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Aroma characteristics of lupin and soybean after germination and effect of fermentation on lupin aroma

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ABSTRACT

Greater human consumption of Australian sweet lupin (*Lupinus angustifolius*) and other legume products such as soybean (*Glycine max*) is often limited due to undesirable beany flavor. The impacts of germination of lupin and soybean seeds and fermentation of lupin flour on aroma profiles were compared by gas chromatography olfactometry using trained sensory assessors. Untreated soybean compared to untreated lupin had significantly higher concentrations of volatiles commonly associated with beany flavor in legumes; (*E*)-2-hexanal, (*E*)-2-octenal, 1-octen-3-one, 1-hexanol and 1-heptanol. After germination of lupin and soybean, 2-methylbutanal was the most abundant volatile, increasing meaty and brothy odor characteristics. Germination of lupin and soybean also increased sweet, woody, mushroom and baked aroma notes. Fermentation of lupin increased mushroom, soil, green and nutty aroma characteristics, however beany odor did not decrease. Germination and to a lesser extent fermentation, may be successful strategies to increase the acceptability of legume flavor.

1. Introduction

Interest in plant-based proteins as meat alternatives and functional foods has increased over recent decades. New non-animal protein sources and novel processing methods can be combined by the food industry to produce nutritious and palatable foods and ingredients to meet changing customer demands (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003). Legumes are of interest due to their high protein content and potential associated health benefits. Soybean (*Glycine max*) and Australian sweet lupin (*Lupinus angustifolius*) are excellent protein sources for human foods (Bartkiene, Krungleviciute, Juodeikiene, Vidmantiene, & Maknickiene, 2015) however, both are underutilized in the average Western diet.

One barrier to greater human consumption of lupin and soy is the flavor, which is often described as “beany”, “green” or “rancid” (Kaneko, Kumazawa, & Nishimura, 2011). The development of beany, green and unpleasant flavors in legumes is attributed to the activities of endogenous lipoxygenase enzymes, which initiate lipid oxidation and the formation of beany volatiles (Boatright & Lei, 1999) (Stephany, Bader-Mittermaier, Schweiggert-Weisz, & Carle, 2015). Several processes have been applied to eliminate undesirable flavors in soybean and pea products including heat treatment or thermal inactivation of lipoxygenase enzymes (Das, Anjaneyulu, & Kondaiah, 2006), enzymatic hydrolysis (Yoo & Chang, 2016), lactic fermentation (Schindler et al., 2011) and germination (Arif et al., 2012). Fermentation is an inexpensive and safe way to improve the nutritional profile of legumes and is one of the oldest methods of producing and preserving foods (Blandino et al., 2003). Lactic fermentation of legumes was shown to increase protein digestibility (Bartkiene et al., 2015), reduce flatulence-producing compounds, reduce trypsin inhibitors and other anti-nutritional components (Granito & Álvarez, 2006) (Refstie, Sahlstrom, Brathen, Baeverfjord, & Krogedal, 2005) and improve sensory properties of soymilk (Blagden & Gilliland, 2005) and reduce and/or mask the off-flavors of lupin (Schindler et al., 2011).

Germination is another inexpensive and effective technology for enhancing the nutritional quality of legumes, by increasing digestibility of proteins, reduction of anti-nutritional factors and increasing antioxidant capacity and vitamin C and E content (Aguilera et al., 2013) (Frias, Miranda, Doblado, & Vidal-Valverde, 2005).

The sensory properties of some germinated and fermented legume products have been investigated to a certain degree,

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however detailed flavor profiles have not previously been reported. Understanding the main flavor changes due to germination and fermentation is necessary to optimize the processes for the best flavor characteristics and acceptability of lupin and soy foods and ingredients.

In this study, the potential of germination and fermentation to modify the flavor and sensory characteristics of lupin and soybean flour were evaluated. Commercially available yogurt culture mix with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophillus* was used for the fermentation of lupin.

2. Material and methods

2.1. Plant material

Australian sweet lupin seeds and lupin flour from the same cultivation year (2015) were obtained from Coorow Seeds Pty Ltd., (Coorow, WA, Australia). Australian grown soybean seeds (Demeter Farm Mill, Australia) and flour (Lotus Organic, Australia) were purchased from a local supermarket.

2.2. Chemicals

Reference volatiles were purchased from Sigma-Aldrich (Castle Hill, Australia): hexanal, 1-hexanol, (*E*)-2-hexenal, octanal, dimethyl trisulfide, 2-pentylfuran, 2,3-butanedione, (E,Z)-2,6-nonadienal, 2-methoxyphenol (guaiacol), 1-octen-3-one, 1-octen-3-ol, 2-phenylethyl alcohol, (*E*)-2-pentenal, (*E,E*)-2,4-heptadienal, benzaldehyde and 4-methyl-1-pentanol (internal standard). Further references were obtained from Givaudan (ex-Quest International): 1-penten-3-ol, (*E*)-2-octenal, (*E*)-2-nonenal, dimethyl sulfide, 2-methylbutanal, 1-penten-3-one, 3-octen-2-one, 3-isobutyl-2-methoxypyrazine and 3-ethyl-2,5-dimethyl pyrazine. Silane treated glass wool and saturated alkanes standard C7–C40 were supplied by Supelco (Bellefonte, USA). Tenax porous polymer adsorbent (Tenax-TA, 60/80 mesh) was supplied by Sigma-Aldrich (Castle Hill, Australia). Hydrogen peroxide solution (food grade) was purchased from Anpros Pty Ltd (Bayswater, Australia). Milli-Q water was used in all experiments (Synergy UV, Millipore, Sydney, Australia).

2.3. Germination of lupin and soybean seeds

After removal of any damaged material, whole soybean and lupin dried seeds were sanitized in 3% hydrogen peroxide solution (30 min) and rinsed with Milli-Q water until a neutral pH was obtained and soaked in purified water for 8 h. After soaking, seeds were placed on trays (290 × 340 mm, Green Harvest, Maleny, Australia) covered with germination paper (Anchor, Saint Paul, United States) and germinated for 72 h in a temperature-controlled room (~22 °C) and relative humidity 50–60% with the access to natural daylight (12 h) and darkness (12 h). Soaked and germinated samples were dried in a cabinet dryer (engineered in-house in the UNSW Chemical Engineering Workshop, Australia) for 18 h at 50 °C and then the temperature was increased by 10 °C every hour until 80 °C. After cooling to room temperature, dried seeds were ground using single-phase rotor mill (RMC, Shanghai Miniature Electrical Machinery Plant, China) to pass a 0.5 μm sieve, stored in sealed air tight packs at 4 °C until analysis. Every treatment was replicated so that two experimental samples were available for statistical analysis. At least two analytical determinations were performed on each experimental sample. Untreated lupin and soybean samples were not subjected to the germination process and taken as a control. The seeds were ground and stored at 4 °C until analysis. It should be noted that the husk of the seeds was not removed in these processes. The flour from the untreated lupin is referred to as lupin untr., the flour from the untreated soy as soy untr. The germinated flours are referred to as lupin germ. and soy germ.

2.4. Fermentation of lupin flour

Glassware, utensils and Milli-Q water were autoclaved before fermentation. Commercially available lupin flour was used; the seed-coating or husk had been removed from these samples prior to milling. Flour (25 g) was mixed with 2% (w/v) lyophilized YO-MIX™ 601 LYO 200 DCU yogurt culture (Danisco, Sydney, Australia), in 50 mL of Milli-Q water. The yogurt mix was chosen because it is commercially available and a cost-effective option for the food industry. The commercial yogurt culture contained *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophillus.* An airlock was inserted to the conical flask to create an anaerobic condition. The sample was incubated for 20 h at 30 °C in an orbital shaker (Bioline, Sydney, Australia). After overnight incubation, the fermented flour samples were placed in a freeze-dryer (Scanvac Coolsafe Labogene, Lynge, Denmark) for 24 h and homogenized using a grinder (Magic Bullet, Sydney, Australia). Every treatment was replicated so that two experimental samples were available for statistical analysis. At least two analytical determinations were performed on each experimental sample.

The moisture content of fermented flour samples was determined using AOAC method (AOAC, 2007) before they were stored in the - 20 °C freezer.

2.5. Dynamic headspace sampling

The flour sample (8 g), Milli-Q-water (50 mL) and 4-methyl-1-pentanol as an internal standard (0.5 μg/g) were added to a 250 mL Schott bottle with a magnetic stirrer. The bottle was closed with a Teflon coated cap with two Luer-lock fittings: the inlet was connected to high purity nitrogen gas (Coregas, Yennora, Australia) and the outlet was connected to a Tenax trap (100 mg) held in place by glass wool via Peek® tubing (Alltech, Australia). Volatiles were extracted under nitrogen flow (110 mL/min) for 40 min at room temperature. To remove residual moisture, traps were purged with nitrogen gas (10 mL/min) for 1 min.

2.6. Thermal desorption and gas chromatography-olfactometry (GC-O)

Tenax traps were desorbed at 260 °C for 5 min using a Short Path Thermal Desorption unit (Model TD-5, Scientific Instrument Services Inc., Ringoes, USA) under a flow of helium gas (0.9 mL/min) into the hot injector (240 °C) of the GC (Varian 3800, Agilent Technologies, Santa Clara, CA, USA). Approximately half of the gas effluent was directed to a sniffing port (ODO-II, SGE, Melbourne, Vic., Australia) and the remaining sample to the ion trap mass detector (Varian 4000, Agilent Technologies, Santa Clara, CA, USA). Volatiles were separated on a polar capillary column (VF-WAXms, 30 m, 0.32 mm, 0.5 μm, Agilent Technologies, Mulgrave, Australia). The column temperature was initially held at 40 °C for 5 min, then increased to 250 °C at 6 K/min and held for 5 min.

Samples were measured in electron ionization (EI) mode between 46 and 200 mass to charge ratio (*m/z*). Selected samples were also measured using methanol chemical ionization (CI) to confirm the molecular mass of volatiles, where applicable. Compounds were identified by comparing the EI mass spectral searches obtained from the National Institute of Standards and Technology (NIST) software (Version 2.0, United States of America, 2002). Further identification was achieved through CI molecular mass confirmation and in many cases through analytical reference