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PROXIMATE COMPOSITION AND ANTI-NUTRITIONAL FACTORS OF TRADITIONALLY PROCESSED WHITE LUPINE (*LUPINUS ALBUS* L.) FABACEAE, GROWN IN ETHIOPIA

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ABSTRACT: Lupine seeds (*Lupinus albus* L.) growing in two different agro-ecological zones of Ethiopia (Dangla and Chagni) were traditionally processed to evaluate the changes in their nutritional status and anti-nutritional factors. The traditional processing methods included roasting followed by soaking; boiling followed by soaking and germination. In all the methods, the whole seed and the kernel were compared. Moisture, crude protein, crude fat, crude fiber, crude ash, utilizable carbohydrates and gross energy for raw seeds which were obtained from Dangla were 6.94%, 37.87%, 9.34%, 11.08%, 2.80%, 38.92% and 391.19 Kcal/100 gm, respectively. The values for seeds from Chagni were 8.04%, 39.71%, 8.79%, 11.07%, 2.90%, 37.56% and 388.12 Kcal/100 gm, respectively. The total alkaloid and phytate contents of the Dangla seeds were 2.46% and 144.33 mg/100 gm and 2.26% and 143.96 mg/100 gm, respectively for Chagni seeds. In roasted and soaked seeds, the alkaloid level was significantly ($p<0.05$) reduced and de-hulling reduced the anti-nutritional factors effectively. Phytate was significantly reduced during germination and generally the levels of protein, fat, and total energy were found to increase.

Key words/phrases: Germination, *Lupinus albus*, Roasting, Soaking.

INTRODUCTION

Lupine belongs to the genus *Lupinus* and family of Genisteae, which is also called Fabaceae or Leguminosae (Uzun *et al*., 2006). Commonly, four lupine species are reported as cultigens in the world. These include *L. albus* L., *L. angustifolius* L., *L. luteus* L. and *L. mutabilis* Sweet (Gladstones *et al.*, 1998; Cowling *et al.*, 1998; Uzun *et al*., 2006; Kurzbaum *et al*., 2008). The common names for these species are white lupine, narrow-leaved (blue) lupine, yellow lupine and pearl lupine, respectively. The two types of lupines that are mainly cultivated today are the white and blue ones and their major sites of production are Europe and Australia, respectively (Yoshie-Stark and Wasche, 2003).

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Lupinus albus is a plant with numerous useful properties. It can be used both as fodder and for soil fertilization (Maknickienė and Asakavičiūtė, 2008) and can recover from severe water deficit conditions (Yoshie-Stark and Wasche, 2003). In addition, it has a strong capability for nitrogen fixation and organic phosphorus release from soil. This has allowed the crop to be used in crop rotation practices during intensive crop production seasons, where this feature of the crop is feasible within nitrate vulnerable zones (Fraser *et al*., 2004; Sujak *et al*., 2006). Moreover, its adaptation to poor soil makes it economically feasible (Trugo *et al.*, 1993; Farrell *et al.*, 1999; Gaultier *et al.*, 2003; Lampart-Szczapa *et al.*, 2003; James *et al.*, 2004; Sujak *et al.*, 2006) and suitable for cultivation in wide climatic range.

Lupine is a high source of protein and is commonly consumed as a snack in the Middle East and is coming into use as a high-protein soy substitute in other parts of the world (Kurzbaum *et al*., 2008). The seeds are used as a complete or partial substitute for soybeans in the production of milk powder and tofu (Australian Health Info Centre, 2009). Food products available in different markets of Europe are lupine snacks, pasta, bread, cookies, lupine coffee and some vegetarian instant meals. The utilization of this plant can be extended to the production of protein concentrates, which can be added to other food products or fodder thereby enriching their nutritional values (Batterham *et al.*, 1986; Guillaume *et al.*, 1987; Marrs, 1996; Dijkstra *et al.*, 2003; Archer *et al.*, 2004; Sujak *et al*., 2006).

The most prominent anti-nutritional factors in lupine seeds are the bitter and toxic quinolizidine alkaloids. Raffinose Family Oligosaccharides (RFOs) also constitute a considerable amount in the seed (Calloway *et al.*, 1971; Taverner *et al.*, 1983; Khalil *et al*., 2006). In contrast to other leguminous plants such as peas and soy beans, *Lupinus albus* contain extremely low amounts of trypsin inhibitors, lecitins, saponins and cyanogens (Jimenez-Martinez *et al.*, 2003; Joray *et al*., 2007; Zraly *et al*., 2007).

Lupinus albus are often referred to as either bitter or sweet. Bitter lupines have high concentrations of alkaloids, while the sweet lupines have low levels (Australia-New Zealand Food Authority, 2001). Alkaloid content in *Lupinus albus* depends on numerous factors such as species variety, developmental stage, environment and geographical location. In parallel with the processing methods, plant breeders have been trying to develop sweet lupine containing low level of alkaloids. These varieties have advantages of having low alkaloid content but they are also less resistant to disease and herbivore attack (Sanchez *et al*., 2005).

In addition to genetic selection for low alkaloid containing lupine seeds, there are some physical and chemical treatments with acids and alkalis for eliminating the anti-nutritional factors (Arslan and Seker, 2002). Beyond removing the unwanted anti-nutritional factors, these processes improve the nutritive value and digestibility (Khalil *et al*., 2006). Some of these processes include soaking, dehulling, germination (Sripriya *et al*., 1997), fermentation (Czarnecka *et al*., 1998), cooking (Kaankuka *et al*., 1996), heat treatment (Mulimani and Paramjyothi, 1994) and irradiation (Joseph and Dikshit, 1993). In spite of all these efforts, sweet lupine varieties are not completely free of alkaloids.

In Ethiopia, four species of lupines have been introduced. These include *Lupinus luteus*, *Lupinus mutabilis*, *Lupinus mexicanus* and *Lupinus albus* (locally known as Gibto). Currently however, the available species in the country are *Lupinus mutabilis* and *Lupinus albus* (Forest Gene Bank of Ethiopia, 2008). *Lupinus albus* has desirable agronomic characteristics, as it is non-shattering, disease-resistant, high-yielding and can grow on marginal soils.

Although, *Lupinus albus* is widely used as a food crop in Ethiopia, there are no reports on the effects of traditional processes on its nutritional status and levels of its anti-nutritional factors. Here, we report the efficiency of some traditional processing techniques in removing the anti-nutritional factors and their effect on the nutritional values.

MATERIALS AND METHODS

Description of the sampling areas

Lupinus albus seeds were collected from two different agro-ecological regions of Ethiopia, namely, Dangla (longitude 11°16' N, latitude 36°50' E and 2,137 m asl) and Chagni (longitude 10°57' N, latitude 36°30' E and 1,583 m asl). Dangla is a moderately cold region while Chagni is hot and both highly favour the production of the crop (CSA, 2007).

Sampling and sample preparation

Seeds of *Lupinus albus* were randomly collected from both sampling sites and were packed in polyethylene bags and transported to the Food Science and Nutrition Laboratory of Addis Ababa University, Ethiopia. Before analysis, samples were proportionally mixed (composite sample) and were cleaned manually to remove all foreign matter, immature and damaged seeds. Clean seeds were then processed following the traditional techniques:

a) Roasting, soaking and de-hulling

Raw seeds of *Lupinus albus* were roasted on a metal pan for 10 minutes together with pre-cleaned sand (i.e. washed using distilled water) to allow uniform roasting. After cooling (for 10 minutes) samples were washed several times and soaked in a bucket of tap water (1:10 ratio). The soaking was done for five days and soaking water was changed every 4 hours. The bitterness was checked by tasting the whole seed as is done traditionally. A portion of the seed was then de-hulled manually and both the whole seed and the kernel were freeze dried at −42°C (FD-18S automatic freeze drier from LABFREEZ instruments) for 48 hours. The dried samples were milled with a sample miller (Mortar Grinder RM 200 from Retsch) having a sieve size of 60 meshes and packed in brown glass containers until analysis.

b) Boiling, soaking and de-hulling

Clean seeds of *Lupinus albus* were boiled in water for 4 hours and soaked in a bucket of water (1:10 ratio). The soaking was done for five days and soaking water was changed every 4 hours. Afterwards, the whole seed was de-hulled manually and both the whole seed and the kernel were freeze dried at −42°C for 48 hours. The dried samples were milled using laboratory sample mill with sieve size of 60 meshes and packed in brown glass until analysis.

c) Germination

Clean seeds of *Lupinus albus* were soaked in tap water for 24 hours. The water was then drained off and the sample covered in castor bean leaf and left to germinate at room temperature for 48 hours. At the end of the germination process, part of the seeds was de-hulled manually. Both the whole seed and the kernel were freeze dried at −42°C for 48 hours. The dried samples were milled using laboratory sample mill with sieve size of 60 meshes and packed in brown glass until analysis.

Analytical methods

All chemicals used for analysis were analytical-grade reagents which were obtained from the Sigma Aldrich Company. The methods used were: moisture AOAC 925.09 (2000), crude protein AOAC 979.09 (2000), crude fat AOAC 4.5.01 (2000), crude fiber AOAC 962.09 (2000), crude ash AOAC 923.03 (2000) and utilizable carbohydrate content was calculated by difference with the exclusion of crude fiber (AOAC, 2000). The hull weight was determined gravimetrically. The whole seed was weighed and soaked in distilled water in 1:10 seed to water ratio for 12 hours. Then, the whole seed

was de-hulled manually. The kernel was dried at 105°C for 3 hours and then weighed until constant reading was observed. Gross energy content was calculated using the following formula:

Gross energy (Kcal) = (9 x crude fat) + (4 x crude protein) + (4 x utilizable carbohydrate).

Phytate content was determined according to the method described by Latta and Eskin (1980) cited in Nam-Soon and Man-Jin (2009), and later modified by Vaintraub and Lapteva (1988). The alkaloid content was determined gravimetrically by the method of Harborne (1973) as cited in Adeniyi *et al*. (2009).

Statistical analysis

Nutritional composition and anti-nutritional factors of the raw and processed samples of *Lupinus albus* were statistically compared using Analysis of Variance (ANOVA) and Least Significant Difference (LSD). The statistical package used was SPSS version 15. Significant differences were determined at $p<0.05$ level. Results were expressed as mean ±SD.

RESULTS AND DISCUSSION

There are variations in the nutritional content of lupine as a result of the characteristics of the growing conditions and soil types (Martinez *et al*., 2006). Growing location was reported to significantly affect fresh yield and contents of moisture, crude fiber, oil and protein in white lupine sprouts whereas cultivar effects were significant only for fresh yield of sprouts and a location with cooler climate and heavier soil are desirable (Harbans *et al*., 2012).

Hull weight proportion

Hull weight for seeds from Dangla and Chagni was found to be 16.22% and 19.30%, respectively. The values for the seeds from the two sites were significantly different ($p<0.05$) and this might be attributed to the difference in agronomic features of the sampling sites.

Proximate composition

The moisture content of the whole seeds from Dangla and Chagni were 6.94% and 8.04%, respectively (Table 1). All the treatments showed a significant reduction in the moisture content of the raw seed in both sample types ($p<0.05$).

Crude fat: The crude fat content of the raw *Lupinus albus* seeds from Dangla and Chagni areas were 9.34% and 8.79%, respectively ($p<0.05$). According to Huyghe (1997), oil content of raw *Lupinus albus* is in the range of 6-13%. All the traditional processing techniques (except germination) enhanced the oil content of seeds from Dangla and Chagni, the highest being in boiled and then soaked kernel (16.51% and 14.28%, respectively). This can be attributed to the inactivation of lipolytic enzyme activity, thereby inhibiting the breakdown of triacylglycerols, leading to an increase in the total fat content. On germination however, crude fat content of the raw seeds decreased. As seeds grow, they require high amount of protein and primary materials used in the synthesis of protein are lipids and carbohydrates (Bau *et al*., 1996). In addition, lipase activity increases during germination processes and lipids are hydrolyzed (Osman, 2007).

Crude protein: The crude protein content of whole seeds from Dangla and Chagni were 37.86% and 39.71% ($p<0.05$), respectively. These values are higher than that reported by Jimenez-Martinez *et al*. (2003), which was 34.40% for the same species of *Lupinus albus*, and are comparable with the crude protein content of other legumes like soybean which were 41.1% (El-Adawy *et al*., 2000). Boiling followed by soaking kernels for five days resulted in a high value of crude protein (52.25% in Dangla seeds and 56.13% in Chagni seeds). The rise in the value of crude protein content during soaking is due to the synthesis of enzyme proteins, the simultaneous removal of nitrogenous compounds such as alkaloids and perhaps non-protein nitrogen and the improvement of cell wall permeability (Bau *et al*., 1996; Jimenez-Martinez *et al*., 2003). High values of crude protein when the seed was de-hulled also indicates that the hull has low crude protein content and substantially diluted the protein content of the grain. The crude protein content increased slightly during germination and this can be attributed to the loss of non-protein mass through respiration.

Crude fiber: The Dangla and Chagni seeds (untreated) exhibited crude fiber contents of 11.08% and 11.07%, respectively. There was no significant difference between the two values ($p>0.05$). Reports show varied values (e.g. 11.7%, 4.5%) for crude fiber contents of *Lupinus albus* (El-Adawy *et al.*, 2000; Jimenez-Martinez *et al*., 2003). Following boiling and then soaking, germination of the whole seed has showed higher crude fiber content and this might be due to the growth of additional plant parts such as shoot and root (Jimenez-Martinez *et al*., 2003). The reduction of the crude fiber in de-hulled seeds might be due to the removal of some water-soluble oligosaccharides along with indigestible carbohydrates.

Crude ash: The crude ash content of *Lupinus albus* from Dangla and Chagni sites were found to be 2.80% and 2.88%, respectively and are less than the reports by Sujak *et al*. (2006) (3.9%), El-Adawy *et al*. (2000) (4%) and Jimenez-Martinez *et al*. (2003) (3.2%). However, the values are higher than the result reported by Erbas *et al*. (2005) (2.65%). The difference in ash content between the two samples in the current study could be attributed to the soil type where the plant is grown (Petterson, 2000).

The ash content in all the processed seeds were lower (up to 1.35% in Dangla seeds and 1.47% in Chagni seeds, both in roasted and then soaked cases) and this can be associated with the extensive soaking and washing processes (Arslan and Seker, 2002). Germination, however, significantly increased the ash content.

Utilizable carbohydrate: The raw samples from Dangla and Chagni exhibited utilizable carbohydrate content of 38.92% and 37.50%, respectively. A study by Jimenez-Martinez *et al*. (2003) reported the value for utilizable carbohydrate of raw *Lupinus albus* to be 26.80%, which was lower than values for the raw samples in the current study. All the treatments applied in the current study reduced the utilizable carbohydrates. These results were in agreement with other studies (Yousef and Abdel-Gawad, 1992; El-Adawy *et al*., 2000; Jimenez-Martinez *et al*., 2003) and the reason behind the observed reductions in the utilizable carbohydrates is due to leaching out of various water-soluble components of carbohydrates.

Gross energy: The two raw samples of *Lupinus albus* from Dangla and Chagni sites had total energy content of 391.19 Kcal/100 gm and 388.12 Kcal/100 gm, respectively. All the treatments, except germination led to significant increase in the total energy content of the raw samples. This increment was in parallel to the improvements in protein and fat content.

Table 1. Proximate composition of raw and processed seeds of *Lupinus albus* grown in two different agro-ecologcial zones of Ethiopia (Dangla and Chagni).

Proximate composition Treatments	Moisture (%)[y]	Crude fat (%)[x,y]	Crude protein (%)[x,y]	Crude fiber (%)[x,y]	Crude ash (%)[x,y]	Utilizable carbohydrate (%)[x,y]	Gross energy (Kcal/100 gm)[x,y]
Dangla seeds							
Whole seed (untreated)	6.94 ± 0.03^{C}	9.34± 0.09^{G}	37.87 ± 0.00^{J}	11.08 ± 0.161^{E}	2.80 ± 0.14^{D}	38.91 ± 0.07^{A}	391.19±0.56^{H}
Roasted then soaked whole seed	6.33 ± 0.03^{E}	14.01 ± 0.19^{C}	44.15 ± 0.03^{F}	11.20 ± 0.205^{E}	1.50 ± 0.01^{E,G,H}	31.31 ± 0.02^{E}	427.92 ±1.78^{D,E}
Roasted then soaked kernel	5.66 ± 0.13^{F}	16.28 ± 0.04^{A}	52.10±0.03^{B}	2.34 ± 0.281^{J}	1.35 ± 0.01^{I}	27.94 ± 0.28^{F}	466.66 ±0.86^{A}
Boiled then soaked whole seed	6.38 ± 0.08^{E}	15.15 ± 0.14^{B}	47.25 ± 0.27^{D}	14.66 ± 0.103^{B}	1.60 ± 0.07^{E,H}	26.09 ± 1.03^{G}	429.75 ±4.34^{D}
Boiled then soaked kernel	5.43 ±0.03^{G}	16.51± 0.07^{A}	52.25 ± 0.35^{B}	7.11 ± 0.928^{F}	1.60 ± 0.002^{E,H}	22.52 ± 1.35^{I}	447.71 ±3.34^{C}
Germinated whole seed	7.71 ±0.14^{B}	7.55 ± 0.14^{J}	41.87 ± 0.93^{G}	14.23 ± 0.256^{C}	2.96 ± 0.01^{B,C}	33.38 ± 1.05^{D}	368.98 ± 1.73^{I}
Germinated kernel	5.64 ±0.02^{G}	9.00 ± 0.09^{H}	47.41 ± 0.24^{D}	3.58 ± 0.078^{G}	3.11 ± 0.02^{A}	36.89 ± 0.24^{B}	418.22 ± 0.88^{F}
Chagni seeds							
Whole seed (untreated)	8.04 ±0.02^{A}	8.79 ± 0.05^{H,I}	39.71 ± 0.06^{I}	11.07 ± 0.06^{E}	2.88 ± 0.01^{D,C}	37.56 ± 0.06^{B}	388.12 ±0.46^{H}
Roasted then soaked whole seed	1.88 ± 0.09^{I}	11.47 ± 0.28^{D}	44.27 ± 0.18^{F}	14.10 ±0.385^{D}	1.54 ± 0.04^{F,H}	28.62 ± 0.33^{F}	394.79±3.12^{G}
Roasted then soaked kernel	2.26±0.02^{H}	13.71 ± 0.46^{C}	56.24 ± 0.03^{A}	2.67 ± 0.041^{I}	1.47 ± 0.02^{F,G}	25.90± 0.49^{G,H}	451.98 ± 2.06^{B}
Boiled then soaked whole seed	1.79 ± 0.08^{I}	11.13 ± 0.05^{E}	45.58± 0.15^{E}	14.95 ± 0.251^{A}	1.70 ± 0.05^{E}	26.63 ± 0.40^{G}	389.05 ± 1.49^{H}
Boiled then soaked kernel	2.17 ± 0.02^{H}	14.28 ± 0.00^{C}	56.13 ± 0.21^{A}	2.91 ± 0.022^{G,H}	1.69 ± 0.01^{E}	24.99 ± 0.23^{H}	453.01 ± 0.12^{B}
Germinated whole seed	5.44 ± 0.14^{G}	8.59±0.19^{I}	41.23 ± 0.05^{H}	11.74 ± 0.194^{E}	2.89 ± 0.06^{C,D}	35.55 ± 0.12^{C}	384.41± 1.97^{I}
Germinated kernel	6.70 ±0.04^{M}	7.89 ± 0.14^{F}	48.99 ± 0.09^{C}	2.86 ± 0.376^{H}	3.01 ± 0.18^{B,A}	35.25 ± 0.43^{C}	425.94 ± 0.09^{E}

[x] Data are reported on dry basis, [y] mean ± standard deviation (SD), means in the same column with different letters are significantly different, (A-J), $p<0.05$

Anti-nutritional factors

Total alkaloids: The concentration of alkaloids in the raw samples was 2.46% and 2.26% for the Dangla and Chagni samples (Table 2), respectively showing a significant difference in the two raw samples ($p<0.05$). All the treatments on *Lupinus albus* seed from Dangla and Chagni sites led to significant decrease (33-71%) in the total alkaloid content. Since alkaloids are water-soluble, soaking in water can easily remove them from the whole seed. However, this depends on the type of soaking solution and permeability of the cell wall of the hull (Jimenez-Martinez *et al*., 2003). This improvement in the cell wall permeability of the hull is in order to facilitate the removal of alkaloids. Adewusi and Falade (1996) reported improvement in the hull permeability of the raw seed of *Lupinus albus* and other legume types after thermal treatment and soaking.

De-hulling led to a significant reduction in the total alkaloid content of the raw seeds ($p<0.05$) in the current study. This might be due to the presence of the alkaloids in the hull as well. However, in the case of germination, since there was no involvement of heat, the permeability of the cell wall was not as effective as the former case to remove the alkaloids. Germination could be an effective method to reduce other anti-nutritional factors (Trugo, 1992; De la Cuadra *et al*., 1994; Cunha-Queda and Beirao daCosta, 1994).

Phytic acid: There was no significant difference in the phytic acid content of the two cultivars ($p>0.05$). The values for Dangla seeds were 144.33 mg/100 gm, while for Chagni seeds were 143.96 mg/100 gm. Phytic acid contents of *Lupinus albus* seeds were reported by Trugo *et al*. (1993) to range between 0.4 to 1.2 gm/100 gm dry matter from various cultivars studied. The values found for the raw samples of this study were below this range. However, the levels observed in the current study were within the range reported for common beans, lentils and peas (Donangelo *et al*., 1986; Harland and Oberleas, 1987).

All the treatments applied on the raw seed affected the phytic acid content significantly and the highest reduction was observed in germinated seeds. The reason behind this reduction might be due to the stable nature of phytic acid on heat treatments. This would imply that heat treatment is an effective method for reducing phytic acid content. Instead, hydration seems to facilitate its removal from the raw seed. This is because during hydration there is an activation of phytase enzyme. This enzyme can help the breakdown of the phytate molecule (Bau *et al*., 1996). The increment in phytase activity in the range of 800 - 2000% has been reported for several

varieties of legumes during the first five days of germination. As the number of germination days increased, the higher will be the reduction in phytate content as the enzyme concentration that digests phytate will be high.

Table 2. Total alkaloids and phytate content of raw and processed *Lupinus albus* grown in two different agro-ecological zones of Ethiopia (Dangla and Chagni).

Anti-nutritional factors / Treatments	**Total alkaloid (%)[x,y]**	**Phytate (mg/100 gm)[x,y]**
Dangla seeds		
Whole seed (untreated)	2.46 ± 0.03^{A}	144.33 ± 1.77^{A}
Roasted then soaked whole seed	0.84 ± 0.01^{K}	133.14 ± 0.98^{C}
Roasted then soaked kernel	0.71 ± 0.00^{L}	129.52 ± 2.14^{E}
Boiled then soaked whole seed	1.19 ± 0.02^{G}	117.91 ± 1.73^{G}
Boiled then soaked whole kernel	1.01 ± 0.04^{I}	112.81 ± 1.14^{H}
Germinated whole seed	1.65 ± 0.03^{C}	107.92 ± 0.24^{I}
Germinated kernel	1.48 ± 0.04^{E}	82.87 ±0.54^{L}
Chagni seeds		
Whole seed (untreated)	2.26 ± 0.01^{B}	143.96 ± 0.40^{B}
Roasted then soaked whole seed	0.79 ± 0.04^{K}	130.01 ± 2.83^{D}
Roasted then soaked kernel	0.67 ± 0.01^{L}	125.94 ± 1.82^{F}
Boiled then soaked whole seed	1.07 ±0 .04^{H}	111.66 ± 1.43^{H}
Boiled then soaked whole kernel	0.89 ± 0.03^{J}	105.16 ± 0.89^{J}
Germinated whole seed	1.54 ± 0.04^{D}	92.68 ± 0.74^{K}
Germinated kernel	1.39 ±0 .01^{F}	78.18 ± 0.88^{M}

[x] Data are reported on dry basis , [y] mean value ± standard deviation, means in the same column with different letters are significantly different (A-M), $p < 0.05$

CONCLUSION AND RECOMMENDATION

Lupinus albus seeds collected from two different agro-ecological zones, namely, Dangla and Chagni, Ethiopia, were subjected to traditional processing techniques (roasting then soaking, boiling then soaking and germination). The parameters analyzed were proximate composition and anti-nutritional factors. The results of the research revealed that the treatments were able to reduce the anti-nutritional factors and to enhance the nutritional value. There appears to be no published information on other available varieties of the genus *Lupinus*. Further research on the determination of the amino acid composition and new products development using *Lupinus* is recommended.

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