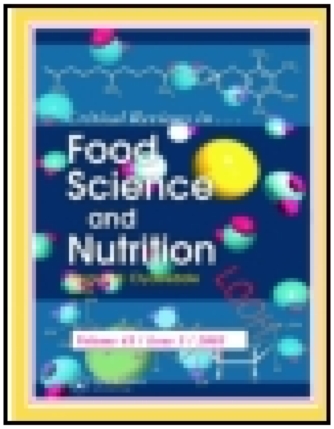

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

Nutritional, Health and Technological Functionality of Lupin Flour Addition to Bread and Other Baked Products: Benefits and Challenges

Villarino C.B.J[a], Jayasena V.[a], Coorey R.[a], Bell S.[bc] & Johnson S.K.[a]
[a] Food Science and Technology Program, School of Public Health, Curtin Health Innovation Research Institute, Curtin University, Bentley, WA 6102
[b] Centre for Grain Food Innovation, 26 Dick Perry Avenue, Kensington, WA 6151, Australia
[c] CSIRO Food Futures National Research Flagship, GPO Box 1600, ACT 2601, Australia
Accepted author version posted online: 12 Feb 2015.

Nutritional, health and technological functionality of lupin flour addition to bread and other baked products: benefits and challenges

Villarino, C.B.J[1]. , Jayasena, V.[1], Coorey, R.[1], Bell, S.[2,3] & Johnson, S.K.[1*]

[1]Food Science and Technology Program, School of Public Health, Curtin Health Innovation Research Institute, Curtin University, Bentley, WA 6102

[2]Centre for Grain Food Innovation, 26 Dick Perry Avenue, Kensington, WA 6151, Australia

[3]CSIRO Food Futures National Research Flagship, GPO Box 1600, ACT 2601, Australia

*Corresponding author: s.johnson@curtin.edu.au

ABSTRACT

Lupin is an undervalued legume despite its high protein and dietary fiber content and potential health benefits. This review focuses on the nutritional value, health benefits and technological effects of incorporating lupin flour into wheat-based bread. Results of clinical studies suggest that consuming lupin compared to wheat bread and other baked products reduce chronic disease risk markers; possibly due to increased protein and dietary fiber and bioactive compounds. However, lupin protein allergy has also been recorded. Bread quality has been improved when 10% lupin flour is substituted for refined wheat flour; possibly due to lupin-wheat protein cross-linking assisting bread volume and the high water binding capacity (WBC) of lupin fiber delaying staling. Above 10% substitution appears to reduce bread quality due to

 ACCEPTED MANUSCRIPT

lupin proteins low-elasticity and the high WBC of its dietary fiber interrupting gluten network development. Gaps in understanding of the role of lupin flour in bread quality include the optimal formulation and processing conditions to maximize lupin incorporation, role of protein cross-linking, anti-staling functionality and stability, and bioactivity of ɔ- conglutin peptide.

Key words: *lupin, wheat flour, bread, proteins, dietary fiber, γ- conglutin, disulphide and dityrosine crosslinking, bread staling, legume, health.*

INTRODUCTION

In this review paper, the term ñbaked productsò includes breads, cakes, pastries, cookies, crackers and other products which use wheat flour as the primary ingredient, and undergo heat processing. Baked products, in particular bread have been an important part of the diet for centuries (Smith et al., 2004) and have remained a staple food across the civilized world (IBISWorld, 2011). It is predicted that the global baked products market will reach a US$ 410 billion by 2015 (Anon, 2011b). However the food industry is pressurised to continuously address dynamic consumer preferences including the demand for healthy and novel foods with whole grains and alternative grains and legumes as substitutes for refined wheat.

Wheat flour is the major component of baked products due to the presence of gluten proteins which provide products with the desired volume and texture (Rosell, 2011). However, health and nutritional issues can arise from the use of wheat flour in bread. For example, celiac disease due to gluten intolerance of which around1% of most populations suffers from (Mandala and Kapsokefalou, 2011) is a growing health concern. In addition, during refining, the removal of bran and germ leads to reduce nutritional value through significant losses in protein, dietary fiber, vitamins, minerals and phytochemicals (Rosell, 2011). These issues related to consumption of refined wheat products have catalysed the search for alternative flour ingredients for baked products including legumes. Legumes (e.g. soybeans, chickpea, and faba beans) are good sources of protein and dietary fiber vitamins and minerals, do not contain gluten and have been added at a rate of 10 to 30% to baked products without reducing quality (Duodu and Minnaar, 2011; Farooq and Boye, 2011).

ACCEPTED MANUSCRIPT

A legume that can potentially address consumersô desire for healthier baked products is lupin. Lupin grain is high in protein and dietary fiber with very little available carbohydrate (Petterson et al., 1997). It also contains vitamins and antioxidants including: tocopherols (Boschin and Arnoldi, 2011); carotenoids (Wang et al., 2008); B-vitamins (Erbas et al., 2005) and phenolic compounds (Oomah et al., 2006b). In addition, lupin is low in anti-nutritional factors such as trypsin inhibitors and saponins compared to many other legumes (Martinez-Villaluenga et al., 2006). Studies have demonstrated that lupin flour can be used to formulate acceptable baked products (Hall and Johnson, 2004), as well as other foods such as pasta (Martínez-Villaluenga et al., 2010), meat products (Drakos et al., 2007), and dairy products (Martinez-Villaluenga et al., 2005). Consumption of these lupin-containing products, in particular bread, has demonstrated through clinical studies, the lowering of risk factors for obesity (Lee et al., 2009), cardiovascular disease (Belski et al., 2011) , type 2 diabetes mellitus (Hall et al., 2005b) and gastrointestinal problems (Johnson et al., 2006).

Despite the potential of lupin as a unique, healthy food ingredient, most of the lupins utilised as stockfeed. The use of lupin as human food, specifically in baked products has been limited due mainly to poor sensory quality (Paraskevopoulou et al., 2010). The continuing rise in food prices, demand for healthier and non-genetically modified (GM) products, and sustainably produced food, suggests the potential for a rapid increase in utilization of lupin as a food ingredient.

This review paper covers the nutritional, health, and technological functionality of lupin flours and the role of lupin protein and dietary fiber. In this review, nutritional functionality refers to the impact on nutritional composition; health functionality refers to health benefits; and

ACCEPTED MANUSCRIPT

technological functionality refers to impacts on product quality (i.e. texture and flavor), and processing efficiency. The terms *lupin, wheat flour, bread, proteins, dietary fiber, γ- conglutin, disulphide and dityrosine crosslinking, bread staling* were used to search *Web of Knowledge, ScienceDirect, Scopus, Wiley Online Library and Google Scholar* from 2000 - present for nutritional and health functionality that only included human studies. For technological functionality, the search period was extended back to the 1980ŝs due to the small number of recent publication.

Information on lupin, lupin flour and its composition, and uses as food ingredient is first presented followed by a brief overview of bread and bread making After which the benefits and challenges related to the nutritional and health functionality of lupin flour addition to bread and other baked products are discussed. The benefits and challenges related to technological functionality as it affects texture and flavour of lupin-flour supplemented bread and baked products are reviewed. A brief discussion on the use of lupin as an ingredient in other food products (apart from wheat-based) is presented towards the end of the review.

LUPIN

Taxonomy, agronomy and general uses

Lupins or lupine belong to the genus Lupinus under the Genisteae tribe of Fabaceae or Leguminosae family (Uzun et al., 2007) to which soybeans, chickpeas and other types of beans also belong. Lupin seeds from different species and varieties vary greatly in size, shape (i.e. round, oval, and flat), and colour (i.e. white, brown, and gray) (Kurlovich et al., 2002).

Wild species of *Lupinus* found in North and South America, the Mediterranean region and northern Africa were introduced to southern Africa and Australia during the early days of colonization (Cowling et al., 1998). Full domestication of a few lupin species (i.e. *L. angustifolius, L. albus, and L. luteus)* for animal feed and human food use occurred in the latter half of the 20th century. Wild lupins have: (a) bitter quinolizidine alkaloids (QA) which renders them unpalatable and potentially toxic, (b) hard seed coats that do not readily imbibe water which allows them to survive in the soil for several seasons prior to germination, and (c) shattering pods that scatter seed on the ground at maturity. On the other hand, domesticated lupins have been selectively bred to have: (a) low alkaloid content, making them edible by domestic animals and humans, (b) softer seeds that immediately germinate in moist soil and (c) non-shattering pods which keep the seeds on the plant giving efficient harvesting.

Lupin grows well in acidic and sandy soils, as for example those found in Western Australia (French et al., 2008). The lupin plant has been used as green manure or forage and as organic material for soil enrichment and stabilization and erosion control (Cowling et al., 1998). Due to its nitrogen fixation ability lupin is a critical rotation crop for the sustainability of some farming system, such as wheat and other cereals in Australia and Europe (French et al., 2008; GL-PRO, 2005).

Species

The genus Lupinus consists of hundreds of species, of which only a few have been domesticated (Foley et al., 2011) including *L. albus, L. angustifolius, L. luteus and L. mutabilis* (Table 1). *L. albus* is grown mainly in Europe (Harzic et al., 2000) while *L. angustifolius* is largely produced in Australia (Cowling and Gladstones, 2000). *L. luteus* is widely distributed in

ACCEPTED MANUSCRIPT

the Mediterranean region (Parra-Gonzalez et al., 2012) while *L. mutabilis* is grown in South America (Erbas, 2010).

L. angustifolius, also known as the Australian sweet lupin (ASL) or the narrow-leaf lupin, is the largest legume crop grown in Australia (Lawrance, 2007). In recent years, interest in the use of ASL as human food has been increasing (Sirtori et al., 2010) in both Australia and Europe due to its potential health benefits. *L.albus* has been used as food since the pre-Roman and Greek times (Cowling et al., 1998). It was soaked and boiled to eliminate the bitter alkaloids (Annicchiarico et al., 2010). *L. luteus* typically has yellow flowers (Cowling et al., 1998), thus its common name, the yellow lupin. There is scarcity of reports on human studies or food applications involving yellow lupins despite according to Petterson et al. (1997), having higher protein and fiber contents than both ASL and *L. albus*. *L. mutabilis* has long been utilised for soil enrichment and as food in the Andean region (Gross et al., 1988). The seeds of this species have the highest protein content among the four commercial species (Trugo et al., 2003) at a similar level to soybeans (Cowling et al., 1998).

Production

According to the Food and Agricultural Organisation of the United Nations (FAO-STAT, 2013) the top five lupin-producing countries in 2011 were Australia, Poland, Chile, Ukraine, and Belarus. Australia produces 85% of the total global supply, the majority of which is ASL. Lupin is lower in cost compared to other grain legumes: for instance in Australia, lupin is currently sold half the price of soybean (igrain.com.au, 2011). However in Australia, lupin is still mainly used as animal feed with only around 4% of the total production processed for human consumption (Lawrance, 2007).

LUPIN FLOUR

Definition and description

Food Standards Australia and New Zealand (FSANZ, 2009), Food Standards Code for Cereals and Cereal Products (Standard 2.1.1) defines flours as "*products of grinding or milling of cereals, legumes or other seeds*". Based on this definition, lupin flour refers to the product from milling lupin seeds. ASL flour has been described as having a pale yellow colour and slight beany flavour (Hall et al., 2005b). According to Australian and UK standards lupin flour should not have more than 200 mg/kg of alkaloids and not more than 0.005 mg/kg of phomopsins (FSANZ, 2011a; MAFF-DOH, 1996).

Lupin flour manufacture

Seeds are first sorted, graded and then cleaned of any foreign objects using a vibrating screen or metal detector. The cleaned whole seeds are passed through a de-huller the hull from the kernel. Hulls are separated by air classification. Since the hull is rarely incorporated into lupin flour for baked products it will no longer be discussed within this review. The split kernels are then milled and sieved to separate into varying particle sizes ranging from <150 to 300 microns (Anon, 2011a). Lupin flour milling process is important in producing flour of optimal quality for specific food applications such as bread. For instance, decreasing the particle size of wheat flour substitutes (i.e bran or whole wheat) used in bread making has been reported to either increase (Moder et al., 1984) or decrease (de Kock et al., 1999) loaf volume. However, no study has been reported on the effects of lupin flour particle size on loaf volume.

Composition

Lupin flours are a rich source of nutrients with ASL flour having higher protein (~40%) and dietary fiber (~40%) but a lower energy value compared to refined wheat flour (Table 2). All lupin flours are also very low in starch, unlike wheat flour in which starch is the major component (Hall and Johnson, 2004). Lupin seeds also contain vitamins such as thiamine, niacin, riboflavin, and tocopherols, as well as minerals including iron, zinc and manganese (Trugo et al., 2003). It was also reported that ASL seeds have high levels of carotenoids compared to *L. luteus and L. albus* (Wang et al., 2008). Lupin flour and protein isolates contain antioxidants (Martínez-Villaluenga et al., 2009) in the form of the polyphenolic tannins and flavonoids (Oomah et al., 2006a). Compared to soybeans and other legumes, lupins have lower levels of anti-nutritional components such as phytate and saponins (Trugo et al., 2003).

Lupin proteins and dietary fiber have the potential to increase the nutritional quality and modify the technological properties of bread and other baked products when wheat flour is substituted by lupin flour.

Protein content and nutritional quality

Protein at an adequate intake and of balanced amino acid composition is an essential dietary component (Rolfes et al., 2009). ASL flour has been reported to contain 41.8% protein (Hall et al., 2005b), however protein content was reported to be affected by both genotype (e.g. variety) and environment (Cowling and Tarr, 2004). Reports on the protein content of flours produced from *L. albus* and *L. luteus* are limited, and these species are not widely utilized as flour on the commercial scale. Dervas et al. (1999) reported that protein content from *L. albus*

 ACCEPTED MANUSCRIPT

flours ranged from 31-36%.The major storage proteins in lupin seed are known as globulins, which are classified into four families: Ǖ- conglutin (11S globulin), ɓ- conglutin (7S globulin), ɔ-conglutin (7S basic globulin) , and ǔ-conglutin (2S sulphur-rich albumin) (Foley, et al., 2011). ɔ-conglutin is a peptide with reported bioactivity and health and pharmaceutical benefits (Duranti et al., 2008).

The main limiting amino acids in lupin are the S-amino acids (methionine and cysteine), valine and tryptophan (Doxastakis et al., 2002). The amounts of lysine, isoleucine, leucine , phenylalanine and tyrosine in lupin are comparable to the Food and Agricultural Organization (FAO) standards for amino acids of ideal reference protein appropriate for adults (FAO/WHO/UNU., 1985). The amino acid profile of lupin complements that of wheat, which is higher in sulphur-containing amino acids (i.e. methionine) but lower in lysine. This is supported by Duodu and Minnaar (2011), who reported that the advantages of using legume flours, including lupin, in combination with wheat flour in baked products are increased protein content and improved amino acid balance of the final product.

Legumes including lupin lack the gluten protein required for desired dough and bread quality. This lack of gluten limits the incorporation rate of lupin in bread and hence limits the improvements in nutritional and health benefits that can be gained through its incorporation (Angioloni and Collar, 2012a). There still remains a lack of information on the effect of genotype x environment on protein quality of lupin flours. In addition, little is known on the levels of the bioactive ɔ- conglutin peptide in different lupin species and varieties nor its stability during food processing.

<u>Dietary fiber content and physical properties</u>

ACCEPTED MANUSCRIPT

Increased dietary fiber intake has been a general dietary recommendation for a healthy diet across the developed world (DHA-NHMRC, 2005; USDA-CNPP, 2012). In whole lupin seeds, both the hull and the kernel contain high levels of dietary fiber (Pfoertner and Fischer, 2001). Since lupin flour is generally manufactured only from the dehulled kernels this review will focus only on dietary fiber in lupin flours. ASL flour has been reported to contain 41.5 % dietary fiber, 11% of which is soluble and 31.5% is insoluble (Hall et al., 2005b). Dietary fiber in lupin flour mainly consists of non-starch polysaccharides located in the thickened endosperm cell walls and raffinose family oligosaccharides such as raffinose, stachyose and verbascose (Evans et al., 1993; Trugo et al., 2003). The non-starch polysaccharide components are composed of a rhamnogalacturonan backbone with galactose and arabinose containing side chains (Pfoertner and Fischer, 2001).

The two main physicochemical properties of the dietary fiber in lupin flour that may influence its nutritional, health and technological properties when lupin flour is used in bread are water binding capacity (WBC) and viscosity. WBC refers to the amount of water a gel system retains within its structure after it is subjected to any form of stress (Tungland and Meyer, 2002); an example being mixing or kneading during bread manufacture. According to Pfoertner and Fischer (2001) lupin fiber has a WBC of 8-10 ml/g. WBC of fibers is related to viscosity that was defined by Dikeman and Fahey (2006) as the ability of some polysaccharides to form gels in the presence of fluids caused by the formation of intermolecular bonds between the polysaccharide components. The addition of lupin flour has great potential to elevate the levels of dietary fiber in baked products. However information is still required on the effect of lupin genotype and production environment on its dietary fiber content and physicochemical

ACCEPTED MANUSCRIPT

properties. This information can then assist in selection of optimal lupin flour for incorporation into consumer acceptable baked and other food products with maximum dietary fiber content.

Other lupin fractions as food ingredients

Lupin flour can be fractionated into protein isolates, purified dietary fiber and water-soluble by-products (i.e. whey proteins and oligosaccharides). Protein isolates are prepared either by isoelectric precipitation (Ruiz and Hove, 1976) or ultrafiltration (Chew et al., 2003) of protein extract of lupin flour. The total protein is conventionally extracted by solubilisation of protein from flour (defatted or non-defatted) at pH 9, centrifugation to remove the insoluble portion (dietary fiber), followed by acid precipitation of the major globulin proteins at pH 4.5 (Sipsas, 2008). The acid-precipitated protein is then separated from the acid-soluble whey fraction by centrifugation. Both the acid-precipitated protein and the fiber fractions are then dried to produce the final dry powder ingredients. The acid-soluble whey fraction was once considered a waste stream but has demonstrated potential as a source of foaming proteins (Wong et al., submitted), bioactive peptides (Sironi et al., 2005) and oligosaccharides with may have prebiotic activity as shown in soybean (Patel and Goyal, 2012). In addition to protein and dietary fiber fractions, oil can be extracted from lupins (Hill, 2005) and has similar free fatty acid (i.e. oleic, linoleic and linolenic) profile with peanut and rapeseed oils (Erbas et al., 2005).

Use of lupin flour in wheat-based food

Lupin flour and its fractions have been investigated as a substitute for wheat flour in bread including: white breads (Doxastakis et al., 2002; Guemes-Vera et al., 2008; Mubarak, 2001; Paraskevopoulou et al., 2010); Chilean breads (Ballester et al., 1988) and; sourdough bread (Bartkiene et al., 2011). Lupin has been used in other baked goods such as muffins, cookies and brownies (Clark and Johnson, 2002; Doxastakis et al., 2002; Hall and Johnson, 2004; Nasar-Abbas and Jayasena, 2012), gluten-free cakes (Levent and Bilgiçli, 2011), and biscuits (Jayasena and Nasar-Abbas, 2011) and other wheat-based foods including instant noodles (Jayasena et al., 2010) and pasta (Clark and Johnson, 2002; Martinez-Villaluenga et al., 2010).

A limited number of commercial breads containing lupin flour are available. Bodhiôs Bakehouse (Fremantle, Australia) produces *Lupin Loaf*, a gluten-free bread which contains 5.6g/100g of protein and 4.2 g/100 g of dietary fiber, and *Wupper Soft with Lupin* which contains 17.5 g/100g of protein and 10.4g/100g of dietary fiber. *Lupin Loaf* (10% lupin flour) is described as having a dense crumb while *Wupper Soft with Lupin* (40% lupin flour) is a sourdough rye bread. These breads are marketed as niche healthy products and have not reached mainstream consumption most likely due to poor consumer acceptability. Consumersô preference for refined white bread is one of the reasons cited for the relatively low consumption of whole-wheat (Bakke and Vickers, 2007) or high-fiber breads. In the case of lupin, published reports demonstrate that a maximum of only 10% lupin flour can be substituted for refined wheat flour before quality is reduced (Doxastakis et al., 2002). This may be attributed to the low elasticity proteins and high WBC of dietary fiber in lupin flour (Turnbull et al., 2005) that weakens the gluten matrix and thus results in poor texture and loaf volume of the bread (Guemes-Vera et al., 2008). There is therefore, a need for research to identify optimal formulations and processing methods to further increase the incorporation of lupin rate of lupin into highly palatable, nutritious bread acceptable to mainstream consumers.

BREAD

Bread is typically formulated from wheat flour, water, yeast and salt (Popper et al., 2006). Ingredients such as non-wheat flour, shortening, sugar, enzymes, dough conditioners, vitamins and minerals may also be added to improve sensory, textural and nutritional quality (Atwell, 2001; Collado-Fernández, 2003). Bread is one of the most commonly eaten food items, with per capita worldwide mean consumption ranging from 41 to 303 kg/year (Rosell, 2011) and thus it is a main source of energy and nutrition for humans (Collado-Fernández, 2003). In America and Australia, breads manufactured from refined wheat flour have been reported to contain 9.2 g/100 of protein and 2.7 g/100 g of dietary fiber (USDA, 2012) and 9.7 g/100 g of protein and 2.8 g/100 g of dietary fiber (FSANZ, 2011b) respectively. Bread is also a good source of available carbohydrates, minerals (i.e potassium, calcium, iron) and B vitamins (Southgate, 2003).

There are different types of bread making processes which vary in their combination of three principal stages kneading of dough (or mixing), fermentation, and baking. Two commonly used methods of bread making are the ñstraight-doughò and the ñsponge and doughò processes (He and MacGregor, 2009). The straight-dough process is the simplest method which involves mixing all ingredients, proofing, punching, shaping, final proofing and baking (Atwell, 2001). The sponge and dough process, involves firstly mixing and fermenting a portion of flour, water and yeast, after which this pre-dough (sponge) is mixed with the rest of the ingredients to form the final dough. The final dough is proofed for a short time before it is divided, rounded, molded, fermented (final proof) and baked (Collado-Fernández, 2003).

The technological aspects of bread making have been thoroughly discussed by several authors (Cauvain and Young, 2007; Collado-Fernández, 2003; Rosell, 2011) and therefore will not be discussed in detail in this review. Table 3 shows the main stages of bread making, the mechanisms involved in each stage, and the related quality parameters that may be affected when non-wheat flours such as lupin flour are substituted to wheat flour. These various stages in bread making are sensitive to the substitution of wheat-flour by non-gluten, low-starch lupin flour in particular the disruption gluten development (Guemes-Vera et al., 2008) and a reduction in carbon dioxide production resulting in bread with poor loaf volume, and hard and crumbly texture (Doxastakis et al., 2002).

NUTRITIONAL AND HEALTH FUNCTIONALITY OF LUPIN FLOUR IN BREAD

Benefits

The review will focus primarily on the impact of lupin protein and dietary fiber on bread quality. Lupin flour addition to results in increasing the nutritional quality and potential health benefits of bread by increasing: (a) protein content and protein nutritional quality; (b) dietary fiber content; (c) carotenoid content and; (d) levels of the peptide ɔ-conglutin. The importance of bread as a vehicle of nutrients is demonstrated by the fact that Australians obtain 45% of their dietary fiber and 25% of their protein from cereals and cereal products, including bread (NHMRC, 2005). Substitution of 20% wheat flour has the potential to add 8 g (~25% of RDI) each of dietary fiber and protein per 100 g of bread (~4 slices).

Beneficial nutritional functionality

The effects of wheat flour substitution by lupin flour in bread and other baked products on protein content, protein nutritional quality and dietary fiber content are presented in Table 4. The findings demonstrate that high levels (30-40%) of substitution of wheat flour by lupin flour can increase the protein (46-352%) and dietary fiber content (106-346). Even low levels substitution (e.g. 3%) can increase protein and dietary fiber levels significantly.

In order to maximize the level of lupin flour incorporation into bread to maximize its nutritional quality, whilst maintaining a consumer acceptable product requires systematic optimization of formulation, processing parameters and their interactions. However this systematic optimization has not been reported in the literature and most studies focused on only a single parameter (e.g. rate of lupin flour incorporation). In addition some published studies used *L. albus* flour some used ASL and some did not specify the species. However, it has been confirmed (V. Jayasena, personal communication, June 06, 2013) that ASL was used in the studies that did not cite the species as presented in tables 4 and 5 (Lee et al., 2006; Lee et al., 2009; Hodgson et al., 2010; Yang et al., 2010; Belski et al., 2011). No investigations have reported the effects of lupin species or variety on the nutritional quality of bread.

Beneficial health functionality

This section will focus on the clinical study evidence than lupin consumption can modify biomarkers for the risk of chronic diseases and other health biomarkers. A summary of relevant studies is presented in Table 5. Research findings have revealed that the consumption of lupin bread and other baked products can help reduce risk factors for obesity, type 2 diabetes mellitus, cardiovascular disease and bowel dysfunction.

Obesity biomarkers

Post-prandial self-reported perception of satiety is a valuable tool to rank foods for their potential ability to reduce overall energy intake and hence risk of obesity (ADA, 2005). It has been reported that lupin-supplemented bread gave high satiety than wheat-only bread when consumed by healthy male and female adults (Lee et al., 2006). This led to a reduction in subsequent energy intake of subsequent meals; effects that the authors attributed to the higher protein and dietary fiber contents of the lupin-containing bread. The increased protein from lupin may have increased plasma amino acids that stimulate the production of the gastrointestinal hormone cholecystokinin sending signals of fullness to the brain (Paddon-Jones et al., 2008).

The high WBC of lupin dietary fiber (Turnbull et al., 2005) may induce satiety by: (a) increasing stomach distension triggering signals of fullness to the brain; (b) delaying gastric emptying, and; (c) prolonging small intestine transit time and absorption rate of nutrients (Kristensen and Jensen, 2011). The role of dietray fiber in the satiating effect of lupin fiber was supported by the findings of Archer et al. (2004) who found that sausage patties in which purified lupin kernel fiber replaced some of the fat where more satiaitng than the full fat.

Another biomarker for appetite is plasma ghrelin, a gut hormone that stimulates appetite leading to increased food intake and thus its suppression leads to onset of satiety (Benelam, 2009; Kirsz and Zieba, 2011). Dietary fiber viscosity, and the release of gut peptide cholecystokinin mediated by proteins can induce delayed gastic emptying which helps regulate ghrelin (Blom et al., 2006; Koliaki et al., 2010). Consumption of high protein and high dietary fiber lupin bread led to decreased post-meal ghrelin levels, increased satiety and lower short-term energy intake compared to wheat bread in a study by Lee et al. (2006).

In contrast, the study by Hall et al. (2005b) showed that consumption of lupin-containing bread did not affect satiety perception and food intake. The authors attributed this lack of effect to the small number of participants resulting to insufficient statistical power.

The evidence that lupin-wheat bread compared to wheat-only bread can increase post-prandial satiety reduction short term energy intake suggest that long term replacement of wheat bread by lupin bread may result in weight loss in overweight or obese people. However, long-term studies have not demonstrated significant effects on lowering body weight or maintenance of weight loss in overweight and obese adults after either a 16- wk (Hodgson et al., 2010) or a 12-mo (Belski et al., 2011b) intervention of regular consumption of lupin-wheat bread compared to wheat-only bread. The authors reasoned that the positive effects of lupin-enriched bread on short-term appetite and energy intake was offset by other dietary, lifestyle and environmental factors that may influence energy balance in the long-term.

Type 2 diabetes mellitus biomarkers

Commonly used biomarkers to rank foods for their potential for reducing risk of development of type 2 diabetes mellitus are post-prandial glycaemia (American Diabetes Association, 2001) and glycaemic index (a property of carbohydrate-containing food that can predict postprandial glycaemia) (Alfenas and Mattes, 2005) and post-prandial insulinaemia and fasting blood glucose and insulin after long-term dietary intervention (Anderson, 2005). Studies investigating the effect of lupin consumption on these biomarkers are presented in Table 5.

The findings of post-prandial studies of lupin bread consumption on glycaemia and insulinaemia are conflicting. Lee et al. (2007) reported lower post-meal plasma glucose and insulin response after consumption of lupin bread compared to wheat bread. The authors

 ACCEPTED MANUSCRIPT

explained that with the matched energy intake at breakfast, lowering the total glycaemic carbohydrate load of the ASL bread breakfast (which is lower in starch components compared to wheat) was the main reason for the lowering effect of lupin on plasma glucose and insulin response. Hall et al (2005b) reported that addition of ASL flour to wheat bread lowered the post-meal plasma glucose response but increased insulin response. The authors attributed the lowering of the glucose levels to the: (1) higher protein content of lupin bread; (2) higher dietary fiber content of lupin bread; (3) presence of phytochemicals in lupin bread that could slow down starch digestion and glucose absorption processes, and (4) lupin bread components such as oligosaccharides, phytic acid, tannins and saponins which may have glycaemia lowering properties. Hall et al (2005b) postulated that the increased insulinaemia after consumption of lupin bread might be due to amino acids such as arginine and phenylalanine and to stearic acid present in ASL.

Beneficial effects reported for lupin on blood glucose may also be due to the presence of the peptide, ɔ-conglutin which accounts for 4-5% of total proteins in mature lupin seed (Duranti et al., 2008). This was reported to reduce blood glucose in humans (Bertoglio et al., 2011). The authors used purified lupin protein with 47% ɔ-conglutin. There is however no study that tested the effects of ɔ-conglutin when incorporated into bread nor the effectseffects of processing (i.e. breadmaking) on ɔ-conglutin.

Johnson et al (2003) reported no differences between the post-prandial plasma glucose and insulin responses of a breakfast containing refined wheat bread with added lupin fiber compared to refined wheat-only bread. This imply that the proteins (and ɔ-conglutin), and perhaps the phytochemicals present in the lupin flour and not the purified lupin fiber, may be responsible for the glycaemia and insulinaemia lowering effects seen in the flour studies (Hall et al., 2005a; Hall et al., 2005b; Lee et al., 2007)

Long-term consumption (i.e 1 and 4 months) of lupin bread did not affect glucose and insulin levels in healthy or overweight/obese subjects (Belski et al., 2011b; Hall et al., 2005a; Hodgson et al., 2010). The authors attributed this lack of observed effect to the difficulty of observing changes in these biomarkers when the baseline values (e.g. fasting glucose) were all within the normal range. Therefore in future studies on the type 2 diabetes protective effect of lupin foods, it is recommended that participants such be at high risk; such as those with insulin resistance. Longer term intervention (12 months) , likewise did not reduce fasting glucose levels but did reduce fasting insulin following weight loss after 4 months (Belski et al., 2011b). The authors suggested that longer term consumption (> 4 months) of lupin bread may lead to improved insulin sensitivity due to its high-protein and dietary fiber contents.

Cardiovascular disease (CVD) biomarkers

Health substantiation studies of functional foods commonly use CVD biomarkers such as total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), HDL-C: LDL-C ratio, triglycerides and blood pressure (Herder et al., 2011). In a study by Hall et al (2005a), consuming meals with lupin kernel fiber-supplemented baked compared to meals with wheat-only baked products for 28 days, resulted in a beneficial decrease in TC, LDL-C, TC: HDL-C ratio, LDL-C: HDL-C, ratio and triglycerides, but did not change HDL-C levels. The beneficial effect of lupin dietary fiber on CVD biomarkers may have been due to its high WBC which may have had a cholesterol-lowering effect by increasing viscosity in the gastrointestinal tract and thus reduced the diffusion rate of bile acids, inhibiting

ACCEPTED MANUSCRIPT

their re-absorption (Zacherl et al., 2011). According to Hall et al (2005a), the residual proteins in the lupin kernel fiber may also have played a role in modifying the CVD biomarkers. A recent review by Cam and Mejia (2012) highlighted the potential role of dietary proteins and peptides, including lupin proteins to reduce CVD risk biomarkers. This was demonstrated in the consumption of food products (i.e. beverage and dietary bars) containing lupin proteins (Naruszewicz et al., 2007; Sirtori et al., 2012).

No significant effects on TC, LDL-C, HDL-C:LDL-C ratio, triglyceride but beneficial decrease in HDL-C were found by Hodgson et al. (2010) after participants consumed lupin flour-supplemented bread compared to wheat-only bread for 16 wk. According to the authors, the lack of effects of lupin on CVD biomarkers may be due to the: (1) inadequate amount of protein contributed by lupin flour to bring beneficial result, and (2) high baseline dietary fiber intake of the subjects. These conflicting results with the findings of Hall et al (2005a) was attributed to the use lupin kernel fiber by Hall et al (2005a) instead of lupin flour, and the difference in the amount of total dietary fiber consumed by the subjects of the conflicting studies. Hodgson et al. (2010) argued that lupin fiber used by Hall et al (2005a) was more effective in improving the CVD biomarkers because: (1) isolation and purification of kernel fiber may have altered its chemical structure and physical properties, and (2) use of purified kernel fiber delivered more total dietary fiber in the diet compared to lupin flour.

Blood pressure is another important CVD biomarker for which the effect of lupin bread consumption has been measured. Consumption of lupin bread for 16 wk resulted to lowered blood pressure compared to wheat-only bread (Belski et al., 2011b; Lee et al., 2009; Yang et al., 2010). The authors suggested that the beneficial effects observed were related to the high protein

 ACCEPTED MANUSCRIPT

content, specifically the amino acid arginine, found at high levels in lupin protein, and polyphenols in lupin.

Colonic health biomarkers

Commonly used biomarkers for effect of functional foods on colonic health include intestinal transit time, frequency of defecation, stool weight and SCFA (Meyer and Stasse-Wolthuis, 2009). Two studies have reported the effect of lupin consumption on biomarkers for colonic health (Table 5). Four week addition of lupin kernel dietary fiber to the diet has demonstrated improvements in bowel function, lowered faecal pH, and increased butyrate levels in faeces.

The authors suggested that these effects were due to fiber fermentation in the colon and high waterï binding capacity of the residual fiber in the faeces (Johnson et al., 2006). Lupin fiber addition to the diet was also reported to increase the levels of the potentially beneficial *Bifidobacterium* spp. in the faeces whilst reducing levels of the potentially pathogenic clostridia group; and thus was classified as a ñprebioticò by the authors (Smith et al., 2006).

Glycaemic index (GI) lowering potential

GI refers to the incremental area under the blood glucose response curve (**AUC**) within a 2- hr period from consuming food (e.g. lupin bread) containing 50 g of available CHO, relative to the AUC produced by 50 g of glucose or white bread (Chiu et al., 2011). It is mainly used for the purpose of labelling food products to guide consumers in their food intake. According to Buyken et al (2010), GI of food in the diet has a direct relationship with risk of type 2-diabetes. Lupin bread has demonstrated lower GI than refined wheat bread (Hall et al., 2005b). However,

 ACCEPTED MANUSCRIPT

caution should be noted in claiming that lupin flour *per se* has low GI because it has a negligible amount of available starch and thus its GI cannot be measured.

Gluten-free

Celiac disease is an autoimmune intestinal disorder caused by permanent intolerance to gluten affecting ~1% of the general population (Niewinski, 2008). The increasing number of diagnosed cases of celiac disease prompted an increase in the demand for gluten-free products such as breads. Lupin, like any other grain legumes, is gluten-free and studies have investigated lupin flour use in gluten-free products such as cakes (Levent and Bilgiçli, 2011), and pasta (Capraro et al., 2008). However, there appears to be no published study reporting the use of lupin flour for gluten-free bread formulation. Lupin could however be a suitable substitute to the genetically-modified, more expensive and high-phytoestrogen soybean flour, in producing gluten-free bread.

Perceived health benefit as a non-genetically-modified (GM) food

One major advantage of lupin compared to other legumes (i.e. soybean) is its non GM status (Dijkink,et al., 2008; Pedersen and Gylling, 2000). Due to the ñperceivedò health and environmental risks of generic modification of food and food ingredients, consumers are now demanding more of natural food products (Bredahl, 2001), such as lupin. The more widespread use of lupin as an ingredient in bread could address the growing consumer desire for non-GM products.

Challenges

The high protein and dietary fiber levels in lupin flour can pose some nutritional and health-related challenges when incorporated into bread. The challenges include: lupin allergenicity; presence of flatulence inducing oligosaccharides; presence of potentially toxic lupin alkaloids and; contamination with phomopsin fungal toxins.

Lupin allergy

Severe allergenic responses to lupin consumption have been recorded (Hieta et al., 2009; Reis et al., 2007; Sanz et al., 2010). A cross-reactivity study using blood samples from 34 subjects with peanut allergy and 5 non-peanut allergenic participants indicated that the allergenicity was due mainly to the Ǔ-conglutin peptide of lupin (Sirtori et al., 2011). Beta-conglutin and ɔ- and ŭ- conglutin peptides, have also been identified as causes of anaphylactic and other allergenic reactions from foods containing lupin (Jappe and Vieths, 2010; Sanz et al., 2010). Several food processing methods have been investigated to reduce the allergenic effects of lupin, including extrusion, autoclaving, boiling and microwave heating (Alvarez-Alvarez et al., 2005) and steam pressure at high temperature and short time (Guillamón et al., 2008). Extrusion, boiling and microwave heating had no significant effect on lupin allergenicity (Alvarez-Alvarez et al., 2005). Autoclaving of lupin seeds at 138^{o}C for 30 min and controlled pressure drop at 6 bar for 3 min destroyed its allergenic potency without affecting acceptance of the lupin bread as judged by an expert panel (Guillamon et al., 2010).

Flatulence

A potential drawback of the use of lupin flour is the presence of high levels of raffinose family of oligosaccharides (RFOs), raffinose, stachyose, and verbascose, which can cause flatulence (Martínez-Villaluenga et al., 2006). It was reported that raw lupin seeds contain 7-15%

of RFOs, the highest level amongst all types of grain legumes (Martinez-Villaluenga, et al., 2005). Oligosaccharides cause flatulence since they are not hydrolysed nor absorbed in the small intestine but instead enter the colon where they are rapidly fermented by colonic microflora (Price et al., 1988). Soaking of legumes, including lupin, has been used as a pre-treatment to reduce their levels of oligosaccharides and hence their flatulence potential (Fernandes et al., 2010)

Quinolizidine alkaloids (QA)

A potential food safety issue of lupin consumption is the presence of bitter QAs (Resta et al., 2008) which can result in moderate acute toxicity (Erbas, 2010). According to Resta et al. (2008), QA intoxication in mammals results in trembling, shaking, excitation and convulsion, and moderate oral toxicity can lead to loss of motor coordination and control. Breeding of varieties low in QAs, the ñsweetò varieties such as ASL, has decreased the QA to safe levels (Pilegaard and Gry, 2008). Australian (FSANZ, 2011a) and Great Britain (MAFF-DOH, 1996) standards state that the QA content of lupin and lupin products (i.e. flour) should not exceed 200 mg/kg. Sujak et al. (2006) reported that lupin seeds may contain 118-650 mg/kg alkaloids, however processing of the seeds can significantly decrease their levels. Traditionally lupins were soaked and boiled to eliminate QAs (Annicchiarico et al., 2010). Defatting and drying of lupin seeds (El-Adawy et al., 2001) and dilution by incorporation into food products (Resta et al., 2008) have been reported to decrease the amount of alkaloids in the final product. Evaluation of lupin food products available in the Swiss market show that all samples tested had alkaloid contents below the maximum levels as legislated in Australia and Great Britain (Reinhard et al., 2006).

ACCEPTED MANUSCRIPT

Phomopsins

Lupins, similar to other grains and grain legumes, may be contaminated with phomopsin, the mycotoxins produced by the fungus *Diaporthe toxica* (known formerly as *Phomopsis leptostromiformis*) (EFSA, 2012). Phomopsin causes the liver disease *lupinosis* in sheep which can cause death (Prieto-Simón et al., 2007), and these compounds may be a potential health risks to humans. Australia and Great Britain have set the limit for phomopsin content in lupin foods at 0.005 mg/kg (FSANZ, 2011a; MAFF-DOH, 1996). Control of phomopsin relies on breeding resistant varieties (Kurlovich et al., 2002), which has translated to phomopsin-free lupin food products in the Swiss market (Reinhard et al., 2006).

TECHNOLOGICAL FUNCTIONALITY OF LUPIN IN BREAD MANUFACTURING

Benefits

The protein and fiber components of lupin flour can have potential to profoundly influence the technological aspect of bread manufacture including bread process efficiency, and dough and sensory acceptability qualities. Published reports have demonstrated that a substitution rate of ~10%, lupin could provide the following beneficial effects during bread making (Table 6): increased dough stability, dough tolerance, loaf volume and weight; decreased mixing time; improved tolerance to mixing and handling during fermentation; delayed staling and bread firmness after 24 h storage. Sensory properties of bread were also not affected at a substitution rate of 9% lupin flour to wheat flour (Mubarak, 2001). Studies on other baked products such as biscuits, gluten-free cakes, and muffins have reported that lupin flour incorporation rates of 20-30% can be achieved without reducing sensory quality and

ACCEPTED MANUSCRIPT

acceptability (Jayasena and Nasar-Abbas, 2011; Levent and Bilgiçli, 2011; Nasar-Abbas and Jayasena, 2012).

Protein crosslinking

The crucial step of gluten matrix formation during bread dough mixing can be explained in part by protein crosslinking, which is the formation of covalent or non- covalent bonds between amino acid side chains in polypeptides, either within a protein or between proteins (Feeney and Whitaker, 1988). Two types of protein crosslinks have been identified during gluten development in bread: disulphide and dityrosine (Gerrard et al., 2005). Disulphide crosslinks are produced from two cysteine residues that are adjacent within a food protein matrix (Lindsay and Skerritt, 1999), while dityrosine crosslinks are formed between while dityrosine crosslinks are formed between two or three tyrosine residues (Tilley et al., 2001).

Lupin does not contain gluten but contains globulins and albumins comprising of cysteine and tyrosine residues. Mean cysteine and tyrosine levels for lupin is 1.6 and 1.83 g/100g protein (Sujak, et al., 2006), while it is 2.2 and 1.4 g/100 g protein for wheat (Shoup et al., 1966), respectively. The availability of cysteine and tyrosine residues in lupin proteins may assist in the development of crosslinks between lupin and gluten proteins and thus form the needed structure for dough and bread.

The amount of disulphide bonds and the strength of these bonds influence the rheological properties of dough (Shewry and Tatham, 1997) and that optimal disulphide crosslinking during dough mixing is important in bread making (Buchert et al., 2010). In contrast, some studies show that the levels of disulphide bonds can either negatively affect (Manu and Prasada Rao, 2008) or have no effect (Poulsen, 1998) on dough and bread quality. Reports on the relationship of dityrosine or tyrosine concentration with dough or bread quality were also conflicting. Amonsou et al. (2012) demonstrated that high levels of tyrosine (9.12 g/100g) in marama protein led to formation of dityrosine crosslinks in the peroxidase-treated dough, which was described as highly viscous and extensible (viscoelastic). However, other investigators showed that dityrosine levels did not influence gluten formation (Pena et al., 2006; Rodriguez Mateos et al., 2006) and consequently dough and bread quality.

The contrasting evidences on how disulphide and dityrosine crosslinks influence wheat dough and bread quality may also apply to lupin-wheat dough and bread. The beneficial results of up to 10% lupin flour incorporation to wheat bread may be attributed to possible crosslinking between wheat and lupin proteins. Bread making processes such as mixing, proofing and baking lead to formation of dityrosine (Rodriguez Mateos et al., 2006) and disulphide (Gerrard et al., 2005) and thus may also affect the influence of these crosslinks to lupin-wheat dough and bread quality.

However, as previously discussed (Table 6) only a maximum 10% substitution of wheat flour by lupin flour can be applied before quality of dough and bread deteriorates. There is therefore a need to investigate how to maximize the potentially beneficial effects of optimizing protein cross-linking in lupin breads. There are no reported studies that have investigated whether disulphide or dityrosine crosslinks are formed in neither lupin dough nor their impact on dough and bread quality.

Most of the reported studies (Table 6) have used the straight-dough method for lupin bread manufacturing. However the sponge-dough process may be a more effective method for incorporating lupin into bread because of its robustness to process fluctuations (i.e. mixing and

 ACCEPTED MANUSCRIPT

proofing). It may be useful to establish separate mixing and proofing parameters (i.e. time and/or temperature) for wheat sponge and lupin sponge given the differences in their rheological properties. This would allow the wheat gluten matrix to develop initially without disruption of the low-elasticity proteins and high water binding of dietary fibers in lupin flour and thus may help reduce the negative effects of lupin flour addition

Anti-staling properties

Ronda and Roos (2011) defined staling in bread as hardening of the crumb mainly caused by starch retrogradation in which water distribution plays a critical role. During storage of bread, moisture is redistributed from gluten to starch allowing for crystallization of starch (i.e. amylopectin retrogradation) and dehydration of gluten both of which result to crumb hardening (Gray and Bemiller, 2003). Moisture migration from crumb to crust can also lead to crumb staling and increase firming rate (Baik and Chinachoti, 2000). According to Hug-Iten et al (2003) that besides the molecular order of starch, the extent of network plasticisation is an important determinant of crumb firmness and water is the most important plasticiser in food; highlighting that high water absorption during mixing, proofing and baking of bread can delay staling. The high WBC of lupin has potential to lead to staling-inhibition through: by preventing gluten dehydration and slow down firming rate by retaining more moisture in the crumb; and providing plasticising function. According to Gray and Bemiller (2003) proteins also have a function in the delay of bread staling. The authors cited that proteins dilute and interact with starch and reduce the extent of starch retrogradation, and serve as a moisture reserve to reduce firming rate. In support of this, substitution of wheat flour with 10% lupin protein isolates has been reported to delay bread firming (Paraskevopoulou, et al., 2010). There is however only limited information on the effects of lupin dietary fiber and protein on bread staling.

Challenges

The main quality problem arising lupin incorporation into wheat bread is low loaf volume and poor texture, mainly due to the low-elasticity of lupin proteins and the high water binding capacity of lupin dietary fiber. Microscopic examination of wheat and lupin flour doughs has revealed that the gluten matrix was less interconnected in the presence of lupin proteins (Güemes-Vera et al., 2004).

There are published studies on how to improve the quality of bread supplemented with gluten-free flours which may be applicable to lupin flour. High-pressure processing of flours and the use of ñbread improversò were found to have positive effects on the quality of the non-wheat flour dough and bread. Angioloni and Collar (2012a) reported that high-pressure treatment of non-wheat flours (i.e. oats, millet and sorghum) resulted in more acceptable breads than untreated flours even at substantial (40-60%) rate of wheat flour substitution. The authors noted that high pressure treatment led to dough strengthening due to the development of protein network and/or intra- and inter-molecular disulphide bonds leading to more acceptable breads compared to untreated samples. This may be due to the pressure-induced denaturation of proteins leading to increased reactivity of sulfhydryl bonds and higher disulphide crosslinking (Galazka et al., 2000). No study however has used high-pressure treatment with the aim of improving the quality of lupin-supplemented bread.

The use of bread improvers has been widely used to improve quality of breads supplemented with non-wheat flours. Joye et al. (2009) summarized the various ñbread improversò which are either chemicals (i.e. Potassium bromate, iodate, chlorine dioxide azocarbonamide, ascorbic acid and peroxides) or enzymes (i.e. Transglutaminase, glucose oxidase, hexose oxidase, and laccase), which promote the formation of covalent bonds between gluten proteins during bread making. The authors also presented the mechanisms which explain how each of these additives helps in improving dough and bread quality. In general, the chemical agents act as oxidants of the cysteine (SH) residues and tyrosine (phenolic) residues to form crosslinks. Enzymes act as catalysts in the oxidation of the same residues to produce disulphide and dityrosine crosslinks, or in the case of transglutaminase, the crosslinking of lysine and glutamine residues.

The use of chemical agents has been a major safety concern for consumers and thus enzymes are considered as safer alternatives in bread making. The use and effects of enzymes in non-wheat flour supplemented- or gluten-free breads which may be applicable to lupin bread, were explored by several investigators (Alaunyte et al., 2012; Gujral and Rosell, 2004; Renzetti and Arendt, 2009; Renzetti et al., 2010; Ribotta et al., 2010; Roccia et al., 2012). There is a need to investigate the use and effects of enzymes for protein-crosslinking in lupin-wheat bread making and the association between crosslinking level and dough and bread quality.

The use of enzymes and bioprocessing techniques has been widely applied to improve the quality of high-fiber baked products and therefore such approach may be applicable to lupin bread. An example is xylanase that degrades and reduces the water binding properties of non-starch polysaccharides (NSP) (Courtin et al., 2001) leading to a redistribution of water from the NSP to the gluten matrix (Shah et al., 2006). Sourdough fermentation can enhance quality of fiber-supplemented breads, in particular lupin-wheat bread. A study by Bartkiene et al. (2011)

ACCEPTED MANUSCRIPT

showed that the sourdough fermentation by *Pediococcus acidilacti* of wheat flour substituted with 10% lupin flour resulted in better bread quality compared to untreated samples. According to Ktenioudaki and Gallagher (In Press), sourdough fermentation alters dough components through acidification, proteolysis of gluten and starch hydrolysis contributing to improve quality in high-fiber breads, such as lupin-wheat bread. Sourdough fermentation of wheat with coarse durum wheat bran, (Rizzello et al., 2012) and composite non-wheat flours (i.e. buckwheat, amaranth, chickpea, and quinoa flours) (Coda et al., 2010) resulted in improved textural, sensory and nutritional properties compared to unfermented samples and may be applicable to lupin. To date, there are very few studies on the potential of sourdough fermentation for high quality lupin-wheat bread making.

The optimization of water incorporation rate is a critical parameter to maximize the quality of dough and bread. Most studies on lupin bread formulation have however have not focused on this aspect (Table 6). In some studies, the amount of water used for the control breads (wheat bread) were the same for the lupin-wheat breads (Guillamon et al., 2010; Pollard et al., 2002). It has been explained that the high water binding capacity of lupin dietary fiber or protein disrupts gluten matrix when substituted for wheat flour in bread. This necessitates the consequent adjustment of added water to compensate for the water tightly bound by the dietary fiber and protein in lupin flour. Likewise, most of the researchers investigated the effects of discrete levels for water and lupin incorporation rates without examining the interactive effects of these two parameters on the quality of lupin-wheat dough and bread. In addition, no in depth process optimization studies have been reported for lupin-wheat bread which has aimed to simultaneously optimize multiple processing parameters such as: mixing time; proofing time and; baking time and temperatures.

A useful methodological and statistical approach for optimization of bread formulation and processing is response surface methodology (RSM). RSM is a collection of mathematical and statistical methods that are efficient in the modelling and analysis of experiments or situations in which an output or response of interest is dependent on several factors, and the aim is to optimize the response (Montgomery, 2009). RSM had been used to optimize formulation and process parameters of other ñhealthyò breads such as wholemeal oat bread (Flander et al., 2007) and gluten-free breads (McCarthy et al., 2005; Sanchez et al., 2004). Likewise, studies have been reported to optimize both the formulation and processing method of breads made from blends of wheat and legume flours using statistical designs including RSM (Angioloni and Collar, 2012b; Jideani and Onwubali, 2009; Yamsaengsung et al., 2010). Similar optimization studies are still required to optimize both the formulation and processing variables in lupin-wheat bread manufacture to maximize lupin addition whilst maintaining acceptable sensory quality and consequently maximize the nutritional and health potential of the bread.

Another challenge for the incorporation of lupin into wheat bread is the potential for undesirable aftertaste. Sensory evaluation of baked products with lupin flour showed that consumers detected aftertaste or unusual taste (Hall and Johnson, 2004). Incorporation of more than 30% lupin flour in muffins and 20% in biscuits lowered flavor acceptance of the products, which was attributed to a beany flavor imparted by lupin (Jayasena and Nasar-Abbas, 2011; Nasar-Abbas and Jayasena, 2012). Lupin flour from *L. angustifolius cv Boregine* has been described as having a grassy, metallic, fatty, hay-like, meat-like and cheese-like odour

ACCEPTED MANUSCRIPT

characteristics (Bader et al., 2009). Volatile compounds were also detected when lupin protein isolate (LPI) was added to bread (Paraskevopoulou et al., 2012).

Studies have been conducted to resolve the issue of off-odours/ flavors in lupin-based foods. It has been reported that roasting lupin seeds may help remove its ñbeanyò flavor (Yañez et al., 1986). A patented method for *L. albus* flour suggests an optional step of heating seeds to destroy lipoxygenase and thus prevent rancidity and render flour a longer shelf-life (Auger and Corre, 1993). De-oiling of lupin flakes with ethanol and 2-propanol resulted in protein isolates with less ñlegume-likeò flavor improved consumer acceptance and functionality (Bader et al., 2011). Volatile compounds produced from lactic acid or sour-dough fermentation of lupin protein extracts (and possibly lupin dough) with sweet, solvent, fungal, musty, earthy, burnt, dusty or cereal-like characteristics masked the undesirable odorants in lupin (Schindler et al., 2011). This was evident in the higher overall and flavor acceptance scores of breads with sourdough fermented lupin flours compared to unfermented samples (Bartkiene et al., 2011).

OTHER FOOD USES OF LUPIN

Aside from wheat-based food products, lupin has been used in a wide range of other foods. Lupin fiber and protein isolates have been used as fat replacers and vegetable protein extenders in meat products such as sausages and frankfurters (Alamanou et al., 1996; Archer et al., 2004). Lupin also has the potential to be used in dairy products such as ice cream (Yap, 2006) and fermented milk (Martínez-Villaluenga & Gómez, 2007). Due to its yellow color and emulsifying properties, lupin may also be used as a substitute to egg yolks in brioche (Kohajdova

ACCEPTED MANUSCRIPT

et al., 2011) or egg glazes (Rodgers, 2004). Jayasena et al (2010) developed a lupin-based tofu analogue. Other Asian fermented foods that have successfully incorporated lupin include tempe (a traditional Indonesian food), miso (Japanese condiment and soup base) and soy sauce (Sipsas, 2008).

CONCLUSIONS

This paper presented a comprehensive analysis of scientific work on the nutritional, health and technological functionality of lupin flour addition to bread and other baked products. Scientific evidences show that incorporation of lupin flour into baked products is accompanied by both benefits and challenges in terms of nutritional, health and technological functionality. The high protein and dietary fiber contents of lupin provide a ñdouble-edgedò effect when substituted for wheat flour in bread. Evidence has been presented that supplementing baked products with lupin flour improves nutritional profile mainly through increased protein and dietary fiber. There is mounting evidence that these lupin products when included in the diet can reduce biomarkers of risk of obesity, cardiovascular diseases, type 2 diabetes mellitus and some forms of cancers. In addition, lupin is gluten-free, low in anti-nutritional factors compared to other legumes, and has antioxidant activities. Lupin may also be a better alternative to soybeans as it is not genetically modified, has lower levels of phytoestrogens and is lower in cost. On the other hand, lupin protein allergens, dietary fiber-induced flatulence, and to a minor extent, alkaloids and phomopsins, pose some health issues in the use of lupin flour in baked products.

Investigations of lupin flour incorporation into baked products demonstrated that a 10% rate substitution to wheat flour resulted in equal or better quality compared to wheat-alone bread.

The positive effects may be due to the increased formation of protein crosslinks (i.e. disulphide and dityrosine) between lupin and wheat proteins. Technological drawbacks such as lowered volume, denser pore structure and firmer crumb in the final product are common when lupin substitution was beyond 10%. These negative effects may be attributed to the low-elasticity of lupin proteins, and high water binding of its dietary fiber; both of which interrupt the development of the desired wheat gluten network. The review highlighted the lack of evidence on the effects of flour derived from different lupin species/varieties when incorporated to bread. Likewise, there is a need to investigate the effects of bread making on the potentially anti-diabetic peptide ɔ- conglutin, and the role of protein crosslinking in lupin-wheat dough and bread and how it can be optimized. There is also a lack of information on the anti-staling function lupin in bread. Lastly, systematic optimization of the formulation and processing parameters of lupin-wheat bread, to maximize lupin incorporation rate and nutritional benefits whilst maintaining or improving quality.

ACKNOWLEDGMENTS

The authors would like to thank the Grains Research and Development Corporation and Curtin University for funding.

 ACCEPTED MANUSCRIPT

REFERENCES

ADA (2005). Position of the American Dietetic Association: fat replacers. *Journal of the American Dietetic Association* **105,** 266-275.

Alamanou, S., Bloukas, J. G., Paneras, E. D., & Doxastakis, G. (1996). Influence of protein isolate from lupin seeds (Lupinus albus ssp. Graecus) on processing and quality characteristics of frankfurters. *Meat Science,* **42**(1), 79-93.

Alaunyte, I., Stojceska, V., Plunkett, A., Ainsworth, P. and Derbyshire, E. (2012). Improving the quality of nutrient-rich Teff (Eragrostis tef) breads by combination of enzymes in straight dough and sourdough breadmaking. *Journal of Cereal Science* **55,** 22-30.

Alfenas, R. C. G. and Mattes, R. D. (2005). Influence of glycemic index/load on glycemic response, appetite, and food intake in healthy humans. *Diabetes Care* **28,** 2123-2129.

Alvarez-Alvarez, J., Guillamon, E., Crespo, J. F., Cuadrado, C., Burbano, C., Rodriguez, J., Fernandez, C. and Muzquiz, M. (2005). Effects of extrusion, boiling, autoclaving, and microwave heating on lupine allergenicity. *Journal of Agricultural and Food Chemistry* **53,** 1294-1298.

American Diabetes Association (2001). Postprandial blood glucose. *Diabetes Care* **24,** 775-778.

Amonsou, E. O., Taylor, J. R. N., Naushad Emmambux, M., Gyebi Duodu, K. and Minnaar, A. (2012). Highly viscous dough-forming properties of marama protein. *Food Chemistry* **134,** 1519-1526.

Anderson, D. C. (2005). Pharmacologic prevention or delay of type 2 diabetes mellitus. *The Annals of Pharmacotherapy* **39,** 102-109.

Angioloni, A. and Collar, C. (2012a). Effects of pressure treatment of hydrated oat, finger millet and sorghum flours on the quality and nutritional properties of composite wheat breads. *Journal of Cereal Science* **56,** 713-719.

Angioloni, A. and Collar, C. (2012b). High legume-wheat matrices: an alternative to promote bread nutritional value meeting dough viscoelastic restrictions. *European Food Research and Technology* **234,** 273-284.

Annicchiarico, P., Harzic, N. and Carroni, A. M. (2010). Adaptation, diversity, and exploitation of global white lupin (Lupinus albus L.) landrace genetic resources. *Field Crops Research* **119,** 114-124.

Anon (2011a). Australian Sweet Lupin Flour Technical Data Sheet, Irwin Valley: Western Australia

Anon (2011b). Global bakery products market to reach US$410 billion by 2015. *Food Australia* **63,** 134.

Archer, B. J., Johnson, S. K., Devereux, H. M. and Baxter, A. L. (2004). Effect of fat replacement by inulin or lupin-kernel fiber on sausage patty acceptability, post-meal perceptions of satiety and food intake in men. *British Journal of Nutrition* **91,** 591-599.

Atwell, W. A. (Ed) (2001). *Wheat Flour*, American Association of Cereal Chemists.

Auger, I. and Corre, V. (1993). Whole flour from lupin, procedure for obtaining and applications thereof. Austalian Patent AU-B-80457/91.

ACCEPTED MANUSCRIPT

Bader, S., Czerny, M., Eisner, P. and Buettner, A. (2009). Characterisation of odour-active compounds in lupin flour. *Journal of the Science of Food and Agriculture* **89,** 2421-2427.

Bader, S., Oviedo, J. P., Pickardt, C. and Eisner, P. (2011). Influence of different organic solvents on the functional and sensory properties of lupin (*Lupinus angustifolius L*.) proteins. *LWT - Food Science and Technology* **44,** 1396-1404.

Baik, M. Y. and Chinachoti, P. (2000). Moisture redistribution and phase transitions during bread staling. *Cereal chemistry* **77,** 484.

Bakke, A. and Vickers, Z. (2007). Consumer liking of refined and whole wheat breads. *Journal of Food Science* **72,** S473-S480.

Ballester, D., Castro, X., Cerda, P., Garcia, E. and Yanez, E. (1988). Bread quality and nutritional value of ôMarraquetaô and ôHallullaô supplemented with full-fat sweet lupin flour (*Lupinus albus cv. Multolupa*). *International Journal of Food Science & Technology* **23,** 225-231.

Bartkiene, E., Juodeikiene, G., Vidmantiene, D., Viskelis, P. and Urbonaviciene, D. (2011). Nutritional and quality aspects of wheat sourdough bread using L. luteus and L. angustifolius flours fermented by Pedioccocus acidilactici. *International Journal of Food Science and Technology* **46,** 1724-1733.

Belski, R., Mori, T. A., Puddey, I. B., Sipsas, S., Woodman, R. J., Ackland, T. R., Beilin, L. J., Dove, E. R., Carly.o.n, N. B., Jayaseena, V. and Hodgson, J. M. (2011b). Effects of lupin-enriched foods on body composition and cardiovascular disease risk factors: a 12-month randomized controlled weight loss trial. *International Journal of Obesity* **35,** 810-819.

Benelam, B. (2009). Satiation, satiety and their effects on eating behaviour. *Nutrition Bulletin* **34,** 126-173.

Bertoglio, J. C., Calvo, M. A., Hancke, J. L., Burgos, R. A., Riva, A., Morazzoni, P., Ponzone, C., Magni, C. and Duranti, M. (2011). Hypoglycemic effect of lupin seed [gamma]-conglutin in experimental animals and healthy human subjects. *Fitoterapia* **82,** 933-938.

Blom, W. A., Lluch, A., Stafleu, A., Vinoy, S., Holst, J. J., Schaafsma, G. and Hendriks, H. F. (2006). Effect of a high-protein breakfast on the postprandial ghrelin response. *The American Journal of Clinical Nutrition* **83,** 211-220.

Boschin, G. and Arnoldi, A. (2011). Legumes are valuable sources of tocopherols. *Food Chemistry* **127,** 1199-1203.

Bredahl, L. (2001). Determinants of consumer attitudes and purchase intentions with regard to genetically modified food ï results of a cross-national survey. *Journal of Consumer Policy* **24,** 23-61.

Buchert, J., Ercili Cura, D., Ma, H., Gasparetti, C., Monogioudi, E., Faccio, G., Mattinen, M., Boer, H., Partanen, R., Selinheimo, E., Lantto, R. and Kruus, K. (2010). Crosslinking food proteins for improved functionality. *Annual Review of Food Science and Technology* **1,** 113-138.

Buyken, A. E., Mitchell, P., Ceriello, A. and Brand-Miller, J. (2010). Optimal dietary approaches for prevention of type 2 diabetes: a life-course perspective. *Diabetologia* **53,** 406-418.

Cam, A. and de Mejia, E. G. (2012). Role of dietary proteins and peptides in cardiovascular disease. *Molecular Nutrition & Food Research* **56,** 53-66.

 ACCEPTED MANUSCRIPT

Capraro, J., Magni, C., Fontanesi, M., Budelli, A. and Duranti, M. (2008). Application of two-dimensional electrophoresis to industrial process analysis of proteins in lupin-based pasta. *Lwt-Food Science and Technology* **41,** 1011-1017.

Cauvain, S. P. and Young, L. S. (Eds) (2007). *Bread-the product in Technology of Breadmaking* Springer Science: NY.

Chew, P. G., Casey, A. J. and Johnson, S. K. (2003). Protein quality and physico-functionality of Australian sweet lupin (Lupinus angustifolius cv. Gungurru) protein concentrates prepared by isoelectric precipitation or ultrafiltration. *Food Chemistry* **83,** 575-583.

Chiu, C. J., Liu, S., Willett, W. C., Wolever, T. M., Brand-Miller, J. C., Barclay, A. W. and Taylor, A. (2011). Informing food choices and health outcomes by use of the dietary glycemic index. *Nutrition reviews* **69,** 231-242.

Clark, R. and Johnson, S. (2002). Sensory acceptability of foods with added lupin (Lupinus angustifolius) kernel fiber using pre-set criteria. *Journal of Food Science* **67,** 356-362.

Coda, R., Rizzello, C. G. and Gobbetti, M. (2010). Use of sourdough fermentation and pseudo-cereals and leguminous flours for the making of a functional bread enriched of ɔ-aminobutyric acid (GABA). *International Journal of Food Microbiology* **137,** 236-245.

Collado-Fernández, M. (2003). BREAD | Breadmaking Processes **In** Encyclopedia of Food Sciences and Nutrition pp. 627-634. Benjamin, C., Ed, , Academic Press, Oxford,

Courtin, C. M., Gelders, G. G. and Delcour, J. A. (2001). Use of two endoxylanases with different substrate selectivity for understanding arabinoxylan functionality in wheat flour breadmaking. *Cereal chemistry* **78,** 564-571.

 ACCEPTED MANUSCRIPT

Cowling, W., Buirchel, B. J. and Tapia, M. E. (1998). Lupin, *Promoting the conservation and use of underutilized and neglected crops,* International Plant Genetic Resources Institute: Western Australia.

Cowling, W. A. and Gladstones, J. S. (2000). Lupin breeding in Australia **In:** Linking Research and Marketing Opportunities for Pulses in the 21st Century, pp. 541-548. Knight, R., Ed., Kluwer Academic Publisher, Dordrecht.

Cowling, W. A. and Tarr, A. (2004). Effect of genotype and environment on seed quality in sweet narrow-leafed lupin (Lupinus angustifolius L.). *Australian Journal of Agricultural Research* **55,** 745-751.

de Kock, S., Taylor, J. and Taylor, J. R. N. (1999). Effect of heat treatment and particle size of different brans on loaf volume of brown bread. *Lebensmittel-Wissenschaft und-Technologie* **32,** 349-356.

Dervas, G., Doxastakis, G., Hadjisavva-Zinoviadi, S. and Triantafillakos, N. (1999). Lupin flour addition to wheat flour doughs and effect on rheological properties. *Food Chemistry* **66,** 67-73.

DHA-NHMRC (2005). Food for Health: Dietary Guidelines for Australians, A guide to healthy eating. Canberra.

Dijkink, B., Miedendorp de Bie, V. and Blom, W. (2008). Altering lupine flour for the industry. *12th International Lupin Conference Fremantle, Western Australia*, pp. 455-458.

Dikeman, C. L. and Fahey, G. C. (2006). Viscosity as related to dietary fiber: a review. *Critical Reviews in Food Science and Nutrition* **46,** 649-663.

Doxastakis, G., Zafiriadis, I., Irakli, M., Marlani, H. and Tananaki, C. (2002). Lupin, soya and triticale addition to wheat flour doughs and their effect on rheological properties. *Food Chemistry* **77,** 219-227.

Drakos, A., Doxastakis, G. and Kiosseoglou, V. (2007). Functional effects of lupin proteins in comminuted meat and emulsion gels. *Food Chemistry* **100,** 650-655.

Duodu, K. G. and Minnaar, A. (2011). Chapter 18 - Legume composite flours and baked goods: mutritional, functional, sensory, and phytochemical Qualities. **In:** Flour and Breads and their Fortification in Health and Disease Prevention, pp. 193-203. Preedy, V.R., Watson, R.R., and Patel, V.B. ,Eds, Academic Press,San Diego,

Duranti, M., Consonni, A., Magni, C., Sessa, F. and Scarafoni, A. (2008). The major proteins of lupin seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients. *Trends in Food Science & Technology* **19,** 624-633.

EFSA (2012). Scientific Opinion on the risks for animal and public health related to the presence of phomopsins in feed and food *EFSA Journal* **10**.

El-Adawy, T. A., Rahma, E. H., El-Bedawey, A. A. and Gafar, A. F. (2001). Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chemistry* **74,** 455-462.

Erbas, M. (2010). The effects of different debittering methods on the production of lupin bean snack from bitter *Lupinus albus L.* seeds. *Journal of Food Quality* **33,** 742-757.

Erbas, M., Certel, M. and Uslu, M. K. (2005). Some chemical properties of white lupin seeds *(Lupinus albus L.*). *Food Chemistry* **89,** 341-345.

Evans, A. J., Cheung, P. C. K. and Cheetham, N. W. H. (1993). The carbohydrate composition of cotyledons and hulls of cultivars of Lupinus angustifolius from Western Australia. *Journal of the Science of Food and Agriculture* **61,** 189-194.

Faki, H., Venkatarama, L. V. and Desikachar, H. S. R. (1984). Effect of processing on the in vitro digestibility of proteins and carbohydrates in some Indian legumes. *Plant Foods for Human Nutrition* **34,** 127-133.

FAO-STAT (2013). Crops-Lupins-Production Quantity 2011, FAO: Rome.

FAO/WHO (1991). Protein quality evaluation. Report of a jointvFAO-WHO expert consultation., FAO: Rome.

Feeney, R. E. and Whitaker, J. R. (1988). Importance of cross-linking reactions in proteins, *Advances in Cereal Science and Technology*: City, pp. 21-43.

Fernandes, A. C., Nishida, W. and Da Costa Proença, R. P. (2010). Influence of soaking on the nutritional quality of common beans (*Phaseolus vulgaris L*.) cooked with or without the soaking water: a review. *International Journal of Food Science & Technology* **45,** 2209-2218.

Flander, L., Salmenkallio-Marttila, M., Suortti, T. and Autio, K. (2007). Optimization of ingredients and baking process for improved wholemeal oat bread quality. *Lwt-Food Science and Technology* **40,** 860-870.

Foley, R. C., Gao, L.-L., Spriggs, A., Soo, L. Y. C., Goggin, D. E., Smith, P. M. C., Atkins, C. A. and Singh, K. B. (2011). Identification and characterisation of seed storage protein transcripts from Lupinus angustifolius. *Bmc Plant Biology* **11**.

French, B., Shea, G. and Buirchell, B. J. (2008). *Introduction and history,* GRDC: South Perth.

FSANZ (2011a). Australia New Zealand Food Standards Code - Standard 1.4.1 - Contaminants and Natural Toxicants.[Online] http://www.comlaw.gov.au/Series/F2008B00618. Accessed [28 April 2012]

FSANZ (2011b). NUTTAB 2010.[Online] http://www.foodstandards.gov.au/consumerinformation/nuttab2010/nuttab2010onlinesearchabledatabase/onlineversion.cfm?&action=getFood&foodID=02B10546. Accessed [28 April 2012]

Galazka, V. B., Dickinson, E. and Ledward, D. A. (2000). Influence of high pressure processing on protein solutions and emulsions. *Current Opinion in Colloid & Interface Science* **5,** 182-187.

Gerrard, J. A., Meade, S. J., Miller, A. G., Brown, P. K., Yasir, S. B. M., Sutton, K. H. and Newberry, M. P. (2005). Protein cross-linking in food. *Annals of the New Y.o.rk Academy of Sciences* **1043,** 97-103.

GL-PRO (2005). Guidelines for growing grain legumes in Europe.[Online] http://www.globalbioenergy.org/uploads/media/0503_AEP_-_Guidelines_for_growing_grain_legumes_in_Europe.pdf. Accessed [09 January 2013]

Gray, J. A. and Bemiller, J. N. (2003). Bread Staling: Molecular Basis and Control. *Comprehensive Reviews in Food Science and Food Safety* **2,** 1-21.

Gross, R., Von Baer, E., Koch, F., Marquard, R., Trugo, L. and Wink, M. (1988). Chemical composition of a new variety of the Andean Lupin (Lupinus mutabilis cv. Inti) with low-alkaloid content. *Journal of Food Composition and Analysis* **1,** 353-361.

Güemes-Vera, N., Arciniega-Ruiz Esperza, O. and Dávila-Ortiz, G. (2004). Structural analysis of the Lupinus mutabilis seed, its flour, concentrate, and isolate as well as their behavior when mixed with wheat flour. *LWT - Food Science and Technology* **37,** 283-290.

Guemes-Vera, N., Pena-Bautista, R. J., Jimenez-Martinez, C., Davila-Ortiz, G. and Calderon-Dominguez, G. (2008). Effective detoxification and decoloration of *Lupinus mutabilis* seed derivatives, and effect of these derivatives on bread quality and acceptance. *Journal of the Science of Food and Agriculture* **88,** 1135-1143.

Guillamón, E., Burbano, C., Cuadrado, C., Muzquiz, M., Pedrosa, M. M., Sánchez, M., Cabanillas, B., Crespo, J. F., Rodriguez, J., Haddad, J. and Allaf, K. (2008). Effect of an instantaneous controlled pressure drop on in vitro allergenicity to lupins (*Lupinus albus var Multolupa*). *International Archives of Allergy and Immunology* **145,** 9-14.

Guillamon, E., Cuadrado, C., Pedrosa, M. M., Varela, A., Cabellos, B., Muzquiz, M. and Burbano, C. (2010). Breadmaking properties of wheat flour supplemented with thermally processed hypoallergenic lupine flour. *Spanish Journal of Agricultural Research* **8,** 100-108.

Gujral, H. S. and Rosell, C. M. (2004). Improvement of the breadmaking quality of rice flour by glucose oxidase. *Food Research International* **37,** 75-81.

Hall, R. S. and Johnson, S. K. (2004). Sensory acceptability of foods containing Australian sweet lupin (*Lupinus angustifolius*) flour. *Journal of Food Science* **69,** snq92-snq97.

Hall, R. S., Johnson, S. K., Baxter, A. L. and Ball, M. J. (2005a). Lupin kernel fiber-enriched foods beneficially modify serum lipids in men. *European Journal of Clinical Nutrition* **59,** 325-333.

ACCEPTED MANUSCRIPT

Hall, R. S., Thomas, S. J. and Johnson, S. K. (2005b). Australian sweet lupin flour addition reduces the glycaemic index of a white bread breakfast without affecting palatability in healthy human volunteers. *Asia Pacific Journal of Clinical Nutrition* **14,** 91-97.

Harzic, N., Shield, I., Huyghe, C. and Milford, G. (2000). Lupinus albus as a European Crop **In:** Linking Research and Marketing Opportunities for Pulses in the 21st Century, pp. 561-574. Knight, R., Ed., Kluwer Academic Publisher, Dordrecht.

He, F. J. and MacGregor, G. A. (2009). A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *Journal of Human Hypertension* **23,** 363-384.

Herder, C., Karakas, M. and Koenig, W. (2011). Biomarkers for the prediction of type 2 diabetes and cardiovascular disease. *Clin Pharmacol Ther* **90,** 52-66.

Hieta, N., Hasan, T., Mäkinen-Kiljunen, S. and Lammintausta, K. (2009). Lupin allergy and lupin sensitization among patients with suspected food allergy. *Annals of Allergy, Asthma & Immunology* **103,** 233-237.

Hill, G. D. (2005). Recent developments in the use of lupins in animal and human nutrition. *Mexico, Where Old and New Lupins Meet 11th International Lupin Conference. Guadalajara, Mexico*, pp. 267-279.

Hodgson, J. M., Lee, Y. P., Puddey, I. B., Sipsas, S., Ackland, T. R., Beilin, L. J., Belski, R. and Mori, T. A. (2010). Effects of increasing dietary protein and fiber intake with lupin on body weight and composition and blood lipids in overweight men and women. *International Journal of Obesity* **34,** 1086-1094.

 ACCEPTED MANUSCRIPT

Hug-Iten, S., Escher, F. and Conde-Petit, B. (2003). Staling of bread: role of amylose and amylopectin and influence of starch-degrading enzymes. *Cereal Chemistry Journal* **80,** 654-661.

IBISWorld (2011). Global bakery goods manufacturing.[Online] http://clients.ibisworld.com/globalindustry/default.aspx?indid=361. Accessed[05 May 2012]

Jappe, U. and Vieths, S. (2010). Lupine, a source of new as well as hidden food allergens. *Molecular Nutrition & Food Research* **54,** 113-126.

Jayasena, V., Khu, W. S., & Nasar-Abbas, S. M. (2010). The development and sensory acceptability of lupin-based tofu. *Journal of Food Quality,* **33**(1), 85-97.

Jayasena, V., Leung, P. P. Y. and Nasar-Abbas, S. M. (2010). Effect of lupin flour substitution on the quality and sensory acceptability of instant noodles. *Journal of Food Quality* **33,** 709-727.

Jayasena, V. and Nasar-Abbas, S. M. (2011). Effect of lupin flour incorporation on the physical characteristics of dough and biscuits. *Quality Assurance and Safety of Crops & Foods* **3,** 140-147.

Jideani, V. A. and Onwubali, F. C. (2009). Optimization of wheat-sprouted soybean flour bread using response surface methodology. *African Journal of Biotechnology* **8,** 6364-6373.

Johnson, S. K., Chua, V., Hall, R. S. and Baxter, A. L. (2006). Lupin kernel fiber foods improve bowel function and beneficially modify some putative faecal risk factors for colon cancer in men. *British Journal of Nutrition* **95,** 372-378.

Johnson, S. K., McQuillan, P. L., Sin, J. H. and Ball, M. J. (2003). Sensory acceptability of white bread with added Australian sweet lupin (Lupinus angustifolius) kernel fiber and its glycaemic and insulinaemic responses when eaten as a breakfast. *Journal of the Science of Food and Agriculture* **83,** 1366-1372.

Joye, I. J., Lagrain, B. and Delcour, J. A. (2009). Use of chemical redox agents and exogenous enzymes to modify the protein network during breadmaking ï A review. *Journal of Cereal Science* **50,** 11-21.

Kirsz, K. and Zieba, D. A. (2011). Ghrelin-mediated appetite regulation in the central nervous system. *Peptides* **32,** 2256-2264.

Kohajdova, Z., Karovicova, J., & Schmidt, S. (2011). Lupin Composition and Possible Use in Bakery- A Review. *Czech Journal of Food Sciences, 29*(3), 203-211.

Koliaki, C., Kokkinos, A., Tentolouris, N. and Katsilambros, N. (2010). The effect of ingested macronutrients on postprandial ghrelin response: a critical review of existing literature data. *International journal of peptides* **2010**.

Kristensen, M. and Jensen, M. G. (2011). Dietary fibers in the regulation of appetite and food intake. Importance of viscosity. *Appetite* **56,** 65-70.

Ktenioudaki, A. and Gallagher, E. (In Press). Recent advances in the development of high-fiber baked products. *Trends in Food Science & Technology*.

Kurlovich, B., Stankevich, A. and Stepanova, S. (2002). Lupins: Geography, Classification, Genetic Resources And Breeding. Intan, St. Petersburg, pp. 11-38

Lawrance, L. (2007). Lupins: Australia's role in world markets. **In:** Australian Commodities, pp. 353-356.Wright, A., Ed, ABARE, Canberra.

Lee, Y. P., Mori, T. A., Puddey, I. B., Sipsas, S., Ackland, T. R., Beilin, L. J. and Hodgson, J. M. (2009). Effects of lupin kernel flour-enriched bread on blood pressure: a controlled intervention study. *American Journal of Clinical Nutrition* **89,** 766-772.

Lee, Y. P., Mori, T. A. and Sipsas, S. (2007). Lupin-enriched bread increases satiety and reduces energy intake acutely. (vol 85, pg 975, 2006). *American Journal of Clinical Nutrition* **85,** 1166-1166.

Lee, Y. P., Mori, T. A., Sipsas, S., Barden, A., Puddey, I. B., Burke, V., Hall, R. S. and Hodgson, J. M. (2006). Lupin-enriched bread increases satiety and reduces energy intake acutely. *American Journal of Clinical Nutrition* **84,** 975-980.

Levent, H. and Bilgiçli, N. (2011). Enrichment of gluten-free cakes with lupin (Lupinus albus L.) or buckwheat (Fagopyrum esculentum M.) flours. *International Journal of Food Sciences and Nutrition*.

Lindsay, M. P. and Skerritt, J. H. (1999). The glutenin macropolymer of wheat flour doughs: structure-function perspectives. *Trends in Food Science & Technology* **10,** 247-253.

MAFF-DOH (1996). ACNFP Annual Report.UK.pp. 14.

Mandala, I. and Kapsokefalou, M. (2011). Chapter 15 - Gluten-free bread: sensory, physicochemical, and nutritional aspects **In:** Flour and Breads and their Fortification in Health and Disease Prevention, pp. 161-169. Preedy, V.R., Watson, R.R., and Patel, V.B. ,Eds, Academic Press,San Diego,.

Manu, B. T. and Prasada Rao, U. J. S. (2008). Influence of size distribution of proteins, thiol and disulfide content in whole wheat flour on rheological and chapati texture of Indian wheat varieties. *Food Chemistry* **110,** 88-95.

Martinez-Villaluenga, C., Frias, J. and Vidal-Valverde, C. (2005). Raffinose family oligosaccharides and sucrose contents in 13 Spanish lupin cultivars. *Food Chemistry* **91,** 645-649.

Martinez-Villaluenga, C., Frias, J., Vidal-Valverde, C., & Gomez, R. (2005). Raffinose Family of Oligosaccharides from Lupin Seeds as Prebiotics: Application in Dairy Products. *Journal of Food Protection, 68*(6), 1246-1252.

Martínez-Villaluenga, C., Frías, J. and Vidal-Valverde, C. (2006). Functional lupin seeds (*Lupinus albus L. and Lupinus luteus L.*) after extraction of alpha-galactosides. *Food Chemistry* **98,** 291-299.

Martinez-Villaluenga, C., Sironi, E., Vidal-Valverde, C. and Duranti, M. (2006). Effects of oligosaccharide removing procedure on the protein profiles of lupin seeds. *European Food Research and Technology* **223,** 691-696.

Martínez-Villaluenga, C., & Gómez, R. (2007). Characterization of bifidobacteria as starters in fermented milk containing raffinose family of oligosaccharides from lupin as prebiotic. *International Dairy Journal,* **17**(2), 116-122.

Martínez-Villaluenga, C., Torres, A., Frias, J. and Vidal-Valverde, C. (2010). Semolina supplementation with processed lupin and pigeon pea flours improve protein quality of pasta. *LWT - Food Science and Technology* **43,** 617-622.

Martínez-Villaluenga, C., Zielinski, H., Frias, J., Piskula, M. K., Kozlowska, H. and Vidal-Valverde, C. (2009). Antioxidant capacity and polyphenolic content of high-protein lupin products. *Food Chemistry* **112,** 84-88.

McCarthy, D. F., Gallagher, E., Gormley, T. R., Schober, T. J. and Arendt, E. K. (2005). Application of response surface methodology in the development of gluten-free bread. *Cereal Chemistry* **82,** 609-615.

Meyer, D. and Stasse-Wolthuis, M. (2009). The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *Eur J Clin Nutr* **63,** 1277-1289.

Moder, G., J., Finney, K. F., Bruinsma, B. L., Ponte, J., J. G. and Bolte, L. C. (1984). Bread-Making Potential of Straight-Grade and Whole-Wheat Flours of Triumph and Eagle-Plainsman V Hard Red Winter Wheats. *Cereal Chem* **61,** 269 - 273.

Montgomery, D. C. (Ed) (2009). Design and Analysis of Experiments, John Wiley and Sons: New Jersey.

Mubarak, A. E. (2001). Chemical, nutritional and sensory properties of bread supplemented with lupin seed (Lupinus albus) products. *Nahrung-Food* **45,** 241-245.

Naruszewicz, M., Nowicka, G., Kosiewicz-Latoszek, L., Arnoldi, A. and Sirtori, C. (2007). Lupin protein (Lupinus albus) intake decrease blood pressure and other cardiovascular risk factors in smokers. A pilot study. *Annals of Nutrition and Metabolism* **51,** 273-273.

Nasar-Abbas, S. M. and Jayasena, V. (2012). Effect of lupin flour incorporation on the physical and sensory properties of muffins. *Quality Assurance and Safety of Crops & Foods* **4,** 41-49.

NHMRC (2005). Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes.Canberra.

Niewinski, M. M. (2008). Advances in Celiac Disease and Gluten-Free Diet. *Journal of the American Dietetic Association* **108,** 661-672.

Oomah, B. D., Tiger, N., Olson, M. and Balasubramanian, P. (2006). Phenolics and antioxidative activities in narrow-leafed lupins (*Lupinus angustifolius L.*). *Plant Foods for Human Nutrition* **61,** 91-97.

Paddon-Jones, D., Westman, E., Mattes, R. D., Wolfe, R. R., Astrup, A. and Westerterp-Plantenga, M. (2008). Protein, weight management, and satiety. *The American Journal of Clinical Nutrition* **87,** 1558S-1561S.

Paraskevopoulou, A., Chrysanthou, A. and Koutidou, M. (2012). Characterisation of volatile compounds of lupin protein isolateenriched wheat flour bread. *Food Research International* **48,** 568-577.

Paraskevopoulou, A., Provatidou, E., Tsotsiou, D. and Kiosseoglou, V. (2010). Dough rheology and baking performance of wheat flour-lupin protein isolate blends. *Food Research International* **43,** 1009-1016.

Parra-Gonzalez, L. B., Aravena-Abarzua, G. A., Navarro-Navarro, C. S., Udall, J., Maughan, J., Peterson, L. M., Salvo-Garrido, H. E. and Maureira-Butler, I. J. (2012). Yellow lupin (Lupinus luteus L.) transcriptome sequencing: molecular marker development and comparative studies. *Bmc Genomics* **13**.

Patel, S. and Goyal, A. (2012). The current trends and future perspectives of prebiotics research: a review. *3 Biotech* **2,** 115-125.

Pedersen, S. M. and Gylling, M. (2000). Lupin proteins for fermentation-economic and technology assessment. *1st World Conference on Biomass for Energy and Industry*. *Sevilla, Spain*, pp. 1127.

 ACCEPTED MANUSCRIPT

Pena, E., Bernardo, A., Soler, C. and Jouve, N. (2006). Do tyrosine crosslinks contribute to the formation of the gluten network in common wheat (*Triticum aestivum L.*) dough? *Journal of Cereal Science* **44,** 144-153.

Petterson, D. S., Sipsas, S. and Mackintosh, J. B. (1997). The Chemical Composition and Nutritive Value of Australian Pulses, Grains Research and Development Corporation.

Pfoertner, H. P. and Fischer, J. (2001). Dietary fibers of lupins and other grain legumes In B.V., M. and L., P. (Eds), *Advanced Dietary Fiber Technology*, Blackwell Science Ltd: Oxford, pp. 361-366.

Pilegaard, K. and Gry, J. (2008). Alkaloids In Edible Lupin Seeds: A Toxicological Review And Recommendations, Nordic Council of Ministers: Copenhagen.

Pollard, N. J., Stoddard, F. L., Popineau, Y., Wrigley, C. W. and MacRitchie, F. (2002). Lupin flours as additives: Dough mixing, breadmaking, emulsifying, and foaming. *Cereal Chemistry* **79,** 662-669.

Popper, L., Schafer , W. and Freund, W. (2006). Future of Flour: A Compendium of Flour Improvement, Muhlenchemie GmbH and Co. KG and Agremedia GmbH: Ahrensburg.

Poulsen, C. (1998). Purification and characterization of a hexose oxidase with excellent strengthening effects in bread. *Cereal chemistry* **75,** 51.

Price, K. R., Lewis, J., Wyatt, G. M. and Fenwick, G. R. (1988). Review article Flatulence-causes, relation to diet and remedies. *Food / Nahrung* **32,** 609-626.

Prieto-Simón, B., Noguer, T. and Campàs, M. (2007). Emerging biotools for assessment of mycotoxins in the past decade. *TrAC Trends in Analytical Chemistry* **26,** 689-702.

Reinhard, H., Rupp, H., Sager, F., Streule, M. and Zoller, O. (2006). Quinolizidine alkaloids and phomopsins in lupin seeds and lupin containing food. *Journal of Chromatography A* **1112,** 353-360.

Reis, A. M., Fernandes, N. P., Marques, S. L., Paes, M. J., Sousa, S., Carvalho, F., Conde, T. and Trindade, M. (2007). Lupin sensitisation in a population of 1,160 subjects. *Allergologia et Immunopathologia* **35,** 162-163.

Renzetti, S. and Arendt, E. K. (2009). Effect of protease treatment on the baking quality of brown rice bread: From textural and rheological properties to biochemistry and microstructure. *Journal of Cereal Science* **50,** 22-28.

Renzetti, S., Courtin, C. M., Delcour, J. A. and Arendt, E. K. (2010). Oxidative and proteolytic enzyme preparations as promising improvers for oat bread formulations: Rheological, biochemical and microstructural background. *Food Chemistry* **119,** 1465-1473.

Resta, D., Boschin, G., D'Agostina, A. and Arnoldi, A. (2008). Evaluation of total quinolizidine alkaloids content in lupin flours, lupin-based ingredients, and foods. *Molecular Nutrition & Food Research* **52,** 490-495.

Ribotta, P., Pérez, G., Añón, M. and León, A. (2010). Optimization of additive combination for improved soyï wheat bread quality. *Food and Bioprocess Technology* **3,** 395-405.

Rizzello, C. G., Coda, R., Mazzacane, F., Minervini, D. and Gobbetti, M. (2012). Micronized by-products from debranned durum wheat and sourdough fermentation enhanced the nutritional, textural and sensory features of bread. *Food Research International* **46,** 304-313.

 ACCEPTED MANUSCRIPT

Roccia, P., Ribotta, P. D., Ferrero, C., Perez, G. T. and Leon, A. E. (2012). Enzymes action on wheat-soy dough properties and bread quality. *Food and Bioprocess Technology* **5,** 1255-1264.

Rodgers, S. (2004). Value adding with functional meals. *Food Service Technology,* **4**(4), 149-158.

Rodriguez Mateos, A., Millar, S., Bhandari, D. and Frazier, R. (2006). Formation of dityrosine cross-links during breadmaking. *Journal of Agricultural and Food Chemistry* **54,** 2761-6.

Rolfes, S. R., Pinna, K. and Whitney, E. (2009). Understanding Normal and Clinical Nutrition, Cengage Learning Inc.: Belmont CA.

Ronda, F. and Roos, Y. H. (2011). Staling of fresh and frozen gluten-free bread. *Journal of Cereal Science* **53,** 340-346.

Rosell, C. M. (2011). The science of doughs and bread quality aspects **In:** Flour and Breads and their Fortification in Health and Disease Prevention, pp. 3-14. Preedy, V.R., Watson, R.R., and Patel, V.B. ,Eds, Academic Press,San Diego,.

Ruiz, L. P. and Hove, E. L. (1976). Conditions affecting production of a protein isolate from lupin seed kernels. *Journal of the Science of Food and Agriculture* **27,** 667-674.

Sanchez, H. D., Osella, C. A. and de la Torre, M. A. (2004). Use of response surface methodology to optimize gluten-free bread fortified with soy flour and dry Milk. *Food Science and Technology International* **10,** 5-9.

Sanz, M. L., de las Marinas, M. D., Fernandez, J. and Gamboa, P. M. (2010). Lupin allergy: a hidden killer in the home. *Clinical and Experimental Allergy* **40,** 1461-1466.

Schindler, S., Wittig, M., Zelena, K., Krings, U., Bez, J., Eisner, P. and Berger, R. G. (2011). Lactic fermentation to improve the aroma of protein extracts of sweet lupin (Lupinus angustzfolius). *Food Chemistry* **128,** 330-337.

Shah, A. R., Shah, R. K. and Madamwar, D. (2006). Improvement of the quality of whole wheat bread by supplementation of xylanase from Aspergillus foetidus. *Bioresource Technology* **97,** 2047-2053.

Shewry, P. R. and Tatham, A. S. (1997). Disulphide bonds in wheat gluten proteins. *Journal of Cereal Science* **25,** 207-227.

Shoup, F. K., Pomeranz, Y. and Dey.o.e, C. W. (1966). Amino acid composition of wheat varieties and flours varying widely in bread-making potentialities. *Journal of Food Science* **31,** 94-101.

Sipsas, S. (2008). Lupin products-concepts and reality. *12th International Lupin Conference. Fremantle, Western Australia*, pp. 506-513.

Sironi, E., Sessa, F. and Duranti, M. (2005). A simple procedure of lupin seed protein fractionation for selective food applications. *European Food Research and Technology* **221,** 145-150.

Sirtori, C. R., Triolo, M., Bosisio, R., Bondioli, A., Calabresi, L., De Vergori, V., Gomaraschi, M., Mombelli, G., Pazzucconi, F., Zacherl, C. and Arnoldi, A. (2012). Hypocholesterolaemic effects of lupin protein and pea protein/fiber combinations in moderately hypercholesterolaemic individuals. *British Journal of Nutrition* **107,** 1176-1183.

Sirtori, E., Resta, D., Arnoldi, A., Savelkoul, H. F. J. and Wichers, H. J. (2011). Cross-reactivity between peanut and lupin proteins. *Food Chemistry* **126,** 902-910.

Smith, J. P., Daifas, D. P., El-Khoury, W., Koukoutsis, J. and El-Khoury, A. (2004). Shelf life and safety concerns of bakery products - A review. *Critical Reviews in Food Science and Nutrition* **44,** 19-55.

Smith, S. C., Choy, R., Johnson, S. K., Hall, R. S., Wildeboer-Veloo, A. C. M. and Welling, G. W. (2006). Lupin kernel fiber consumption modifies fecal microbiota in healthy men as determined by rRNA gene fluorescent in situ hybridization. *European Journal of Nutrition* **45,** 335-341.

Southgate, D. A. T. (2003). Bread | dietary importance. **In**: Encyclopedia of Food Sciences and Nutrition. pp. 642-647. Benjamin, C., Ed, Academic Press: Oxford,

Sujak, A., Kotlarz, A. and Strobel, W. (2006). Compositional and nutritional evaluation of several lupin seeds. *Food Chemistry* **98,** 711-719.

Tilley, K. A., Benjamin, R. E., Bagorogoza, K. E., Okot-Kotber, B. M., Prakash, O. and Kwen, H. (2001). Tyrosine cross-links:ۦmolecular basis of gluten structure and function. *Journal of Agricultural and Food Chemistry* **49,** 2627-2632.

Trugo, L. C., von Baer, D. and von Baer, E. (2003). Lupin. **In**: Encyclopedia of Food Sciences and Nutrition. pp. 3623-3629. Benjamin, C., Ed, Academic Press: Oxford.

Tungland, B. C. and Meyer, D. (2002). Nondigestible oligo- and polysaccharides (dietary fiber): their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety* **1,** 90-109.

Turnbull, C. M., Baxter, A. L. and Johnson, S. K. (2005). Water-binding capacity and viscosity of Australian sweet lupin kernel fiber under in vitro conditions simulating the human upper gastrointestinal tract. *International Journal of Food Sciences and Nutrition* **56,** 87-94.

USDA-CNPP (2012). Dietary Guidelines for Americans, 2010: Washington.

USDA (2012). National Nutrient Database for Standard Reference Release 25, Agricultural Research Service National Agricultural Library. [Online] http://ndb.nal.usda.gov/ndb/foods/show/5375?fg=&man=&lfacet=&format=&count=&max=25&offset=&sort=&qlookup=bread. Accessed [12 Nov 2012]

Wang, S., Errington, S. and Yap, H. H. (2008). Studies on carotenoids from lupin seeds. *12th International Lupin Conference*. *Fremantle, Western Australia*.

Wong, A., Pitts, K., Jayasena, V. and Johnson, S. (Submitted). Isolation and foaming functionality of acid-soluble protein from lupin (*Lupinus angustifolius*) kernels. *Journal of the Science of Food and Agriculture*.

Yamsaengsung, R., Schoenlechner, R. and Berghofer, E. (2010). The effects of chickpea on the functional properties of white and whole wheat bread. *International Journal of Food Science & Technology* **45,** 610-620.

Yañez, E., Lobos, P., Diaz, G. and Ballester, D. (1986). Effect of roasting on the chemical composition and protein quality of lupin seeds (*Lupinus albus cv Multolupa*). *Journal of Food Science* **51,** 1235-1238.

Yang, X., Croft, K. D., Lee, Y. P., Mori, T. A., Puddey, I. B., Sipsas, S., Barden, A., Swinny, E. and Hodgson, J. M. (2010). The effects of a lupin-enriched diet on oxidative stress and

 ACCEPTED MANUSCRIPT

factors influencing vascular function in overweight subjects. *Antioxidants & Redox Signaling* **13,** 1517-1524.

Yap, L. S. (2006). *Determination of the Functionality of Novel Lupin Based Ingredients in Ice Cream.* Curtin University of Technology.

Zacherl, C., Eisner, P. and Engel, K.-H. (2011). In vitro model to correlate viscosity and bile acid-binding capacity of digested water-soluble and insoluble dietary fibers. *Food Chemistry* **126,** 423-428.

Table 1. Taxonomic and common names of some commercially grown lupin species

Species	Common names
L. albus	White lupin, Egyptian lupin, tremoo, altramuz
L. angustifolius	Blue lupin, narrow-leafed lupin
L. luteus	Yellow lupin, tremosilla
L. mutabilis	Tarwi, tauri, tarhui, chocho, Andean lupin

[1] Trugo *et al.*, (2003)

Table 2. Nutritional composition of Australian sweet lupin (ASL) and refined wheat flours[1]

Composition	ASL Flour	Wheat Flour
Energy (kJ/100g)	981	1416
Protein (g/100g)	42	12
Fat (g/100g)	7	1
Total dietary fiber (g/100g)	42	3
Soluble dietary fiber (g/100g)	*11*	*1*
Insoluble dietary fiber (g/100g)	*31*	*2*
Available carbohydrate (g/100g)	1	69

[1]Hall et al. (2005a)

 ACCEPTED MANUSCRIPT

Table 3. Summary of the main stages of bread making, the mechanisms involved and related dough and bread quality parameters that may be affected when non-wheat flours (i.e. legume flours) are substituted to wheat flour

Stage	Mechanisms involved	Related quality parameters
Mixing and kneading	❖ Hydration of wheat proteins and starch ❖ Energy generated to develop gluten matrix through covalent bonds (protein cross-linking) ❖ Incorporation of air bubbles ❖ Enzymatic production of sugars from starch	❖ Water absorption ❖ Dough development time ❖ Viscosity ❖ Stability ❖ Elasticity ❖ Extensibility ❖ Gas retention
Fermentation	❖ Yeasts ferment sugars to produce carbon dioxide ❖ Bubbles surrounded by gluten expand	❖ Loaf volume ❖ Crumb cell structure ❖ Textural properties
Baking	❖ Rate of fermentation increases in initial stages expanding dough ❖ Proteins are denatured and gluten matrix becomes rigid ❖ Yeasts and enzymes are heat inactivated ❖ Starch gelatinizes and stabilises structure ❖ Maillard reaction gives browning of the crust ❖ Formation of bread flavors	❖ Loaf volume ❖ Crumb cell structure ❖ Textural properties ❖ Colour and flavor properties

 ACCEPTED MANUSCRIPT

Table 4. Studies on wheat flour substitution by lupin flour of baked products - effect on protein content and dietary fiber content.

Lupin species/variety	Lupin fraction	Lupin incorporation rate (% wheat flour)	Product	Increase (%)	Reference
Not cited	Flour	40	Pan bread	Protein: 110, Dietary fiber: 106	Belski et al. (2011)
		30	Biscuit	Protein: 352, Dietary fiber : 211	
L.albus	Wholegrain flour	10, 20, 30, 40	Gluten-free cakes	Protein : 26-103	Levent and Bilgiçli (2011)
Not cited	Flour	40	Pan bread	Protein: 108, Dietary fiber: 346	Hodgson et al. (2010)
Not cited	Flour	40	Pan bread	Protein: 108, Dietary fiber: 341	Lee et al (2009)
Not cited	Flour	40	Pan bread	Protein: 65, Dietary fiber: 252	Lee et al (2007)
L. angustifolius	Flour	10	Pan bread	Protein: 14, Dietary fiber: 112	Hall and Johnson (2004)
		60	Muffins	Protein: 46, Dietary fiber: 294	
		28	Choc chip cookies	Protein: 51, Dietary fiber: 316	
L. albus	Flour, protein concentrate and isolate	3,6, 9, 12	Bread	Protein:11- 53, Dietary fiber: 7-44	Mubarak (2001)

 ACCEPTED MANUSCRIPT

L. albus cv. Multolupa	Full-fat flour	3, 6, 9, 12	Bread	Protein: 20-23,	Ballester et al. (1988)
L. angustifolius	Lupin kernel fiber	9 24	Pan bread Muffins	Dietary fiber: 132 Dietary fiber: 285	Clark and Johnson (2002)

Table 5. Clinical human studies on the effect of lupin baked products on biomarkers of various diseases.

Lupin species/variety	Lupin fraction	Product	Inclusion levels (% by wt. of wheat flour)	Specification of test population	Design	Effects of lupin treatment	Reference
A. Obesity biomarkers							
Postprandial							
Not cited	Flour	Bread	40	Healthy male and female subjects (n=16) ,fasting blood glucose of Ò5.6 mmollï 1. Mean age : 58.6±7.2	• Randomized controlled crossover trial • Lupin bread vs. wheat bread at breakfast (toast) and lunch (sandwich) • Dose of lupin within each treatment :cannot be determined • Energy intake at breakfast controlled (1655KJ) • Outcome	• Increased self-reported satiety • Lowered energy intakes • Decreased plasma ghrelin •	Lee et al. (2007)

 ACCEPTED MANUSCRIPT

					measures : self-reported satiety, energy intake, plasma ghrelin		
L. angustifolius	Flour	Bread	10	Healthy male and female subjects (n=11), 25-45 y.o.	• Post-meal study • Dose of lupin: 1.9g/100 g per breakfast • Total available carbohydrate was controlled • Outcome measure: self-reported satiety and post-meal food intake	• No effect on satiety and food intake	Hall et al. (2005b)
Long-term							
Not cited	Flour	Bread	40	Overweight and obese male and female subjects (n=88), 21-70 y.o. with fasting blood glucose of Ò5.6 mmollï1.	• Randomized • controlled parallel-design trial for 16 weeks • Lupin bread vs. wheat bread (to replace 15-20% of	• No effect on body weight and composition, plasma leptin and adinopectin	Hodgson et al. (2010)

					daily energy intake) • Dose of lupin : cannot be determined (24% of lupin bread) • Total fat and saturated, monounsaturated and polyunsaturated fat, protein derived from wheat (gluten) and sodium were controlled • Outcome measures: body weight and composition, plasma leptin and plasma adinopectin		
Not cited	Flour	Bread Biscuit	40 30	Overweight and obese male and female subjects (n=131), 21-71 y.o. with	• Randomized, controlled, double-blind parallel design for 12 months • Lupin	Did not enhance weight loss or improve maintenance of weight loss	Belski et al (2011)

ACCEPTED MANUSCRIPT

				fasting blood glucose of Ò6 mmollï 1.	bread and biscuit vs. wholemeal wheat bread and biscuit • Dose of lupin: not cited • Energy, fat and sodium were controlled • Outcome measures; body weight and composition		

B. Type 2 diabetes mellitus biomarkers

Postprandial

Not cited	Flour	Bread	40	Healthy male and female subjects (n=16) ,fasting blood glucose of Ò5.6 mmollï 1. Mean age : 58.6±7.2	• Randomized controlled crossover trials • Lupin bread vs. Wheat bread at breakfast (toast) and lunch (sandwich) • Dose of lupin : cannot be determined	Reduced postprandial glucose and insulin levels	Lee et al. (2007)

 ACCEPTED MANUSCRIPT

					(24% of lupin bread) • Energy intake (1655KJ) at breakfast was controlled • Outcome measures: serum glucose and insulin		
L. angustifolius	Flour	Bread	10	Healthy male and female subjects (n=11), 25-45 y.o.,	• Post-meal study • Lupin bread breakfast vs. wheat bread breakfast • Dose of lupin: 1.9g/100 g breakfast • Total available carbohydrate was controlled • Outcome measures: plasma glucose and serum insulin	Reduced glycaemic index but increased insulinaemic index of bread	Hall et al. (2005b)
L. angustifolius	Fiber	Bread	5.4 and 10.7 of total dry	Healthy male and female subjects	• Single-blind, randomized design	No effect on plasma glucose and	Johnson et al. (2003)

 ACCEPTED MANUSCRIPT

			ingredients	Mean age: 28.9 ± 8.2	• Lupin bread breakfast vs. wheat bread breakfast • Dose of lupin: 2.6 g/100 g of breakfast • Macronutrient composition except for dietary fiber was controlled • Outcome measures: plasma glucose and insulin	insulin levels, and GI Reduced incremental areas under curves (IAUC) for insulin	
Long-term					•		
Not cited	Flour	Bread	40	Overweight and obese male and female subjects (n=88), 21-70 y.o. with fasting blood glucose of Ò5.6 mmoll^{ï1}.	• Randomized • controlled parallel-design trial for 16 weeks • Lupin bread vs. wheat bread (to replace 15-20% of daily energy intake) • Dose of lupin : cannot be determined	No effect on glucose and insulin levels	Hodgson et al. (2010)

					(24% of lupin bread) • Total fat and saturated, monounsaturated and polyunsaturated • fat, protein derived from wheat (gluten) and sodium were controlled • Outcome measures: serum glucose and serum insulin		
Not cited	Flour	Bread Biscuit	40 30	Overweight and obese male and female subjects (n=131), 21-71 y.o. with fasting blood glucose of Ò6 mmollï 1.	• Randomized, controlled, double-blind parallel design for 12 months • Lupin bread and biscuit vs. wholemeal wheat bread and biscuit • Dose of lupin: not cited	Lowered insulin concentrations No effect on glucose levels	Belski et al. (2011)

ACCEPTED MANUSCRIPT

					• Energy, fat and sodium were controlled • Outcome measures: serum glucose and insulin		
L. angustifolius	Fiber	Bread Muffin Chocolate brownie	7.5 5.7 7.1 *(by weight of product)*	Healthy male and female subjects (n=11), 25-45 y.o.,	• Single-blind, randomized, crossover, dietary intervention design for 28 days • Lupin baked products vs. control • Dose of lupin: 7.5 g/ 100 in bread; 5.7 g/100 g in muffin; 7.1 g/100 g in chocolate brownie • Macronutrient composition except for dietary fiber was controlled • Outcome measures: plasma glucose	No effect on plasma glucose or fasting serum insulin levels	Hall, et al. (2005a)

					and fasting serum insulin		

C. Cardiovascular diseases biomarkers

Long-term

Not cited	Flour	Bread	40	Overweight and obese male and female subjects (n=88), 21-70 y.o. with fasting blood glucose of Ò5.6 mmollï 1.	• Randomized controlled parallel-design trial for 16 weeks • Lupin bread vs. wheat bread (to replace 15-20% of daily energy intake) • Dose of lupin : cannot be determined (24% of lupin bread) Total fat and saturated, monounsaturated and polyunsaturated • fat, protein derived from wheat (gluten) and sodium were	No effect on TC, LDL-C and triglycerides Decreased HDL-C	Hodgson et al. (2010)

					controlled • Outcome measures: serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), triacylglycerides		
Not cited	Flour	Bread	40	Overweight and obese male and female subjects (n=88), 21-70 y.o. with fasting blood glucose of Ò5.6 mmollï 1.	• Randomized controlled parallel-design trial for 16 weeks • Lupin bread vs. wheat bread (to replace 15-20% of daily energy intake) • Dose of lupin : cannot be determined (24% of lupin bread) Total fat and saturated, monounsat	Lowered blood pressure	Yang et al. (2010)

					urated and polyunsaturated • fat, protein derived from wheat (gluten) and sodium were controlled • Outcome measures: blood pressure		
Not cited	Four	Bread	40	Overweight and obese non-smoking male and female (n=88), 20-70 y.o. with fasting blood glucose of Ò5.6 mmoll$^{ï 1}$	• Randomized controlled parallel-design trial for 16 weeks • Lupin bread vs. wheat bread (to replace 15-20% of daily energy intake) • Dose of lupin : cannot be determined (24% of lupin bread) • Total fat and saturated, monounsaturated and	Lowered blood pressure	Lee et al. (2009)

					polyunsaturated fat, protein derived from wheat (gluten) and sodium were • Outcome measures: blood pressure		
Not cited	Flour	Bread Biscuit	40 30	Overweight and obese male and female subjects (n=131), 21-71 y.o. with fasting blood glucose of Ò6 mmollï 1.	• Randomized, controlled, double-blind parallel design for 12 months • Lupin bread and biscuit vs. wholemeal wheat bread and biscuit • Dose of lupin: not cited • Energy, fat and sodium were controlled • Outcome measures: blood pressure	Lowered blood pressure	Belski et al. (2011)
L.	Fiber	Bread	7.5	Healthy	• Single-	Decreased	Hall et

 ACCEPTED MANUSCRIPT

angustifolius	Muffin Chocolate brownie	5.7 7.1 *(by weight of product)*	male and female subjects (n=11), 25-45 y.o.,	blind, randomized, crossover, dietary intervention design for 28 days • Lupin baked products vs. control • Dose of lupin: 7.5 g/ 100 in bread; 5.7 g/100 g in muffin; 7.1 g/100 g in chocolate brownie • Macronutrient composition except for dietary fiber was controlled • Outcome measures: serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), HDL-C:LDL-C,	TC, LDL-C, TC: HDL-C and LDL-C:HDL-C No effects on HDL-C and triacylglycerol	al. (2005a)

					and triacylglycerols,		

D. Colonic health biomarkers

Long-term

L. angustifolius	Fiber	Bread Muffin Chocolate brownie	7.5 5.7 7.1 *(by weight of product*	Healthy male subjects (n=38), 24-64 y.o.	• Single-blind, randomized, crossover, dietary intervention design • Lupin baked products vs. control • Dose of lupin: 7.5 g/ 100 in bread; 5.7 g/100 g in muffin; 7.1 g/100 g in chocolate brownie • Macronutrient composition except for dietary fiber was controlled • Outcome measures: bowel function self-perception, frequency of defecation,	Improved bowel function e.g. increased frequency of defecation and decreased transit time	Johnson et al. (2006)

					transit time, faecal output, pH and moisture, faecal levels of SCFA and ammonia, faecal bacterial β-glucuronidase activity		
L. angustifolius	Fiber	Bread Muffin Chocolate brownie	7.5 5.7 7.1 *(by weight of product*	Healthy male subjects (n=18), 24-64 y.o, metabolism	• Single-blind, randomized, crossover, dietary intervention design • Lupin baked products vs. control • Dose of lupin: 7.5 g/ 100 in bread; 5.7 g/100 g in muffin; 7.1 g/100 g in chocolate brownie • Macronutrient composition except for dietary fiber was controlled • Outcome measures:	Stimulated colonic bifidobacteria growth	Smith et al. (2006)

		amount of colonic bacteria

ACCEPTED MANUSCRIPT

Table 6. Studies investigating the effects on dough and loaf quality of lupin incorporation into bread

Lupin species/variety	**Lupin fraction**	**Product**	**Lupin Incorporation rate (% of wheat flour)**	**Water incorporation rate (% of product)**	**Bread making process**	**Positive effects**	**Negative effects**	**Reference**
L. luteus *L. angustifolius*	Wholemeal flours	Sourdough dome bread	10	Based on reference moisture content of raw materials, water absorption and required humidity of the end product.	Straight dough	Fermenting the wholemeal flour with *Pediococcus acidilacti* lessened negative effects on quality	Decrease loaf specific volume and porosity Increased crumb hardness	Bartkiene et al. (2011)
L. albus cv. Multolupa	Flour (raw and heat treated)	Pan bread	10	31.4	Straight dough	Panel favoured bread made using autoclaved lupin flour compared to non-heated	Decreased volume and increased density	Guillamon (2010)

						flour due to texture and taste		
L. albus ssp. Graecus	Protein isolates (albumin and globulin)	Pan bread	5, 10	Farinograph value 500 BU	Straight dough	Increased dough stability; Good handling behaviour and tolerance during fermentation stage; Delayed staling	Increased development time; Decreased dough elasticity and bread volume; Increased hardness, gumminess and chewiness	Paraskevopoulou et al. (2010)
L. mutabilis	Flour Protein concentrate Protein isolate	Pan read	5, 10, 15, 20 2.5, 5.0, 7.5, 10 0.5, 1.0, 3.0, 4.0	Not cited	Straight dough	Decreased firmness after 24 h Increased specific volume Texture were rated as good in general	Increased firmness after baking	Guemes-Vera et al. (2008)
L. mutabilis	Flour Protein concentrate Protein isolate	Dough	5, 10, 15, 20 2.5, 5.0, 7.5, 10 0.5, 1.0, 3.0, 4.0	Not cited	Straight dough		Gluten matrix was shown to be less interconnected by microsco	Guemes-Vera et al. (2004)

							py due to the presence of lupin proteins	
Lupinus albus ssp. Graecus	Full-fat flour	Pan bread	5,10	Based on Farinograph value of 500 BU	Straight dough	Increased stability and tolerance of dough at 5 and 10%	Lowered volume as% lupin flour increased	Doxastakis et al. (2002)
L. albus L. angustifolius	Flour	Pan bread	2, 5, 10, 15, 20	35.7	Straight dough	L. albus decreased mixing time L. angustifolius allowed for greater tolerance to over mixing	Decreased dough strength and loaf heightô Increased darkness of crust and crumb	Pollard et al. (2002)
L. albus	Flour Protein concentrate Protein isolate	Pan bread	3, 6, 9 and 12	Based on farinograph value	Straight dough	Flour and protein concentrate can be added up to 6% while protein isolate can be added	Increased dough development time and dough weakening; Decreased dough stability and loaf volume	Mubarak (2001)

						up to 9% without detrimental effects on sensory properties		
Lupinus albus ssp. Graecus	Flour (full-fat, concentrated, defatted and concentrated	Pan bread	5, 10, 15	Based on Farinograph value of 500 BU	Straight dough	Increased stability and tolerance of dough at 5%	Decreased dough strength at 15%; Lowered volume as % lupin flour increased	Dervas et al. (1999)
L. albus cv. Multolupa	Full-fat flour	Rolled bread	0, 3, 6, 9, 12	Not cited	Straight dough	Increased loaf volume for all substituted bread		Ballester et al. (1988)