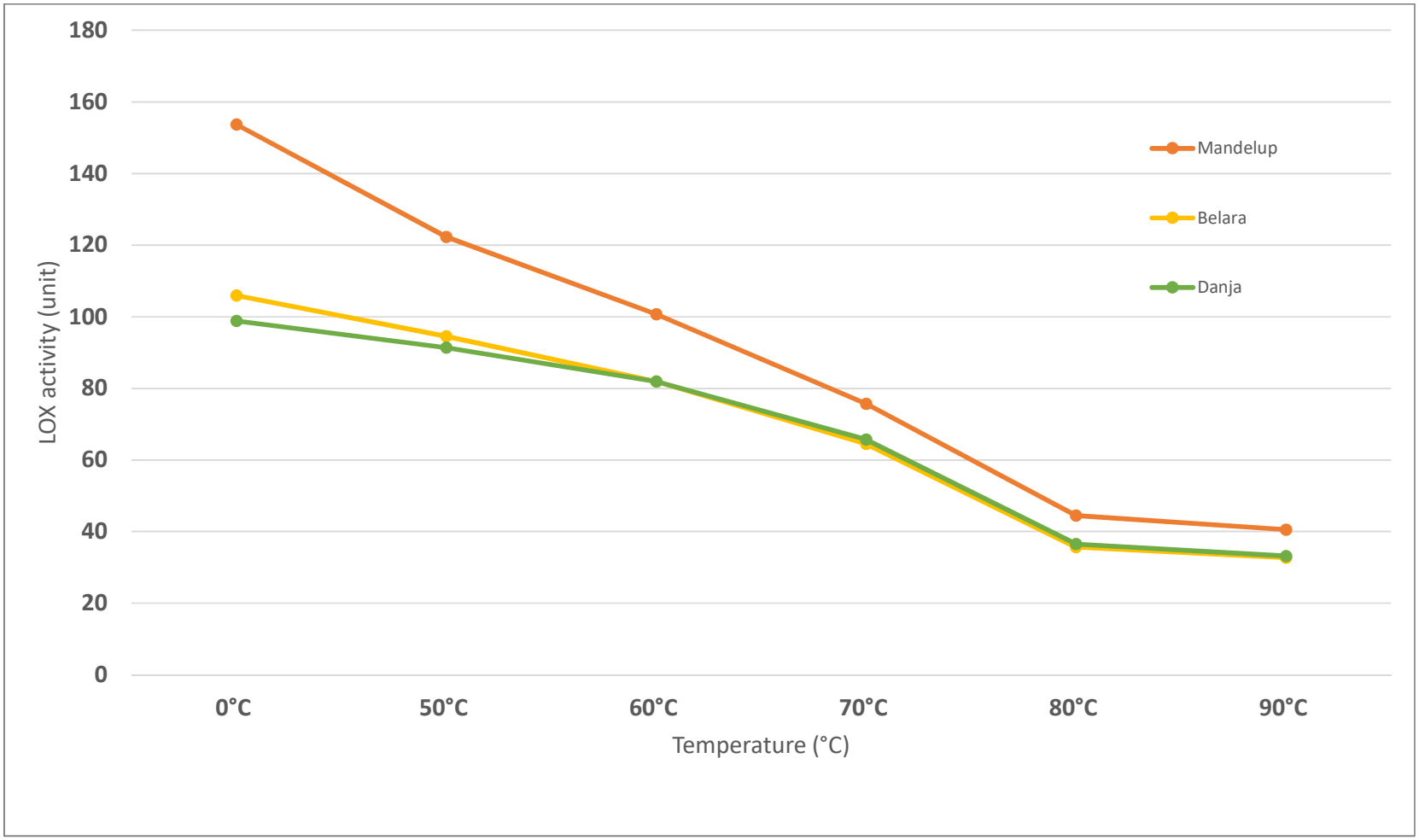

Figure 25 : Effect of temperature on LOX activity in oven heat treatment

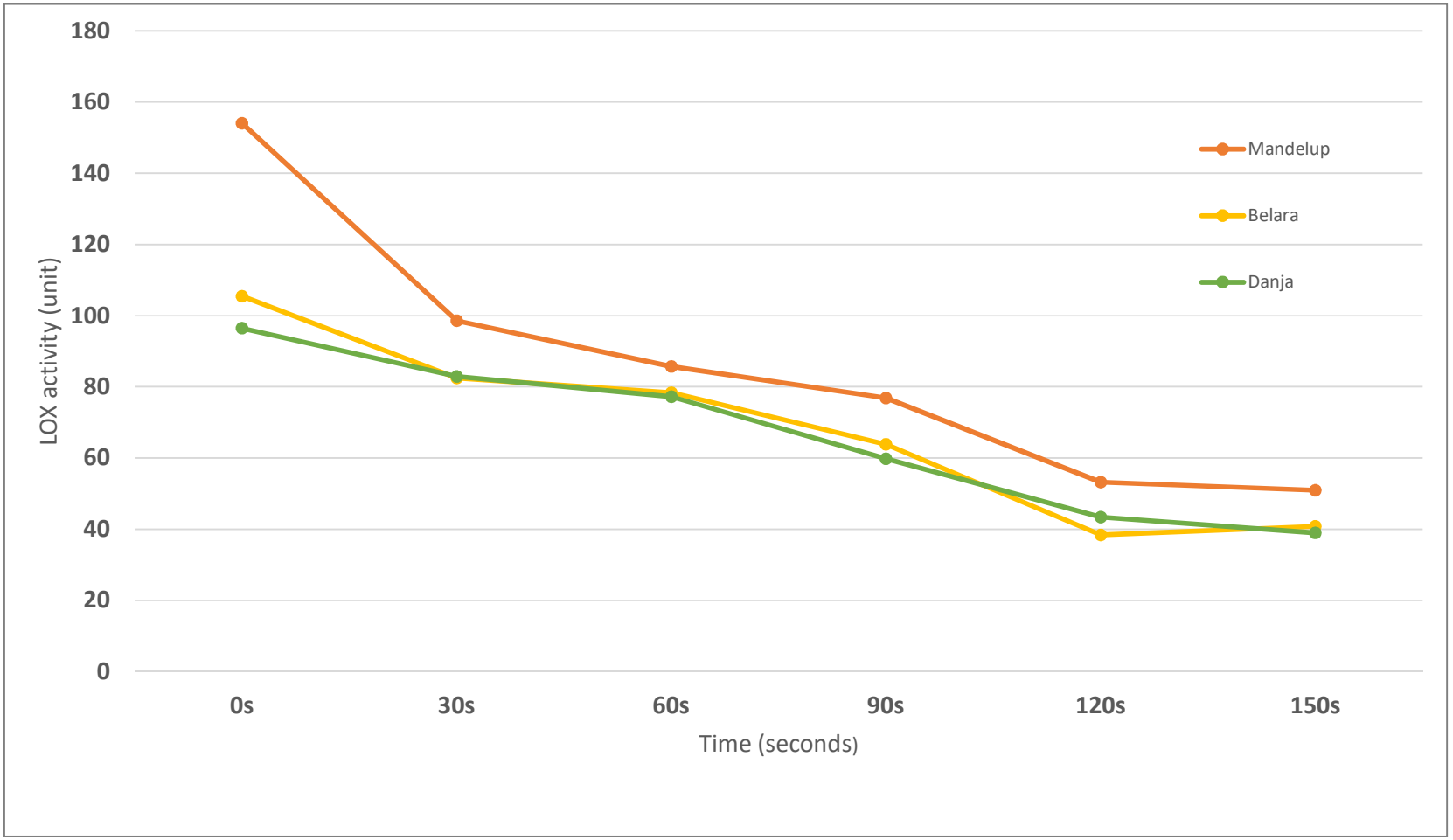

Figure 26 : Effect of microwave heat treatment on LOX activity

4.2.3.3 Effect of pressure cooking time

Table 24 shows the effect of pressure cooking time on lupin LOX activity. There is a significant decrease in the LOX activity of lupin with the increase in time of pressure cooking. To date, there are no published data on the effect of pressure cooker heat treatment on LOX activity in lupin. Heating lupin in pressure cooker resulted in a slightly moist lupin which could give an unappealing texture, therefore it was decided not to incorporate in the product development. Figure 27 also shows the decreasing pattern of lupin LOX with increasing time for all three varieties.

Table 24 : Effects of pressure cooking time on LOX level (variety: Mandelup)

No.	Time (minutes)	LOX activity (mean ± SD)	Reduction compared to the control (%)
1	0	155.58 ± 5.08^{a}	0
2	1	96.96 ± 2.54^{b}	38
3	2	88.66 ± 2.43^{c}	43
4	3	81.79 ± 2.84^{d}	47
5	4	66.06 ± 3.70^{e}	58
6	5	52.12 ± 1.63^{f}	66.5

*Different letters in the same column indicates a significant difference ($P < 0.05$).

**(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

From the three different heat treatments studied, oven heat treatment was selected for the next stage of the study of food applications due to several factors. Oven heat treated flour had the lowest LOX activity at 80

°C

among all treatments. Heat treatment using oven also did not cause burnt spots in the lupin flour or develop undesirable texture. Conventional ovens are also readily available in most food plants and lupin flour can be heat treated in bulk with minimum investment.

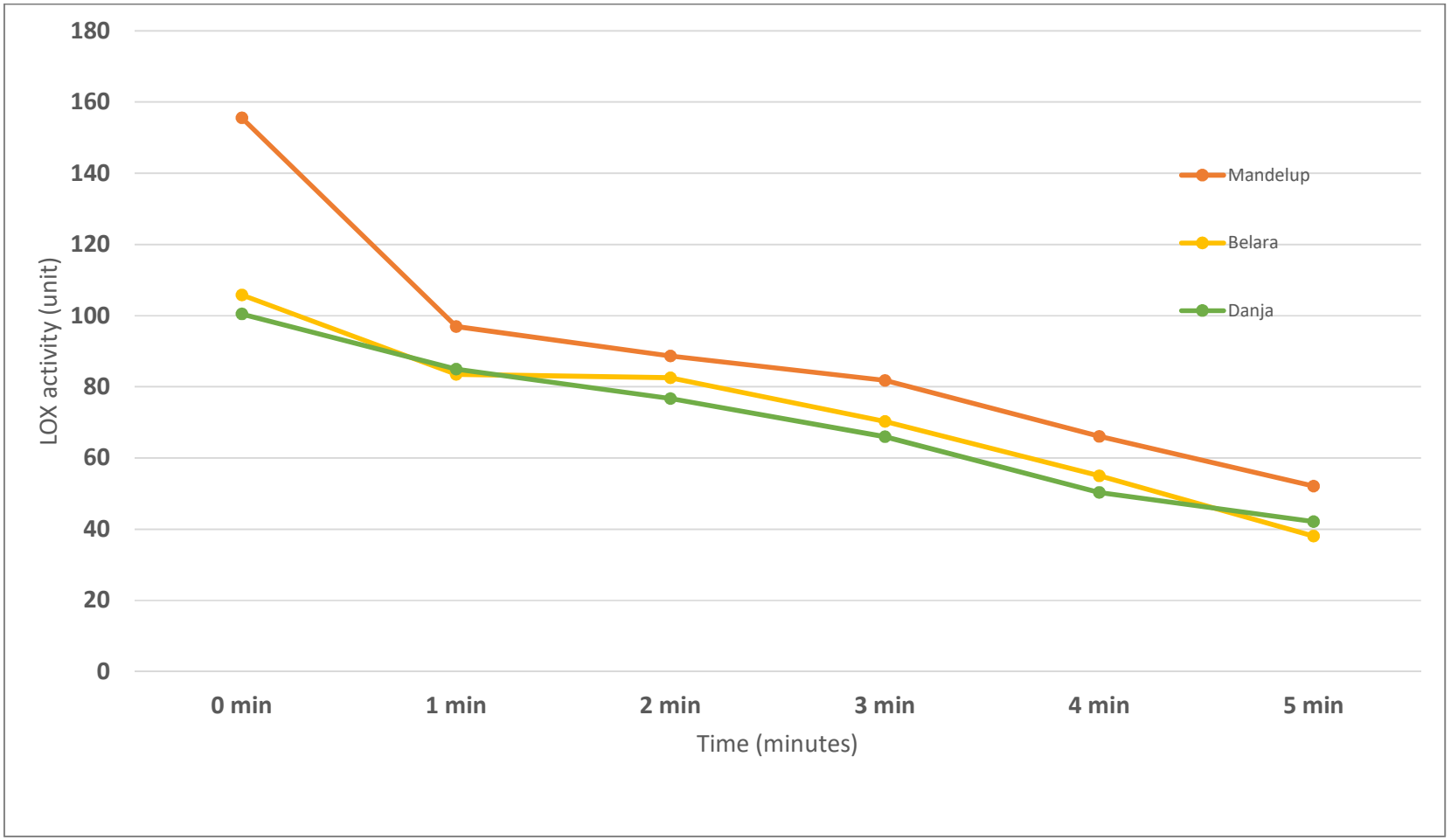

Figure 27 : Effect of pressure cooking time on LOX activity

4.2.4 Effect of heat treatment on colour

Colour measurement of the 25 heat treatment combinations between different processing stages (seed, kernel and flour) are shown in Table 25 for the value L* (lightness, extending from 0 (black) to 100 (white), Table 26 for the value a* (redness, positive value is red and negative value is green) and Table 27 for the value b* (yellowness, positive value is yellow and negative value is blue). Values are means ± standard deviations of the 3 replicates.

The different heat treatments did not have a significant impact on the colour (L*, a* and b* values) of the lupin seed, kernel and flour, indicating that any of the tested heating methods can be used to inactivate LOX in lupin with no effect on the final food product. (Table 25, Table 26 and Table 27). Varieties with high levels of LOX had higher yellow readings while lower LOX varieties had lower yellow readings. This shows that heat treatment does not affect the colour of lupin even though there were changes in LOX. It can be concluded that although there was correlation between the colour and activity of LOX in its natural form found in lupin, the colour does not change even though the heat changed the LOX activity of the lupin.

Table 25 : Effect of heat treatment combinations on colour of lupin (L*) between different stages

No	Heat Treatment			L* value		
	Type	Condition	Duration	Seed	Kernel	Flour
0	OH	0°C	0	89.17 ± 0.52^{a}	89.01 ± 0.62^{a}	89.15 ± 0.53^{a}
1	OH	50°C	5 minutes	89.03 ± 0.53^{a}	89.22 ± 0.71^{a}	89.45 ± 0.32^{a}
2	OH	50°C	10 minutes	89.32 ± 0.51^{a}	89.30 ± 0.38^{a}	88.96 ± 0.40^{b}
3	OH	50°C	15 minutes	89.07 ± 0.35^{a}	89.09 ± 0.29^{a}	89.31 ± 0.31^{a}
4	OH	60°C	5 minutes	88.86 ± 0.52^{a}	89.68 ± 0.32^{b}	89.48 ± 0.28^{b}
5	OH	60°C	10 minutes	89.42 ± 0.42^{a}	89.47 ± 0.52^{a}	89.04 ± 0.31^{b}
6	OH	60°C	15 minutes	89.05 ± 0.31^{a}	89.26 ± 0.52^{a}	88.64 ± 1.60^{a}
7	OH	70°C	5 minutes	89.31 ± 0.51^{a}	89.48 ± 0.38^{a}	88.79 ± 0.24^{b}
8	OH	70°C	10 minutes	88.92 ± 0.60^{a}	89.26 ± 0.53^{a}	88.88 ± 0.55^{a}
9	OH	70°C	15 minutes	89.07 ± 0.51^{a}	88.87 ± 0.58^{a}	88.76 ± 0.53

No	Heat Treatment			L* value		
	Type	Condition	Duration	Seed	Kernel	Flour
10	OH	80°C	5 minutes	89.10 ± 0.35	88.90 ± 0.56	88.82 ± 0.52
11	OH	80°C	10 minutes	89.06 ± 0.39[a]	89.05 ± 0.42[a]	88.64 ± 0.36[b]
12	OH	80°C	15 minutes	88.93 ± 0.42[a]	88.97 ± 0.60[a]	88.72 ± 0.39[a]
13	OH	90°C	5 minutes	89.16 ± 0.18[a]	88.99 ± 0.49[a]	88.95 ± 0.80[a]
14	OH	90°C	10 minutes	89.13 ± 0.36[a]	89.18 ± 0.40[a]	89.05 ± 0.33[a]
15	OH	90°C	15 minutes	88.93 ± 0.32[a]	88.69 ± 0.52[a]	88.54 ± 0.50[a]
16	MW	900 watts	30 seconds	89.43 ± 0.20[a]	89.36 ± 0.16[a]	89.44 ± 0.21[a]
17	MW	900 watts	60 seconds	89.33 ± 0.36[a]	89.41 ± 0.17[a]	89.32 ± 0.35[a]
18	MW	900 watts	90 seconds	89.14 ± 0.45[a]	88.89 ± 0.38[a]	89.10 ± 0.45[a]
19	MW	900 watts	120 seconds	88.84 ± 0.38[a]	88.55 ± 0.28[b]	88.97 ± 0.45[a]
20	MW	900 watts	150 seconds	89.09 ± 0.35[a]	89.30 ± 0.29[a]	89.22 ± 0.23[a]
21	PC	70 KPa	1 minute	89.24 ± 0.55[a]	89.20 ± 0.28[a]	89.04 ± 0.47[a]
22	PC	70 KPa	2 minutes	88.92 ± 0.46[a]	89.13 ± 0.46[a]	89.25 ± 0.32[a]
23	PC	70 KPa	3 minutes	88.87 ± 0.46[a]	88.73 ± 0.40[a]	89.43 ± 0.32[b]
24	PC	70 KPa	4 minutes	88.85 ± 0.51[a]	88.92 ± 0.52[a]	89.42 ± 0.24[b]
25	PC	70 KPa	5 minutes	88.83 ± 0.47[a]	89.05 ± 0.43[a]	89.06 ± 0.49[a]

* OH – Oven; MW – Microwave; PC – Pressure Cooker

**Different letters in the same row indicates a significant difference (P <0.05).

***No significant difference between the types of heat treatments (P>0.05)

***(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

Table 26 : Effect of heat treatment combinations on colour of lupin (a*) between different stages

No	Heat Treatment			a* value		
	Type	Condition	Duration	Seed	Kernel	Flour
0	OH	0°C	0	-0.04 ± 0.40[a]	-0.06 ± 0.34[a]	-0.18 ± 0.30[a]
1	OH	50°C	5 minutes	-0.06 ± 0.31[a]	-0.02 ± 0.38[a]	-0.31 ± 0.20[a]
2	OH	50°C	10 minutes	-0.25 ± 0.27[a]	-0.10 ± 0.24[a]	-0.07 ± 0.17[a]
3	OH	50°C	15 minutes	0.02 ± 0.22[a]	0.10 ± 0.23[a]	-0.21 ± 0.20[b]
4	OH	60°C	5 minutes	0.04 ± 0.20[a]	-0.33 ± 0.25[b]	-0.24 ± 0.15[b]
5	OH	60°C	10 minutes	-0.24 ± 0.24[a]	-0.13 ± 0.33[a]	-0.13 ± 0.24[a]
6	OH	60°C	15 minutes	-0.12 ± 0.31[a]	0.13 ± 0.32[b]	-0.12 ± 0.31[c]

No	Heat Treatment			a* value		
	Type	Condition	Duration	Seed	Kernel	Flour
7	OH	70°C	5 minutes	-0.16 ± 0.41[a]	-0.15 ± 0.32[a]	-0.03 ± 0.25[a]
8	OH	70°C	10 minutes	-0.18 ± 0.23[a]	-0.01 ± 0.48[a]	-0.11 ± 0.19[a]
9	OH	70°C	15 minutes	-0.16 ± 0.28[a]	0.17 ± 0.46[b]	-0.09 ± 0.17[a]
10	OH	80°C	5 minutes	-0.15 ± 0.28[a]	0.22 ± 0.32[b]	-0.03 ± 0.17[a]
11	OH	80°C	10 minutes	-0.16 ± 0.28[a]	0.10 ± 0.26[b]	-0.01 ± 0.25[a]
12	OH	80°C	15 minutes	-0.07 ± 0.21[a]	0.18 ± 0.28[b]	-0.10 ± 0.20[a]
13	OH	90°C	5 minutes	-0.21 ± 0.18[a]	0.12 ± 0.31[b]	-0.16 ± 0.26[a]
14	OH	90°C	10 minutes	-0.15 ± 0.27[a]	-0.08 ± 0.29[a]	-0.30 ± 0.19[a]
15	OH	90°C	15 minutes	-0.14 ± 0.21[a]	0.26 ± 0.27[b]	-0.15 ± 0.27[a]
16	MW	900 watts	30 seconds	-0.24 ± 0.34[a]	-0.24 ± 0.32[a]	0.01 ± 0.37[a]
17	MW	900 watts	60 seconds	-0.21 ± 0.30[a]	-0.06 ± 0.38[a]	-0.27 ± 0.25[a]
18	MW	900 watts	90 seconds	-0.09 ± 0.31[a]	-0.25 ± 0.31[a]	-0.09 ± 0.36[a]
19	MW	900 watts	120 seconds	-0.15 ± 0.30[a]	-0.19 ± 0.31[a]	-0.20 ± 0.29[a]
20	MW	900 watts	150 seconds	-0.16 ± 0.31[a]	-0.11 ± 0.35[a]	-0.09 ± 0.36[a]
21	PC	70 KPa	1 minute	0.09 ± 0.40[a]	-0.04 ± 0.37[a]	-0.23 ± 0.31[a]
22	PC	70 KPa	2 minutes	-0.25 ± 0.27[a]	-0.13 ± 0.35[a]	-0.07 ± 0.41[a]
23	PC	70 KPa	3 minutes	-0.09 ± 0.36[a]	-0.18 ± 0.33[a]	-0.15 ± 0.36[a]
24	PC	70 KPa	4 minutes	0.09 ± 0.33[a]	-0.11 ± 0.32[a]	-0.15 ± 0.31[a]
25	PC	70 KPa	5 minutes	-0.18 ± 0.36[a]	-0.22 ± 0.36[a]	-0.04 ± 0.33[a]

* OH – Oven; MW – Microwave; PC – Pressure Cooker

**Different letters in the same row indicates a significant difference (P <0.05).

***No significant difference between the types of heat treatments (P>0.05)

***(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

Table 27 : Effect of heat treatment combinations on colour of lupin (b*) between different stages

No	Heat Treatment			b* value		
	Type	Condition	Duration	Seed	Kernel	Flour
0	OH	0°C	0	31.21 ± 0.91[a]	31.06 ± 0.96[a]	31.17 ± 0.90[a]
1	OH	50°C	5 minutes	31.62 ± 0.58[a]	30.65 ± 0.73[b]	31.51 ± 0.23[a]
2	OH	50°C	10 minutes	30.84 ± 0.68[a]	30.57 ± 0.72[a]	32.00 ± 0.80[b]
3	OH	50°C	15 minutes	31.13 ± 0.38[a]	30.87 ± 0.77[a]	31.79 ± 0.56[b]
4	OH	60°C	5 minutes	31.48 ± 0.58[a]	30.45 ± 0.52[b]	31.38 ± 0.71[a]

No	Heat Treatment			b* value		
	Type	Condition	Duration	Seed	Kernel	Flour
5	OH	60°C	10 minutes	31.10 ± 0.65[a]	30.38 ± 0.85[b]	32.28 ± 0.60[c]
6	OH	60°C	15 minutes	31.51 ± 0.52[a]	30.71 ± 0.60[b]	32.58 ± 0.71[c]
7	OH	70°C	5 minutes	31.24 ± 0.74[a]	30.74 ± 0.89[b]	32.86 ± 0.47[c]
8	OH	70°C	10 minutes	31.45 ± 0.73[a]	30.81 ± 0.97[b]	32.25 ± 0.54[c]
9	OH	70°C	15 minutes	31.44 ± 0.50[a]	31.18 ± 1.02[a]	32.80 ± 0.53[b]
10	OH	80°C	5 minutes	31.48 ± 0.57[a]	31.15 ± 0.91[a]	32.64 ± 0.66[b]
11	OH	80°C	10 minutes	31.43 ± 0.81[a]	30.78 ± 0.80[a]	32.47 ± 2.30[b]
12	OH	80°C	15 minutes	31.38 ± 0.78[a]	30.90 ± 0.77[b]	32.96 ± 0.49[c]
13	OH	90°C	5 minutes	31.49 ± 0.29[a]	31.05 ± 1.01[b]	32.17 ± 0.39[c]
14	OH	90°C	10 minutes	31.07 ± 0.73[a]	30.94 ± 1.04[a]	32.03 ± 0.67[b]
15	OH	90°C	15 minutes	31.09 ± 0.44[a]	31.36 ± 0.91[a]	33.23 ± 1.16[b]
16	MW	900 watts	30 seconds	30.88 ± 0.66[a]	30.85 ± 0.73[a]	30.76 ± 0.76[a]
17	MW	900 watts	60 seconds	31.15 ± 0.79[a]	31.14 ± 0.76[a]	31.12 ± 0.79[a]
18	MW	900 watts	90 seconds	31.43 ± 0.61[a]	31.42 ± 0.52[a]	31.42 ± 0.57[a]
19	MW	900 watts	120 seconds	31.32 ± 0.76[a]	31.35 ± 0.79[a]	31.32 ± 0.82[a]
20	MW	900 watts	150 seconds	31.42 ± 0.22[a]	31.43 ± 0.20[a]	31.47 ± 0.27[a]
21	PC	70 KPa	1 minute	30.78 ± 0.83[a]	30.57 ± 0.79[a]	30.52 ± 0.82[a]
22	PC	70 KPa	2 minutes	31.49 ± 0.51[a]	31.44 ± 0.51[a]	31.53 ± 0.39[a]
23	PC	70 KPa	3 minutes	31.53 ± 0.67[a]	31.65 ± 0.64[a]	31.56 ± 0.66[a]
24	PC	70 KPa	4 minutes	31.33 ± 0.80[a]	31.30 ± 0.81[a]	31.34 ± 0.82[a]
25	PC	70 KPa	5 minutes	31.07 ± 0.60[a]	31.08 ± 0.44[a]	31.06 ± 0.51[a]

* OH – Oven; MW – Microwave; PC – Pressure Cooker

**Different letters in the same row indicates a significant difference (P <0.05).

***No significant difference between the types of heat treatments (P>0.05)

***(Unit: One unit of LOX represents an increase in absorbance at 234nm of one unit of absorbance per minute, per gram of lupin on dry basis)

4.3 Sensory evaluation heat treated Lupin flour in Chapatti

Based on the results, Mandelup variety from the year 2011 was chosen due to being the most commercially grown and has one of the highest LOX activity. The reason for choosing a high LOX activity variety was it was expected that the beany flavour would be very high. Hence, if the heat treatment was able to reduce the LOX activity in Mandelup and incorporated into chapatti with good sensory acceptability, this shows

that the other 24 varieties with a lower LOX activity than Mandelup can surely be acceptable to be used in food application. The processing stage chosen is kernel since there was no significant difference between seed, kernel and flour. Heat treating of kernel is easier and more cost effective than heat treating lupin flour. Oven heat treatment was chosen due to its efficient LOX lowering effect. Heat treated lupin was incorporated into chapatti at 20%, 30% and 40% respectively.

The objective was to gauge acceptability especially in terms of taste which in this case, the "beany" flavour, to determine if there is any correlation between beany flavour and the LOX activity that could impact product acceptability. Chapatti is a simple product with minimum ingredient that would not mask the lupin flavour, hence its choice for the sensory evaluation (Figure 28) Baked product such as muffins with added ingredients such as sugar or flavour compounds were deemed unsuitable for this type of experiment given the objective in mind.

Figure 28 : Image of 20% heat treated lupin chapatti (left) and normal chapatti made of 100% Atta flour (right)

Both heat treated and non-heat treated lupin flour chapatti samples from Figure 28, had a yellower appearance, which was as expected given that lupin has a distinct yellow colour. However, between lupin chapatti samples of 20%, 30% and 40% heat treated lupin chapatti and non-heat treated lupin chapatti respectively, they were initially observed by the author to be of similar yellow colour. Hence it was imperative

to measure the colour and texture and also the sensory acceptability of the samples to see if there were significant differences between them.

4.3.1 Sensory evaluation of chapatti samples

Sensory evaluation of lupin chapatti samples was conducted with 60 panellists randomly recruited for the study (as per the requirement of the standard ISO 8586:2012) (ISO, 2012). The panellists' demographic data were collected beforehand to get a better understanding of the panellists' background and the possibility of their background impacting the results of this particular sensory evaluation. In a study done by Torres-Penaranda et al. (1998) on sensory evaluation of lipoxygenase free and normal soy products, panellists from United States, Japan and China had significant differences when rating the acceptability of the beany flavour in the products. The demographic data of panellists who attended the sensory evaluation are shown in Figure 29, Figure 30 and Figure 31 respectively. The demographic questions consisted of the panels' gender, their age, their descent which is listed as origin/nationality and whether they were vegetarian or non-vegetarian (Appendix C for the questionnaire).

Figure 29 shows the gender of panellists who participated in the sensory evaluation. The majority of the panellists were female (76.7%) compared to only 23.3% male. In a sensory study involving hedonic scale done by Cristovam, Russell, Paterson, and Reid (2000), there was a significant difference between the type of coffee rated and preferred between different genders. With the high percentage of women in this study, there is a possibility that results could be different if the gender of the panellists were balanced.

Age distribution of panellists are shown in Figure 30. The majority of the panellists were between the age of 20 – 29 years (60%). This is expected as the sensory evaluation study was done at a university where the majority of the panellists were students and were of this particular age group.

Figure 31 shows the origin/nationality of the panellists. The majority of the panellists were of Malay/Indonesian descent (40%) followed by Chinese/Asian (18%) and Indian/South Asian (17%) respectively. This totals to 75% Asians that became the

panellists. There weren't many Caucasians attending the sensory evaluation which might be due to the product not being a staple food in the Western society. Chapatti is commonly consumed in the Asian subcontinent, being an Indian food, and thus attracts more Indian/South Asian and Malay/Indonesian panellists, where those of Indian decent has taken up residency and has an influence on the regions' food.

In a study done by Yeh et al. (1998) on the use of 9 point hedonic scale on Americans, Thai, Chinese and Korean, there were a significant difference on how the panellists from different nationality respond to the hedonic scale. The hedonic scale was mostly used in the middle scale for the ethnic groups as compared to the Americans. This could be due to the cultural behaviour of being polite and not producing negative responding and to avoid extreme responding. Since the panellists are mostly Asians in the current study (75%), and the hedonic scale results are mostly in the middle score, there is a possibility of this. However, given that the product itself is an Asian (South and South East Asian) staple, with the study being aimed towards a greater Asian population, hence the results of this study should be acceptable. Several measures have been taken in this current study to control all confounding factors that might possibly affect the result of study to represent the overall acceptability of the general population. Such controls included detailed explanations to the panellists as to how to answer the questionnaire, to be honest, including giving negative feedback as it would be helpful and that there is no right or wrong answer.

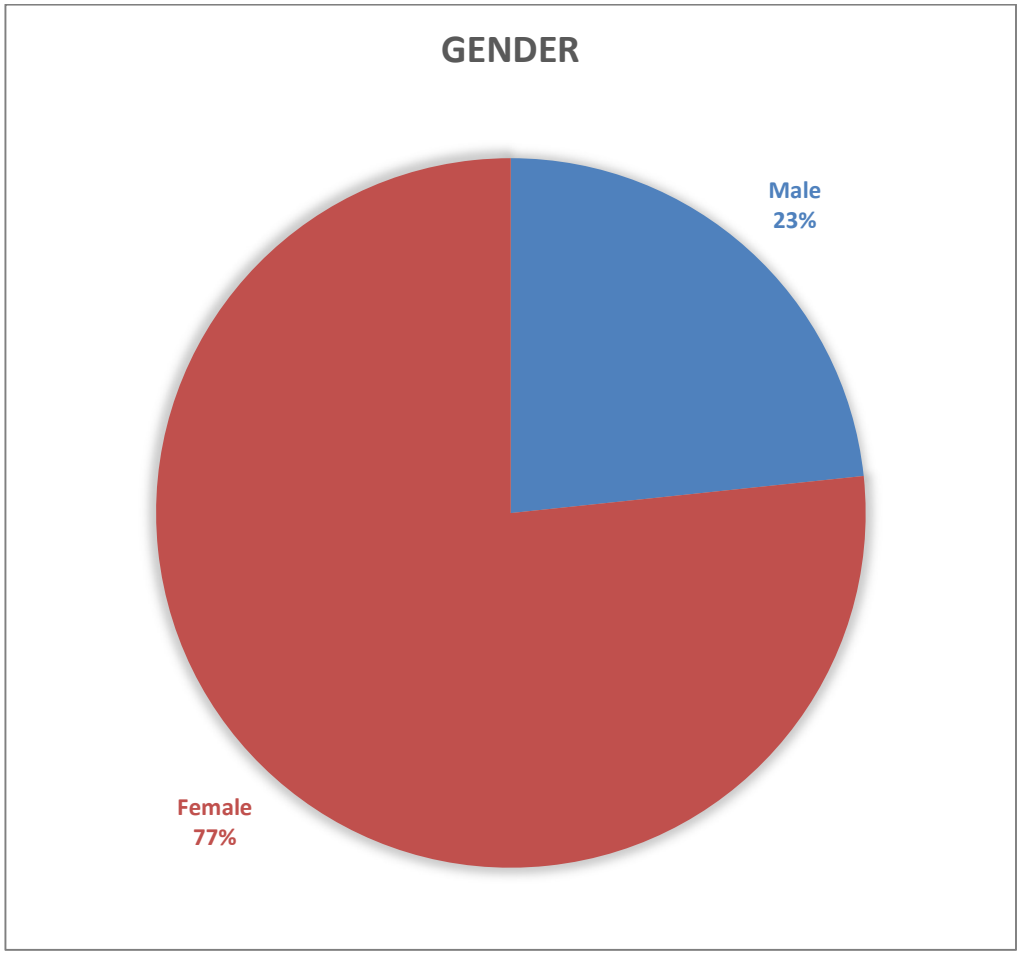

Figure 29 : Gender of panellist

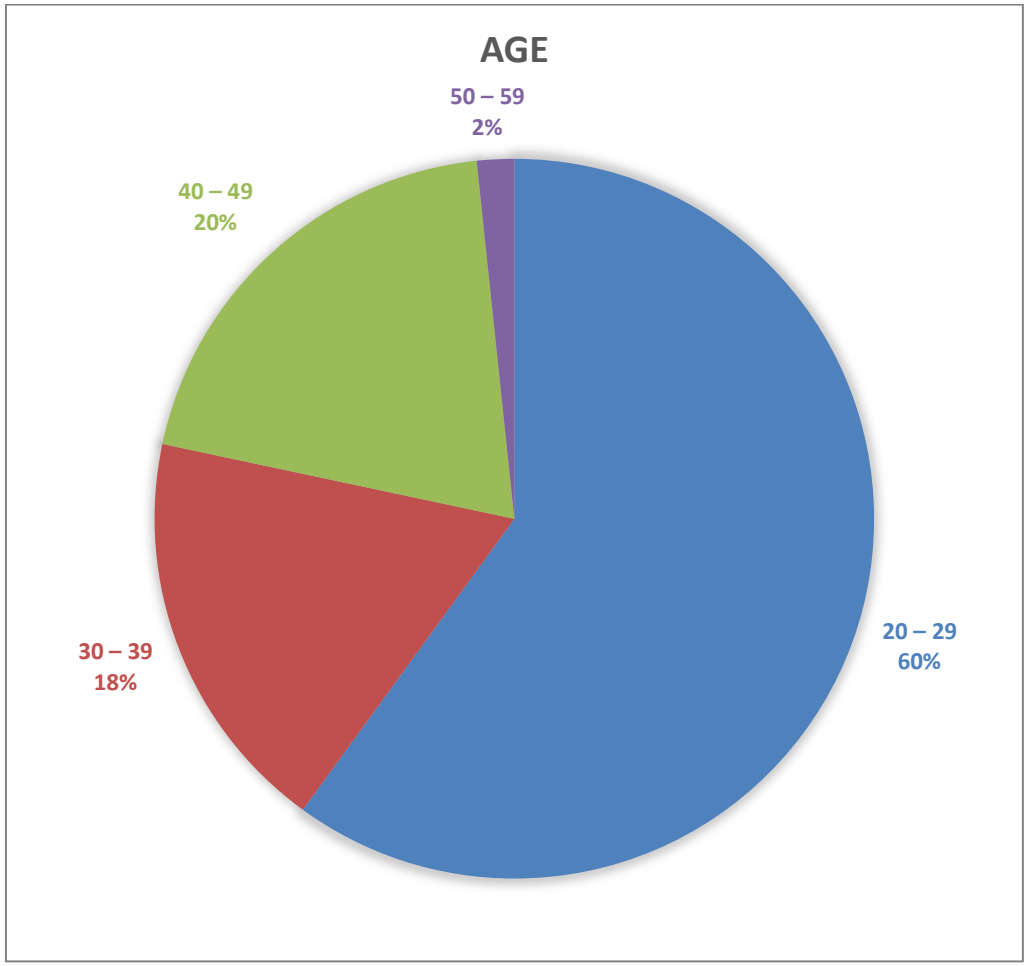

Figure 30 : Panellist age distribution

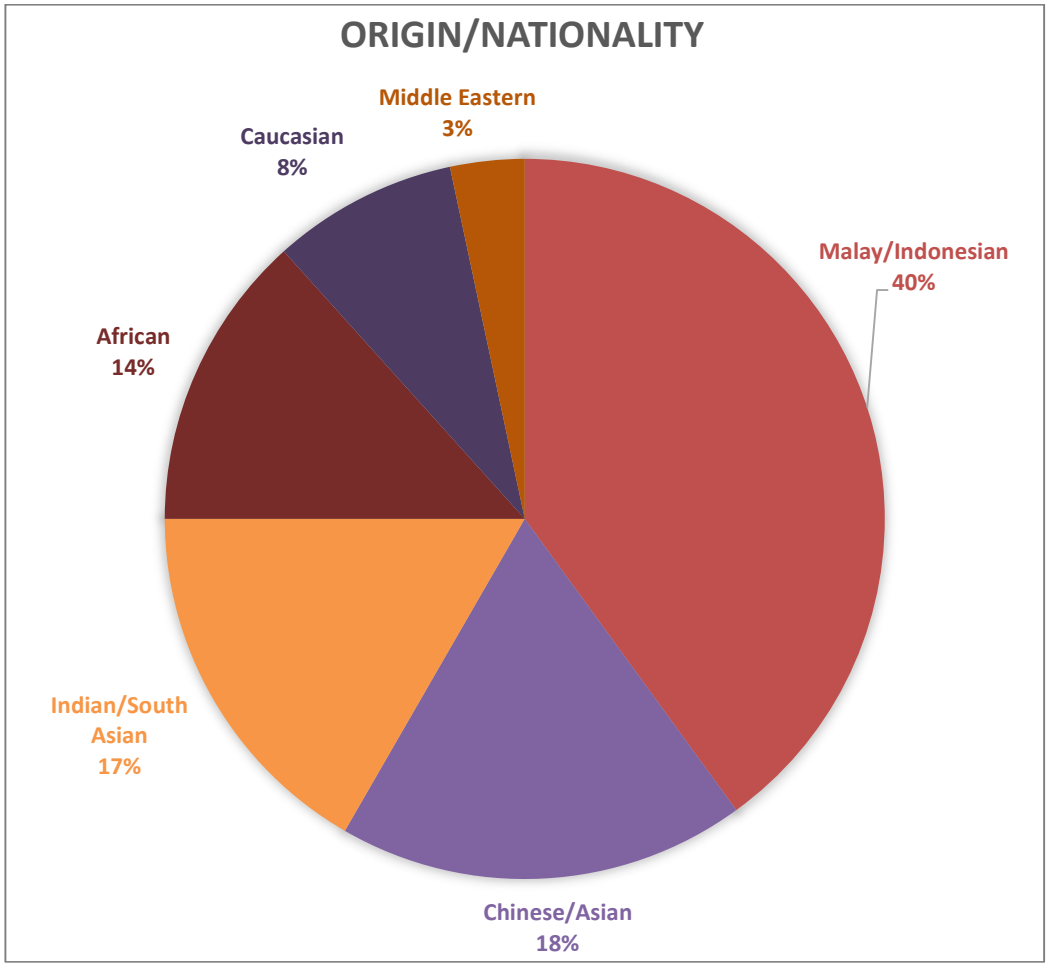

Figure 31 : Origin/nationality of panellist

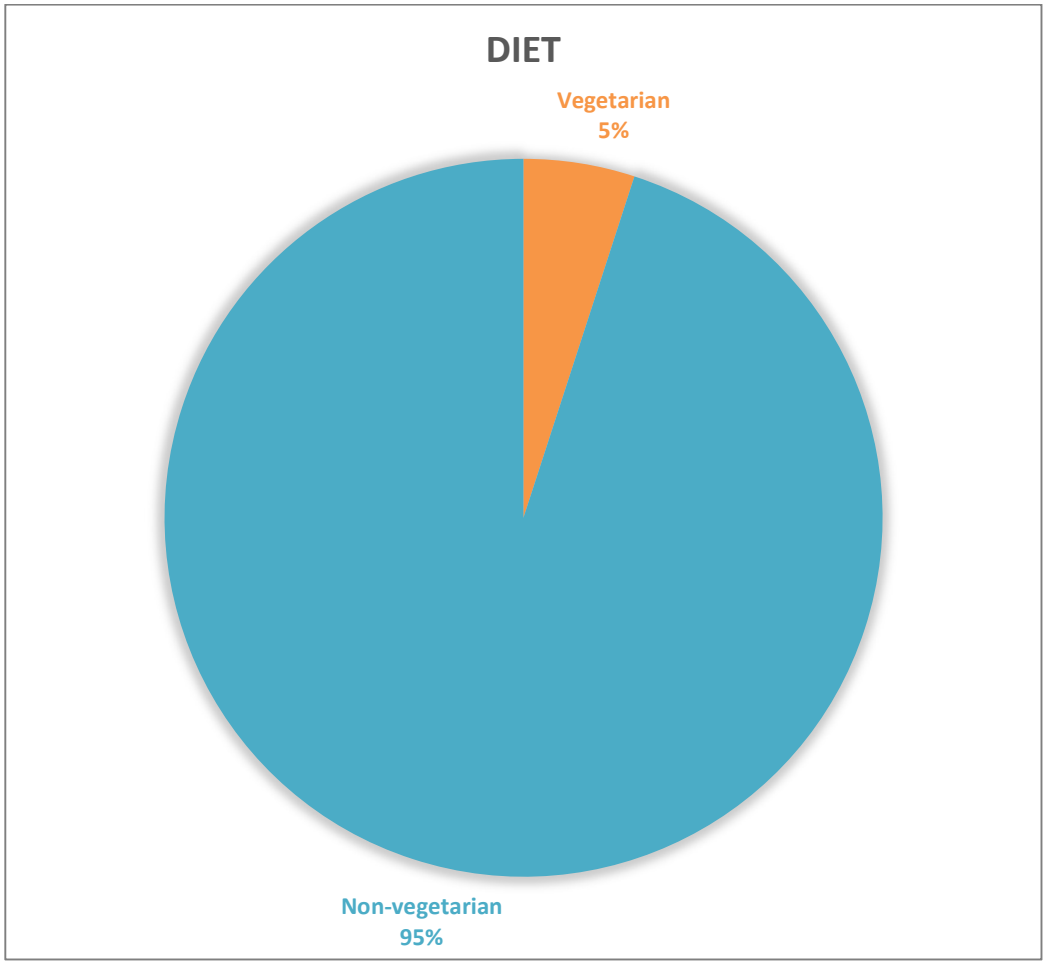

Figure 32 : Diet of panellist

As shown in Figure 32, the majority of the panellists were non vegetarian, which should not affect the results as the product is normally consumed by vegetarian and non-vegetarians alike. Appendix D shows the two questionnaires that the panellists were asked to use in evaluating the samples. Question 1 was designed to determine the frequency of the participants' chapatti consumption. There were 41.7% panellists that ate chapatti at least once a week and 61.7% at chapatti at least once a month which indicate that the majority of the panellists have eaten chapatti quite frequently and were aware of the required characteristics of the products (Figure 33).

Question 2 was designed to determine whether the chapatti consumed was eaten as it was traditionally consumed, which was with a vegetable or meat/fish dish and the majority (95%) did consume it in the traditional manner (Figure 34). In the sensory evaluation, the chapatti was served in the traditional manner.

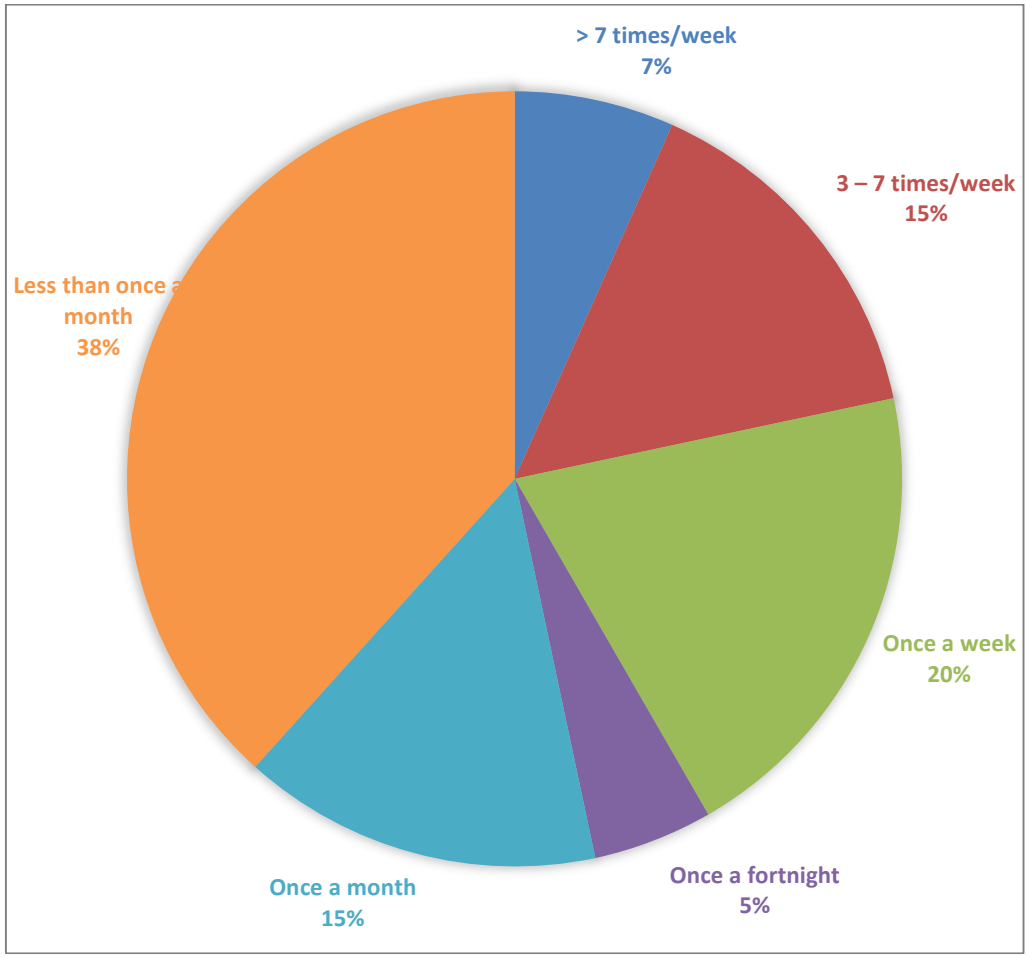

Figure 33 : Frequency of chapatti consumption in panellist

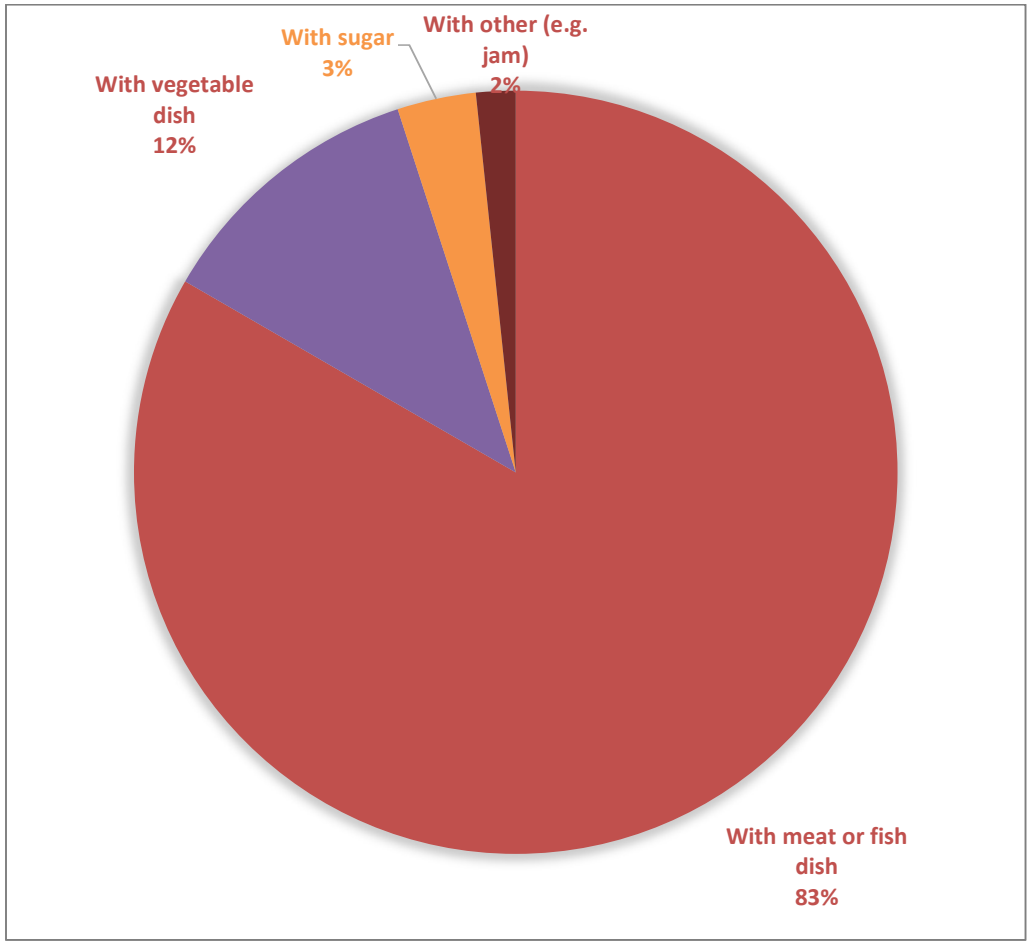

Figure 34 : Pattern of chapatti consumption in panelist

Table 28: Panellist sensory acceptability of non-heat treated and heat - treated lupin flour incorporated chapatti (N = 60)

Lupin (%)	Heat treatment	Appearance	Colour	Texture	Flavour	Overall
0 (Control)	No	6.17 ± 1.96^{a}	6.17 ± 1.84^{a}	6.22 ± 1.88^{c}	6.07 ± 1.89^{bc}	6.22 ± 1.78^{ab}
20	No	5.98 ± 1.72^{a}	6.30 ± 1.50^{a}	5.70 ± 1.96^{abc}	6.08 ± 1.85^{bc}	6.08 ± 1.76^{ab}
30	No	5.98 ± 1.36^{a}	6.23 ± 1.28^{a}	6.00 ± 1.41^{bc}	6.02 ± 1.66^{abc}	6.18 ± 1.32^{ab}
40	No	5.72 ± 1.55^{a}	5.70 ± 1.53^{a}	5.63 ± 1.65^{ab}	5.93 ± 1.73^{abc}	5.85 ± 1.64^{a}
20	Yes	6.27 ± 1.41^{a}	6.40 ± 1.45^{a}	6.13 ± 1.44^{bc}	6.55 ± 1.48^{c}	6.67 ± 1.30^{b}
30	Yes	5.75 ± 1.54^{a}	5.85 ± 1.40^{a}	5.50 ± 1.67^{ab}	5.82 ± 1.67^{ab}	5.87 ± 1.43^{a}
40	Yes	5.60 ± 1.53^{a}	6.02 ± 1.44^{a}	5.33 ± 1.81^{a}	5.30 ± 2.02^{a}	5.63 ± 1.75^{a}

*Different letters in the same row indicates a significant difference ($P < 0.05$).

Table 28 shows the results of sensory acceptability of non-heat treated and heat treated lupin flour incorporated chapatti. Samples were evaluated for their appearance, colour, texture, flavour and overall acceptability. Chapatti samples were evaluated with a number scale ranging from 1 (dislike extremely) to 9 (like extremely). The panellists scores for overall acceptability for all samples ranged from 5 (neither like nor dislike) to 6 (like slightly) (Figure 35). Overall acceptability of 20% heat – treated lupin (6.67 ± 1.30) were significantly higher than 40% non – heat treated lupin (5.85 ± 1.64), 30% heat – treated lupin (5.87 ± 1.43) and 40% heat – treated lupin (5.63 ± 1.75) respectively. However, there were no significant difference between 20% heat – treated lupin and control sample and other non – heat treated samples. In term of comparing control chapatti sample and all lupin incorporated samples, there were no significant difference in overall acceptability, hence it can be concluded that lupin can be incorporated into chapatti up to 40% without affecting its overall acceptability.

In terms of appearance, there were no significant difference between the control and lupin incorporated chapatti samples. It can be concluded that heat treatment does not affect the appearance acceptability of the chapatti samples.

There was no significant difference in colour between the samples. This shows that despite the more distinct yellow colour (Table 28) of the lupin incorporated chapatti compared to control chapatti, it was still acceptable and appeal to the panellists. There have been previous sensory evaluation studies on lupin incorporated food products that have reported on lupin's yellow colour in food that reported increase in colour acceptability score in noodles (Jayasena, Leung, & Nasar-Abbas, 2010), tofu (Jayasena, Khu, et al., 2010) and biscuits (Jayasena & Nasar-Abbas, 2011). This could be due to chapatti is traditionally known to be of a light brown colour whereas the other products mentioned such as noodle benefited immensely with a more distinct yellowish colour.

In terms of texture, there was no significant difference between the control (6.22 ± 1.88) and other samples except 30% heat - treated lupin flour (5.50 ± 1.67) and 40% (5.33 ± 1.81) samples and 40% non – heat treated sample (5.63 ± 1.65). This shows

that the increased amount of lupin incorporated into sample, regardless of heat treatment, had reduced the sensory acceptability of the product. Unlike colour, lupin, being high in protein and fibre, have been known to reduce the texture acceptability when incorporated into food products (Jayasena, Leung, et al., 2010; Jayasena & Nasar-Abbas, 2011; Nasar-Abbas & Jayasena, 2012) except in tofu where there was no significant difference (Jayasena, Khu, et al., 2010). In products such as tofu, gluten development is not required to achieve product textural quality, hence no significant differences would be detected. Lupin fibre can absorb added moisture making it unavailable for gluten development, which is required for products such as chapatti, hence it is possible to find significant differences in such food.

In terms of flavour acceptability, which is the most important aspect evaluated in this study, 20% heat - treated lupin flour (6.55 ± 1.48) is significantly different than 30% heat - treated lupin (5.82 ± 1.67) and 40% heat - treated lupin (5.30 ± 2.02). Heat - treated lupin could be incorporated up to 20 - 30% with no significant difference compared to the control. However, at 40%, heat - treated lupin appears to have a significant difference with control in terms of flavour but there was no significant difference in terms of overall acceptability with control sample. When comparing flavours between heat - treated lupin and non-heat treated lupin of the same percentage, there appears to be no significant difference, which shows that at 30% and 40% incorporation, there are no significant difference between heat - treated lupin flour and non-heat treated lupin flour containing chapatti.

In terms of comparing between heat treated lupin flour and non-heat treated lupin flour, there is no significant difference when the samples were incorporated into chapatti. It is believed that during the chapatti cooking process, the high temperature used (320 °C), which could have lowered the LOX activity and reduced the beany flavour of the non-heat treated lupin flour to similar levels. This means that when making chapatti, the high temperature involved in the chapatti making process itself has the ability to lower LOX levels and reduce the beany flavour without having to use heat treatment prior to cooking to remove the beany flavour.

In terms of comparing between control and 30% and 40% heat treated lupin, there was no significant difference between the overall acceptability scores, so it can be concluded that for chapatti, both heat treated and non-heat treated lupin flour can be incorporated up to 40% without affecting the overall acceptability of chapatti. This is a rather high incorporation percentage, as apart from lupin incorporated in tofu at 40% (Jayasena, Khu, et al., 2010) normally the highest known lupin percentage incorporation was 20% in baked product (James et al., 2007; Jayasena & Nasar-Abbas, 2011) and noodle (Jayasena, Leung, et al., 2010). Lupin flour has been previously proven to increase nutritional value significantly and also dietary fibre content (Jayasena, Leung, et al., 2010) hence the addition of up to 40% will improve the nutritional value of the chapatti. Previous study has shown that baked products resulted in lower incorporation rate of 20% compared to the current study finding of 40% incorporation in chapatti. This could be because to produce a savoury food like chapatti, there was no vanilla essence or sugar used to mask the 'beany' flavour of the product such as in muffins and biscuits which has a sweeter flavour profile.

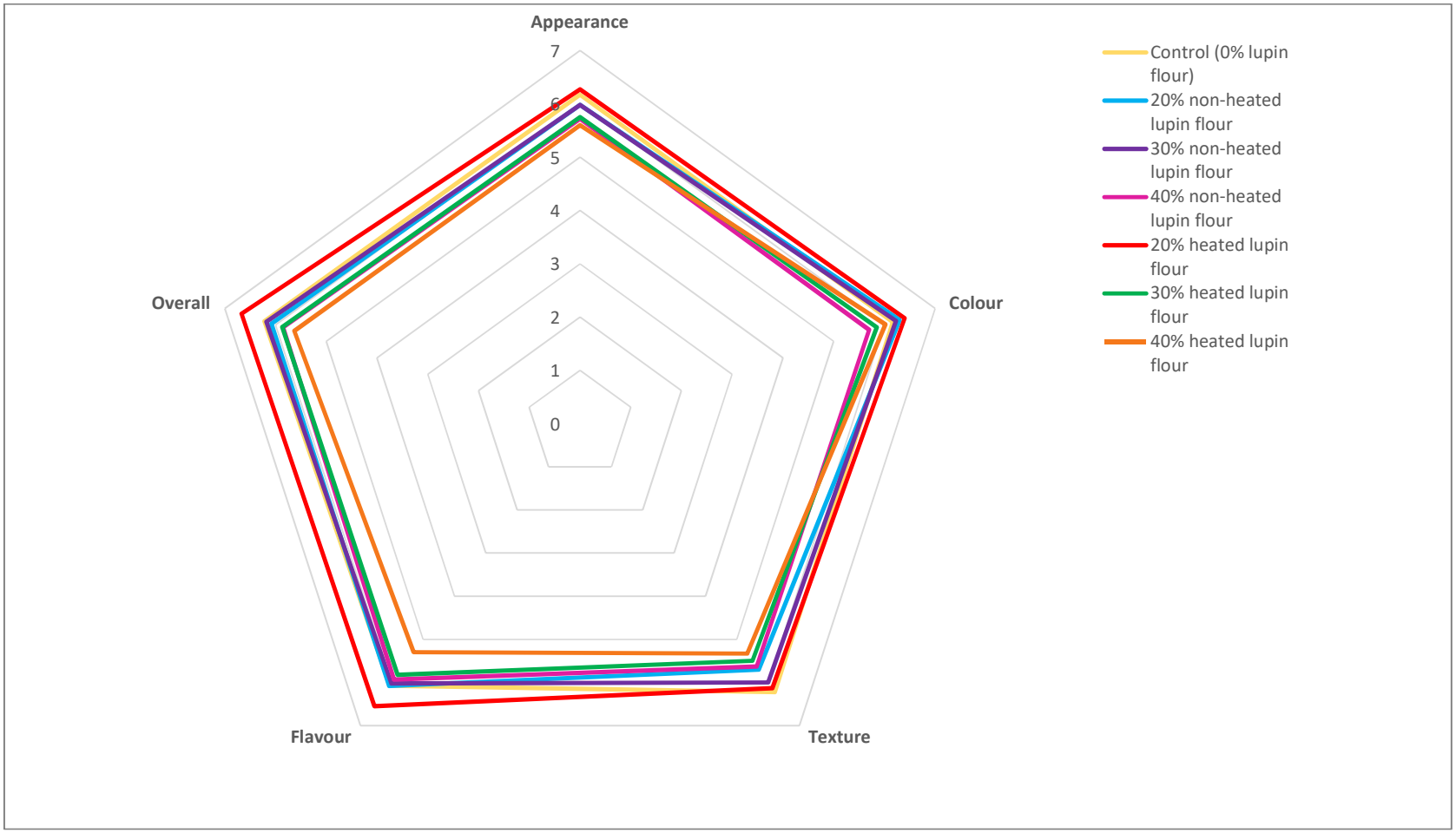

Figure 35 : Spider chart of sensory acceptability of lupin flour incorporated chapatti

4.3.2 Effect of heat treatment on instrumental texture of lupin Chapatti

Table 29 presents the measurement of texture for lupin incorporated chapatti samples. The texture of chapatti was measured in terms of its extensibility as it is a key quality trait of chapatti which includes how it tears and folds, how it feels in the hands and in the mouth (Fenton et al., 2011). It was found that 40% heat - treated lupin flour chapatti sample had the least amount of force needed (1021.1 ± 203.1) to tear. Figure 36 shows the trends of texture (Force) between samples.

Table 29 : Texture (Force and texture acceptability) of lupin flour incorporated chapatti samples

Samples	Control (0%)	Non-heat treated lupin flour			Heat - treated lupin flour		
		20%	30%	40%	20%	30%	40%
Texture (Instrumental) (Newton)	1241.35 ± 269.80^{bc}	1372.23 ± 238.88^{c}	1156.76 ± 274.58^{ab}	1125.66 ± 140.95^{ab}	1191.85 ± 181.23^{b}	1140.63 ± 233.35^{ab}	1021.07 ± 203.14^{a}
Texture (sensory)	6.22 ± 1.88^{c}	5.70 ± 1.96^{abc}	6.00 ± 1.41^{bc}	5.63 ± 1.65^{ab}	6.13 ± 1.44^{bc}	5.50 ± 1.67^{ab}	5.33 ± 1.81^{a}

* Different letters in the same row indicates a significant difference (P <0.05).

All samples were comparable to the texture of control chapatti except 40% heat - treated lupin flour. This could be because in normal chapatti sample, the developing dough produced gluten matrix. However, with the inclusion of lupin flour, the lupin flour interacts and reduced the formation of gluten matrix. The higher fibre content in lupin will absorb moisture making it unavailable for the gluten development. The increasing amount of lupin flour introduced in the samples would reduce the gluten matrix development even further, resulting in a denser and easier to tear chapatti unlike normal chapatti.

Another reason for this could be that the fibre profile of the heat- treated lupin could have been affected, which in turn would affect the texture. Lupin fibre have good water-binding capacity (Turnbull et al., 2005) which can improve the texture of food

products. However, heating could change the fibre profile affecting its water binding capacity (Chang & Morris, 1990) thereby affecting its water binding capacity which then would affect the texture of the chapatti.

According to a recent study published by Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015) on the effect of lupin varieties on wheat bread, Mandelup variety (20%) wheat bread was the hardest, and chewiest compared to the other ASL lupin variety wheat breads used. In this present study, Mandelup, which was shortlisted due to being among the highest LOX activity, did not significantly differ with the texture profile of the control chapatti except at heat treated 40% incorporation level. However, when comparing with the texture of the samples compared to control, the results were lower. According to Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015), there is a significant difference between texture of lupin incorporated bread between samples and it was suggested that Belara, Coromup, Gungurru, Jenabillup and Tanjil were suitable in producing the desired bread properties including texture. Hence, there is a possibility that by using other lupin varieties, the texture profile and texture acceptability of the lupin incorporated chapatti could be improved.

When comparing between heat treated samples and non-heat treated samples, there is a trend whereby the heat - treated lupin samples had lower texture scores than the non-heat treated lupin flour chapatti. This could be due to the heat treatment which have affected the protein structure in the lupin samples, thus further affecting the gluten matrix in chapatti dough, compared to the non-heat treated samples. This conforms to the findings of Villarino, Jayasena, Coorey, Chakrabarti-Bell, and Johnson (2015) that the lipid and protein components of the lupin flour used in that particular study may have assisted the ASL-wheat breads to achieve some of the texture profile properties of the wheat-only bread.

In terms of comparison between the texture measurement score and texture acceptability, with the exception of 20% lupin flour, there are correlations between the texture measured and the acceptability of the samples. Texture with the lowest texture scores such as 30% and 40% lupin flour have lower acceptability score. This could be

because the texture measurement only measures one aspect of chapatti and not the full texture profile.

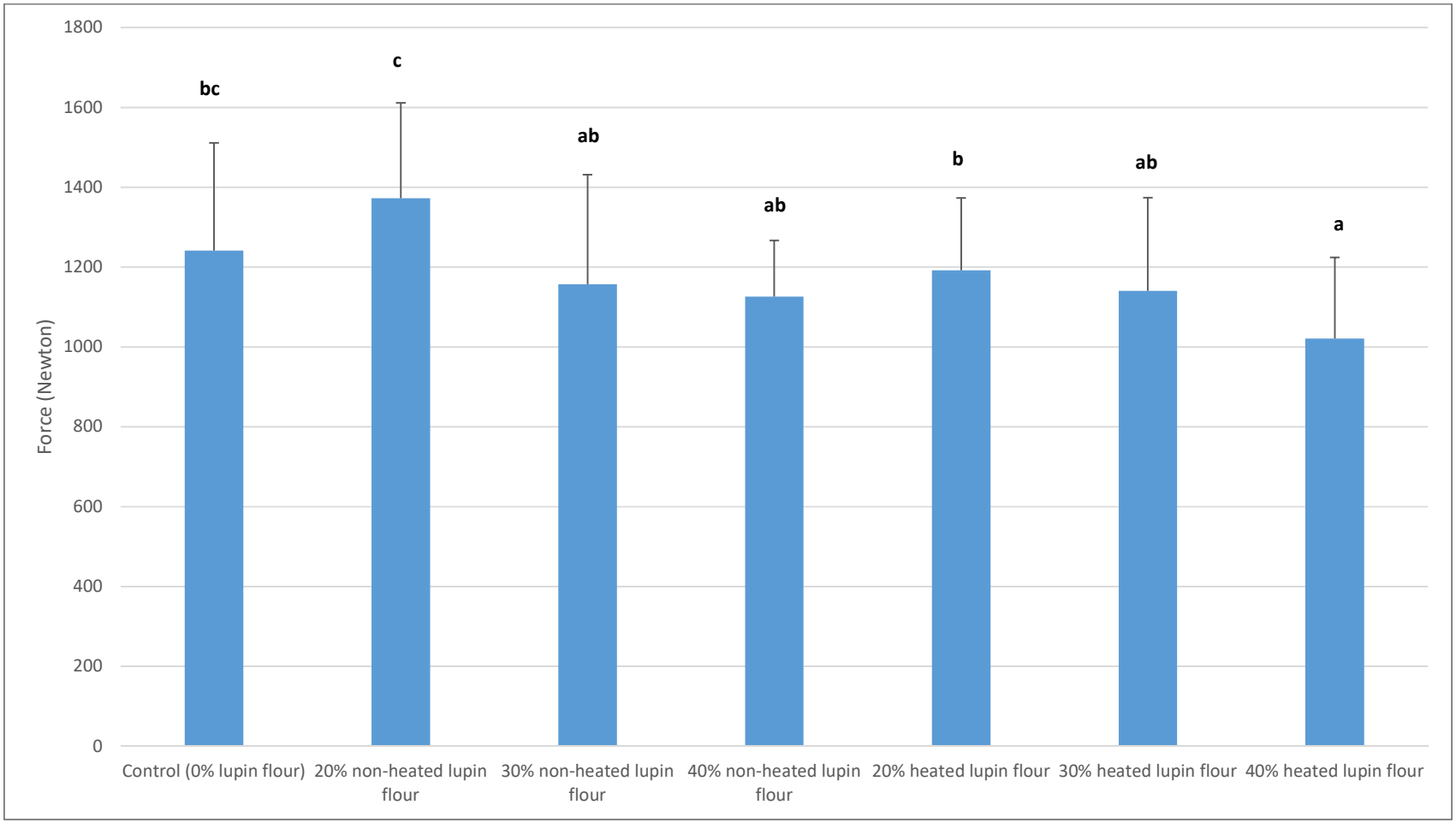

Figure 36 : Texture (force) of lupin incorporated chapatti

4.3.3 Effect of heat treatment on colour of Chapatti

Table 30 presents the colour of control, heat - treated and non-heat treated lupin incorporated chapatti samples with values of L* (lightness, extending from 0 (black) to 100 (white), a* (redness, positive value is red and negative value is green) and b* (yellowness, positive value is yellow and negative value is blue). Values are means ± standard deviations of measurements of 3 replicates (Figure 37).

Table 30: Colour of control, non-heat treated and heat - treated lupin flour incorporated chapatti samples

Samples		Control (0%)	Non-heat treated lupin flour			Heat - treated lupin flour		
			20%	30%	40%	20%	30%	40%
Colour values	L*	69.30 ± 3.26^{a}	67.67 ± 3.45^{a}	68.94 ± 3.47^{a}	68.64 ± 3.05^{a}	68.78 ± 2.69^{a}	68.20 ± 3.04^{a}	67.67 ± 1.44^{a}
	a*	3.12 ± 0.58^{a}	3.32 ± 1.20^{a}	4.03 ± 1.52^{a}	3.13 ± 0.39^{a}	3.20 ± 0.52^{a}	3.42 ± 0.36^{a}	3.45 ± 0.48^{a}
	b*	22.52 ± 0.91^{a}	30.26 ± 1.48^{bc}	33.43 ± 1.73^{d}	35.51 ± 1.93^{e}	29.29 ± 2.17^{b}	31.65 ± 1.25^{cd}	33.13 ± 1.41^{d}
Colour acceptability		6.17 ± 1.84^{a}	6.30 ± 1.50^{a}	6.23 ± 1.28^{a}	5.70 ± 1.53^{a}	6.40 ± 1.45^{a}	5.85 ± 1.40^{a}	6.02 ± 1.44^{a}

* Different letters in the same row indicates a significant difference (P <0.05).

There was no significant difference for the values of L* and a* between all samples. The highest b* reading recorded, which was the yellowest sample was from 40% non – heat treated lupin sample (35.51 ± 1.93) and the lowest was the control (22.52 ± 0.91).

There is a correlation between the amount of lupin incorporated into the sample with the yellowness of the chapatti. The 40% non-heat treated lupin sample had a higher score for b* than 40% heat - treated lupins which means yellower. This would mean that, at a higher inclusion of 40%, there is a possibility that heat treatment significantly

reduced the yellowness of the lupin chapatti. It would be interesting to see if there would be a similar trend should heat – treated lupin be incorporated into other types of food products.

However, when comparing colour value b* which is the yellowness of the lupin chapatti and the sensory colour acceptability of the samples as shown in Table 30, there is no correlation between the two. This is because there were no significant differences for all samples in colour acceptability, however there was significant difference between 40% heat - treated lupin flour and control in the colour measurement via spectrophotometer. This shows that although instrumental colour measurement could detect significant differences between samples, human eyes perceived the samples to be of similar colour.

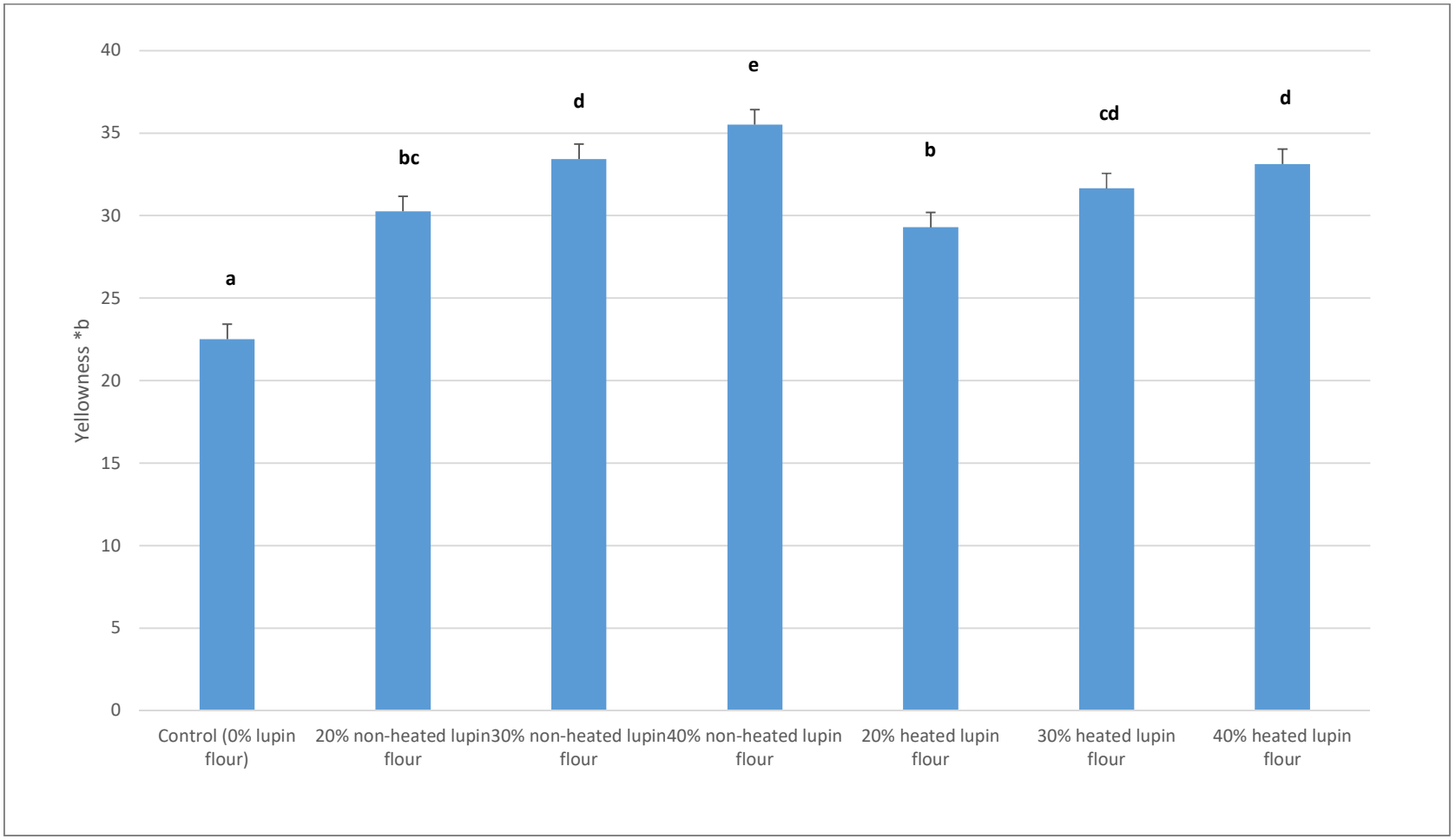

Figure 37 : Yellowness (*b) of lupin incorporated chapatti samples

5.0 Conclusions

- There are significant differences between lupin varieties in terms of LOX activity with Coromup, Mandelup and Moonah have higher LOX activities and Warrah has lowest LOX activity. Given this varietal effect, consideration need to be given when selecting lupin varieties for different food application.

- Environmental conditions have a significant impact on LOX activity in lupin. It is important to note that there is no significant difference in the years with no drought (year 2009 and 2011).

- There is a significant difference between the 25 lupin varieties for yield percentages, ash, protein and fat contents. Even though varieties such as Coromup and Mandelup has higher protein contents, they might not be suitable for some food applications due to their high LOX activities. However, these high LOX varieties can be used for other purposes such as protein extraction for use as a food ingredient.

- Colour measurement (L*, a* and b*) differ significantly between varieties with b* (yellowness) being the most relevant in comparing the colour of lupin samples. There is a positive correlation between the yellowness of the lupin flour with the level of LOX activity. Even though high yellow variety when incorporated into food product such as pasta and noodle can be appealing due to the yellowness, however due to the high LOX level, it deems to be unsuitable for some food applications due to beany flavour. These varieties can be used for other applications such as extraction of yellow pigment for food colouring.

The best heat treatment to reduce LOX activity is heating in a conventional oven at 80 °C

for 5 minutes. There is no significant difference in LOX activity when comparing heat treated lupin at different processing stages (seed, kernel and flour). Heating samples more than 5 minutes has no additional effect on LOX activity. A heat - treated lupin flour with low LOX activity (less beany flavour) can be produced for food application by heating it at 80
°C

- for 5 minutes.

- The different heat treatments have no significant impact on the colour (L*, a* and b* values) of the lupin seed, kernel and flour. While there is a correlation between the colour and LOX activity in its natural form in lupin, heating had no effect on the colour values. Colour may be a predictor of LOX activity in its raw form but not once heat treated.

Both heated and non-heated lupin flour can be incorporated into chapatti products up to 40% inclusion without affecting the overall acceptability. It is believed that the high temperature (320
°C

-) applied in the chapatti cooking process could lower the LOX activities of the chapatti and reducing the beany flavour. Heat treatment on lupin flour prior to cooking chapatti or similar products to remove beany flavour may not be required.

- For texture, the chapatti with the heat - treated lupin flour was significantly lower at 40% compared to the non-heat treated lupin flour containing products. This could be due to the fibre profile of the heat - treated lupin could have been affected, which in turn affecting the texture. Fibre can bind water and alter the texture of a food product.

In conclusion, outcomes of this study would have many potential benefits such as:

- Lupin breeders should be encouraged to develop lupin varieties with low LOX activities that meet the sensory requirements in food product development.
- Farmers could be encouraged to plant selected lupin varieties based on recommendations from lupin breeder thus benefit through increased demand in lupin as a food product.
- The food industry will benefit from the high quality and highly palatable lupin that can be incorporated into food products which could be healthier.
- Consumer will have an alternative food product with high nutritional value and health benefits that is highly palatable and low in cost.
- Health professionals may benefit from having new lupin incorporated products that are available to be recommended to their clients who have heart diseases, prone to diabetes type 2, digestive problems and high blood pressure.
- Due to the availability of nutritious food products and the expected increase in their consumption, it could be expected to lower the incidents of non-communicable diseases such as CVD there by lowering the burden on the health system

6.0 Recommendation For Future Studies

- It is suggested that future research focus on the LOXs activation mechanism so as to prevent or slow down its activity during harvest, storage and processing.
- Determine storage conditions and their impact on LOX activation/activities in lupin. Thereby storage conditions could be developed by the lupin industry through harvest and distribution chain to prevent the development of the beany flavour.
- In terms of LOX assay method, it is suggested in the future to develop a rapid LOX determination method that can be used in the food industry as a regular quality assurance tool when incorporating lupin into food products.
- As for heat treatment, the determined critical time and temperature combination which has successfully reduced LOX activity in lupin varieties can be used as a starting point for future lupin incorporated food quality investigations, especially in food products that do not undergo high temperature processing.
- Recent studies have shown that Mandelup appears to have an undesirable texture when incorporated into other bread products (which also undergo high temperature treatment), hence it is recommended that future studies investigate other low LOX lupin varieties besides Mandelup when producing lupin incorporated foods.
- In terms of product development, it is suggested that heat treated lupin be incorporated into food products such as bread to determine the impact of heat treated lupin flour in different food matrix and the impact of LOX on its sensory acceptability and shelf life. It is suggested to determine the health benefits of consuming heat - treated lupin in different food products as the food matrix itself may have an impact especially with the dietary fibre profile.

It is suggested that where possible high temperature (e.g. 320
°C
) could be applied to lupin incorporated food products, to reduce the impact on its sensory properties. Current research shows that the high temperature (320
°C

-) applied to the dough in the chapatti cooking process lower the LOX activity of the chapatti and reduced the beany flavour.

7.0 References

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8.0 Appendices

Appendix A : Consumer acceptability questionnaire used

SENSORY EVALUATION OF CHAPATTI

Instructions: Please assess each sample individually in the order presented on the following form and place a mark in the box that represents your opinion. There is no right or wrong answers; it is your honest opinion that is most important.

Please cleanse your palate with a bite of cracker and a sip of water before you begin tasting each sample.

Please taste the **Sample No. 838**

How do you find the Appearance of this sample?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How do you find the Colour of this sample?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How do you find the Texture of this sample?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How do you find the Flavour of this sample?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How do you find the Overall acceptability of this sample?

❑	❑	❑	❑	❑	❑	❑	❑	❑
Dislike extremely	Dislike very much	Dislike moderately	Dislike slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Appendix B : Consent Form

Sensory Evaluation Consent Form

1. I have received a statement explaining this project and given the chance to ask any questions relating to the project.
2. I consent to participate in this project held at the School of Public Health, Curtin University.
3. I understand;
   (a) The purpose of this research
   (b) That all information given by myself will be kept confidential and only be used in this research only.
   (c) That I may withdraw at any point in time from the project and any unprocessed data previously supplied will be destroyed.
4. **I do NOT have any food allergies related to legumes or any other food allergies.**

Participant's Consent

Name: _____ Date: ___/ ___/ ___ (dd/mm/yy)

Signature: _____

Appendix C : Demographic data of panelist

Demographic data	Number	Percentage (%)
Gender		
- Male	14	23.33
- Female	46	76.67
Age		
- 20 – 29	36	60.00
- 30 – 39	11	18.33
- 40 – 49	12	20.00
- 50 – 59	1	1.67
Origin/Nationality		
- Indian/South Asian	10	16.67
- Caucasian	5	8.33
- Malay/Indonesian	24	40.00
- Chinese/Asian	11	18.33
- Middle Eastern	2	3.33
- African	8	13.33
Diet		
- Vegetarian	3	5.00
- Non-vegetarian	57	95.00

Appendix D: Questions and percentage (%) of the answers

Questions	Number	Percentage (%)
Q1: How often do you eat chapatti?		
- > 7 times/week	4	6.67
- 3 – 7 times/week	9	15.00
- Once a week	12	20.00
- Once a fortnight	3	5.00
- Once a month	9	15.00
- Less than once a month	23	38.33
Q2: How do you eat chapatti?		
- Vegetable dish	7	11.67
- Meat or fish dish	50	83.33
- With sugar	2	3.33
- With other (jam)	1	1.67

Appendix E : Statistical Analysis example used throughout the study (ANOVA)

Descriptives

L

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
seed	18	89.0317	.52504	.12375	88.7706	89.2928	88.21	89.69
kernel	18	89.2239	.70971	.16728	88.8710	89.5768	88.00	90.11
flour	18	89.4517	.31997	.07542	89.2926	89.6108	89.00	89.94
Total	54	89.2357	.55932	.07611	89.0831	89.3884	88.00	90.11

Test of Homogeneity of Variances

L

Levene Statistic	df1	df2	Sig.
6.605	2	51	.003

ANOVA

L

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.591	2	.796	2.707	.076
Within Groups	14.989	51	.294		
Total	16.581	53			

Robust Tests of Equality of Means

L

	Statistic[a]	df1	df2	Sig.
Welch	4.308	2	30.722	.022

a. Asymptotically F distributed.

Multiple Comparisons

Dependent Variable: L

	(I) Processing stage	(J) Processing stage	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	seed	kernel	-.19222	.18071	.541	-.6285	.2440
		flour	-.42000	.18071	.061	-.8562	.0162
	kernel	seed	.19222	.18071	.541	-.2440	.6285
		flour	-.22778	.18071	.424	-.6640	.2085
	flour	seed	.42000	.18071	.061	-.0162	.8562
		kernel	.22778	.18071	.424	-.2085	.6640
Games-Howell	seed	kernel	-.19222	.20808	.630	-.7041	.3196
		flour	-.42000*	.14492	.019	-.7785	-.0615
	kernel	seed	.19222	.20808	.630	-.3196	.7041
		flour	-.22778	.18349	.441	-.6865	.2309
	flour	seed	.42000*	.14492	.019	.0615	.7785
		kernel	.22778	.18349	.441	-.2309	.6865

*. The mean difference is significant at the 0.05 level.