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

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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

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 γ -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 'baked products' 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 around 1% of most populations suffers from (Mandala and Kapsokafalou, 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).

A legume that can potentially address consumers' desire for healthier baked products is lupin. Lupin grain is high in protein and dietary fiber with very little available carbohydrate (Pettersson et al., 1997). It also contains vitamins and antioxidants including: tocopherols (Boschin and Arnoldi, 2011); carotenoids (Wang et al., 2008); B-vitamins (Erbaş 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 (Martínez-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 (Martínez-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

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 1980s 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

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*

flours ranged from 31-36%. The major storage proteins in lupin seed are known as globulins, which are classified into four families: α -conglutin (11S globulin), β -conglutin (7S globulin), γ -conglutin (7S basic globulin), and δ -conglutin (2S sulphur-rich albumin) (Foley, et al., 2011). α -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 α -conglutin peptide in different lupin species and varieties nor its stability during food processing.

Dietary fiber content and physical properties

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

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 which 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 (Erbaş 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. Bodhi's 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. Consumers' 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 straight-dough and the sponge and dough 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 of 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 α -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 dietary 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 were more satiating 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 gastric 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

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, α -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% α -conglutin. There is however no study that tested the effects of α -conglutin when incorporated into bread nor the effects of processing (i.e. breadmaking) on α -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 implies that the proteins (and α -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

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

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 water-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 prebiotic 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,

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 perceived 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 phomopsis 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 allergic participants indicated that the allergenicity was due mainly to the α -conglutin peptide of lupin (Sirtori et al., 2011). Beta-conglutin and β - and γ -conglutin peptides, have also been identified as causes of anaphylactic and other allergic 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°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 'sweet' 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).

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

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 two 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

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 bread improvers 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 bread

improversö 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)

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 'healthy' 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

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 òbeanyö 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 òlegume-likeö 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

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 "double-edged" 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 - 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.

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

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

¹ Trugo *et al.*, (2003)

Table 2. Nutritional composition of Australian sweet lupin (ASL) and refined wheat flours¹

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
<i>Soluble dietary fiber (g/100g)</i>	<i>11</i>	<i>1</i>
<i>Insoluble dietary fiber (g/100g)</i>	<i>31</i>	<i>2</i>
Available carbohydrate (g/100g)	1	69

¹Hall et al. (2005a)

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	<ul style="list-style-type: none"> ❖ 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 	<ul style="list-style-type: none"> ❖ Water absorption ❖ Dough development time ❖ Viscosity ❖ Stability ❖ Elasticity ❖ Extensibility ❖ Gas retention
Fermentation	<ul style="list-style-type: none"> ❖ Yeasts ferment sugars to produce carbon dioxide ❖ Bubbles surrounded by gluten expand 	<ul style="list-style-type: none"> ❖ Loaf volume ❖ Crumb cell structure ❖ Textural properties
Baking	<ul style="list-style-type: none"> ❖ 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 	<ul style="list-style-type: none"> ❖ Loaf volume ❖ Crumb cell structure ❖ Textural properties ❖ Colour and flavor properties

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	
<i>L. albus</i>	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)
<i>L. angustifolius</i>	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	
<i>L. albus</i>	Flour, protein concentrate and isolate	3,6, 9, 12	Bread	Protein:11- 53, Dietary fiber: 7-44	Mubarak (2001)

<i>L. albus</i> cv. Multolupa	Full-fat flour	3, 6, 9, 12	Bread	Protein: 20-23,	Ballester et al. (1988)
<i>L. angustifolius</i>	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 5.6 mmol l^{-1} . Mean age: 58.6 ± 7.2	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • Increased self-reported satiety • Lowered energy intakes • Decreased plasma ghrelin 	Lee et al. (2007)

					measures :			
					self-reported satiety, energy intake, plasma ghrelin			
<i>L. angustifolius</i>	Flour	Bread	10	Healthy male and female subjects (n=11), 25-45 y.o.	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • 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 ≥ 5.6 mmol ⁶¹ .	<ul style="list-style-type: none"> • Randomized controlled parallel-design trial for 16 weeks • Lupin bread vs. wheat bread (to replace 15-20% of 	<ul style="list-style-type: none"> • No effect on body weight and composition, plasma leptin and adiponectin 	Hodgson et al. (2010)	

					daily energy intake)		
					<ul style="list-style-type: none"> • 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 adiponectin 		
Not cited	Flour	Bread Biscuit	40 30	Overweight and obese male and female subjects (n=131), 21-71 y.o. with	<ul style="list-style-type: none"> • 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)

fasting
blood
glucose
of $\bar{O}6$
mmol⁶¹.

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
compositio
n

B. Type 2 diabetes mellitus biomarkers

Postprandial

Not cited	Flour	Bread	40	Healthy male and female subjects (n=16) ,fasting blood glucose of $\bar{O}5.6$ mmol ⁶¹ . Mean age : 58.6 ± 7.2	<ul style="list-style-type: none">• Randomize d controlled crossover trials• Lupin bread vs. Wheat bread at breakfast (toast) and lunch (sandwich)• Dose of lupin : cannot be determined	Reduced postprandi al glucose and insulin levels	Lee et al. (2007)
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					(24% of lupin bread)		
					<ul style="list-style-type: none"> • Energy intake (1655KJ) at breakfast was controlled • Outcome measures: serum glucose and insulin 		
<i>L. angustifolius</i>	Flour	Bread	10	Healthy male and female subjects (n=11), 25-45 y.o.,	<ul style="list-style-type: none"> • 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)
<i>L. angustifolius</i>	Fiber	Bread	5.4 and 10.7 of total dry	Healthy male and female subjects	<ul style="list-style-type: none"> • Single-blind, randomized design 	No effect on plasma glucose and	Johnson et al. (2003)

				ingredie nts	Mean age: 28.9 ± 8.2	<ul style="list-style-type: none"> • 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 5.6 mmol ⁶¹ .	<ul style="list-style-type: none"> • 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)		
					<ul style="list-style-type: none"> • 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 ≥ 6 mmol/l ⁶¹ .	<ul style="list-style-type: none"> • 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)

<i>L. angustifoli us</i>	Fiber	Bread Muffin Chocol ate browni e	7.5 5.7 7.1 (<i>by weight of product</i>)	Healthy male and female subjects (n=11), 25-45 y.o.,
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- Energy, fat and sodium were controlled
 - Outcome measures: serum glucose and insulin
 - 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 ≥ 5.6 mmol ⁶¹ .	<ul style="list-style-type: none"> • 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)
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Not cited	Flour	Bread	40	Overweight and obese male and female subjects (n=88), 21-70 y.o. with fasting blood glucose of ≥ 5.6 mmol ⁶¹ .	<ul style="list-style-type: none"> controlled Outcome measures: serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), triacylglycerides 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 	Lowered blood pressure	Yang et al. (2010)
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Not cited	Four	Bread	40	Overweight and obese non-smoking male and female (n=88), 20-70 y.o. with fasting blood glucose of ≥ 5.6 mmol/l ⁶¹	<ul style="list-style-type: none"> urated and polyunsaturated fat, protein derived from wheat (gluten) and sodium were controlled Outcome measures: blood pressure 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)
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					polyunsaturated fat, protein derived from wheat (gluten) and sodium were			
					<ul style="list-style-type: none"> • 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 ≥ 6 mmol ⁶¹ .	<ul style="list-style-type: none"> • 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	<ul style="list-style-type: none"> • Single- 	Decreased	Hall et	

<i>angustifolius</i>	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	TC, LDL-C, TC:HDL-C and LDL-C:HDL-C	al. (2005a)
				<ul style="list-style-type: none"> • 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, 	No effects on HDL-C and triacylglycerol	

and
triacylglycerols,

D. Colonic health biomarkers

Long-term

<i>L. angustifolius</i>	Fiber	Bread Muffin Chocolate brownie	7.5 5.7 7.1 (by weight of product)	Healthy male subjects (n=38), 24-64 y.o.	<ul style="list-style-type: none"> • 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)
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<i>L. angustifoli us</i>	Fiber	Bread	7.5	Healthy male subjects (n=18), 24-64 y.o., metabolis m	<p>transit time, faecal output, pH and moisture, faecal levels of SCFA and ammonia, faecal bacterial - glucuronid ase activity</p> <ul style="list-style-type: none"> • 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)
		Muffin	5.7				
		Chocolate	7.1				
		brownie	(by weight of product)				

amount of
colonic
bacteria

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

<i>Lupin species/variety</i>	<i>Lupin fraction</i>	<i>Product</i>	<i>Lupin Incorporation rate (% of wheat flour)</i>	<i>Water incorporation rate (% of product)</i>	<i>Bread making process</i>	<i>Positive effects</i>	<i>Negative effects</i>	<i>Reference</i>
<i>L. luteus</i> <i>L. angustifolius</i>	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 <i>Pediococcus acidilacti</i> lessened negative effects on quality	Decrease loaf specific volume and porosity Increased crumb hardness	Bartkiene et al. (2011)
<i>L. albus</i> cv. <i>Multolupa</i>	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		
<i>L. albus</i> <i>spp.</i> <i>Graecus</i>	Protein isolates (albumin and globulin)	Pan bread	5, 10	Farinogr aph value 500 BU	Strai ght doug h	Increase d dough stability ; Good handlin g behavio ur and toleranc e during ferment ation stage; Delayed staling	Increased develop ment time; Decrease d dough elasticity and bread volume; Increased hardness, gummine ss and chewines s	Paraskevo poulou et al. (2010)
<i>L.</i> <i>mutabilis</i>	Flour Protein concentr ate 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	Strai ght doug h	Decreas ed firmnes s after 24 h Increase d specific volume Texture were rated as good in general	Increased firmness after baking	Guemes- Vera et al. (2008)
<i>L.</i> <i>mutabilis</i>	Flour Protein concentr ate Protein isolate	Dough	5, 10, 15, 20 2.5, 5.0, 7.5, 10 0.5, 1.0, 3.0, 4.0	Not cited	Strai ght doug h		Gluten matrix was shown to be less interconn ected by microscop e	Guemes- Vera et al. (2004)

<i>Lupinus albus ssp. Graecus</i>	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	py due to the presence of lupin proteins Doxastakis et al. (2002)
<i>L. albus</i> <i>L. angustifolius</i>	Flour	Pan bread	2, 5, 10, 15, 20	35.7	Straight dough	<i>L. albus</i> decreased mixing time <i>L. angustifolius</i> allowed for greater tolerance to over mixing	Decreased dough strength and loaf height Increased darkness of crust and crumb	Pollard et al. (2002)
<i>L. albus</i>	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 detrime ntal effects on sensory properti es		
<i>Lupinus albus ssp. Graecus</i>	Flour (full-fat, concentr ated, defatted and concentr ated	Pan bread	5, 10, 15	Based on Farinogr aph value of 500 BU	Strai ght doug h	Increase d stability and toleranc e of dough at 5%	Decrease d dough strength at 15%; Lowered volume as % lupin flour increased	Dervas et al. (1999)
<i>L. albus cv. Multolup a</i>	Full-fat flour	Rolled bread	0, 3, 6, 9, 12	Not cited	Strai ght doug h	Increase d loaf volume for all substitu ted bread		Ballester et al. (1988)