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Application of microwave and infrared treatment to reduce antinutrients in lupine seeds

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Abstract. Lupins are group of legumes that produce seeds containing up to 40% protein, making them an excellent source of food or feed. However, the nutritional value of legumes can be adversely affected by the presence of various anti-nutrients, including phytic acid, alkaloids etc. The aim of this study was to evaluate the potential of microwave and infrared radiation to reduce the levels of anti-nutrients - alkaloids and urease activity - in lupine seeds. The seed treatment was carried out using two different applications - microwave with time regimes 2 minutes or 6 minutes and infrared toplight with time regimes: 10 minutes at 25 °C and 30 minutes at 35 °C. Content of protein, fat, amino acids, alkaloids and urease activity were determined. The results show that in the samples of lupins the alkaloid content decreased from 0.07% to 0.05% after 6 min of microwave treatment and from 0.07% to 0.03% after 10 min of infrared treatment. Concluded that both treatments reduced the content of alkaloids and urease activity in lupine seeds, without significantly changing either the protein content or the amino acid composition and can be used for antinutrient reduction without loss of nutrients.

Key words: plant protein, alkaloids, food, feed.

INTRODUCTION

Obtaining plant-based protein from local sources is becoming increasingly important, especially in countries like Latvia and others in northern Europe where soy cultivation is limited. An assessment of protein crop production costs - both per tonne of product and per tonne of protein - has shown that locally grown peas, field beans, and lupines are more cost-effective protein sources compared to imported GM soybean meal (The competitiveness of locally sourced protein-based raw materials for inclusion in pig feed recipes. In Soy and barley grown in Latvia - Innovation in pig feeding, 2021).

Lupine is a valuable legume that contains up to 40% protein in its seeds, making it an excellent feed source for short supply chains and promoting self-sufficiency within farms. The crude protein content in white lupines ranges from 33% to 37%, with an average of 35% across sources. Yellow and narrow-leaved lupines contain an average of 43% and 30% protein, respectively. Additionally, the protein and amino acid digestibility of lupines is approximately 85% (Hess, 2017; Devi et al., 2019).

Lupin seeds have a wide range of applications and are increasingly used not only as animal feed but also in the food, cosmetics, and pharmaceutical industry. Studies show, that lupine seed extract improves the structure of low-fat yogurt (Ahmed et al., 2024). Lupin flour is largely used as eggs substitute in cakes, biscuits, and has been added to spaghetti, pasta and bread (Kohajdova et al., 2011). Additionally, lupin (*Lupinus angustifolius*) protein hydrolysate is a potential source for nutraceuticals and functional foods (Tapadia et al., 2021; Kamran et al., 2023), can be used as a valuable source of high value-added compounds for diverse food products (Lemus-Conejo et al., 2023), and is suitable for inclusion in the diet of rainbow trout (Serrano et al., 2024). Cabello-Hurtado et al. (2016) highlighted the potential of lupine seed proteins as nutraceuticals in the pharmaceutical industry, while Caramona et al. (2024) noted numerous positive effects in cosmetology, including improved skin condition.

However, the nutritional value of legumes can be adversely affected by the presence of various anti-nutritional factors, including phytic acid, saponins, tannins, alkaloids, polyphenols, protease inhibitors, oxalates, lectins and others (Embaby, 2010). These substances reduce the nutritional value of food or feed by chelating minerals, proteins and other nutrients, thereby inhibiting their digestibility and utilization.

Different *Lupinus* species possess unique alkaloid profiles, typically consisting of 4–5 major alkaloids and several minor ones. The alkaloid composition of a plant is useful for taxonomic purposes (Frick et al., 2017). The predominant alkaloids in narrow-leaved lupine seeds are lupanine (65–75% of the total alkaloid content), angustifoline (10–15%) and 13-hydroxylupanine (10–15%). Minor amonts of sparteine and lupinine are also present. These values may vary depending on the genotype and its locality. The concentration of alkaloids in plant organs, as well as their correlations, can change under different growing conditions (Vishnyakova et al., 2020). Even in low-alkaloid cultivars, their content can vary significantly, sometimes exceeding threshold limit values (Romanchuk & Anokhina, 2018). Lupines grown at high latitudes have been found to contain fewer alkaloids than those grown in southern regions (Gresta et al., 2017). By the seed ripening stage, the alkaloid content in seeds is 5–10 times higher than in green biomass (Akritidou et al., 2015). The alkaloid content in lupine seeds varies across different varieties and is influenced by variety, cultivation system and environmental factors such as temperature. Lupine grown in an organic cultivation system had lower alkaloid content; however, ambient temperatures during seed formation led to higher alkaloid levels (Jansen et al., 2009; Jansen et al., 2015). Genetic selection for alkaloid removal has led to the development of sweet lupin varieties, which are more attractive for animal feeds, thereby increasing their use in commercial feeds for both monogastric and ruminant species (Hess, 2017). However, sweet lupine grows poorly in Latvia, with plant diseases and root rot often observed. Therefore, there is a need to eliminate these toxic substances through processing methods.

One type of processing that can reduce anti-nutritional factors and plays an important role in improving the nutritive value of legumes is heat treatment (Devi et al., 2019; Schryvers et al., 2023). Hefnawy (2011) found that lentil seeds cooked in microwave oven for 15 min could reduce 93.2% trypsin inhibitor and 100% raffinose. Other methods to reduce anti-nutritional compounds are soaking, germination, infrared treatment. Yalcin & Basman (2015) reported that the level of trypsin inhibitor in soybean decreased from 53.25 TIU mg^{-1} to 2.66 TIU mg^{-1}after infrared treatment, although the process parameters (infrared power, treatment time) were not specified in the study.

Wiriyaumpaiwong et al. (2004) investigated the effects of infrared treatment on the urease activity of soybean at a single power level, using a laboratory-scale infrared dryer equipped with two 650 W infrared bulbs. Treatment at 160 °C for 10 minutes was sufficient for urease inactivation, resulting in a pH difference of less than 0.3.

One of the less-studied methods for processing grains and legumes is short-term microwaving, which raises the temperature of the product and provides the possibility of reducing its anti-nutrient content. However, research data are contradictory, and there is no information on the effect of microwaves and infrared treatment on the amino acid composition and other nutrients.

The aim of the study is to obtain data on the potential of microwave and infrared radiation to reduce the levels of anti-nutrients – alkaloids and urease - in lupine seeds and to evaluate the effect of treatment on the chemical composition of the seeds.

MATERIALS AND METHODS

Two experiments were carried out under laboratory conditions:

1. Processing in a household use microwave (MW) oven GoldStar ER-6460D (continuous exposure) was carried out for lupine seeds of the variety 'Derliat' in two modes, with power 700W:

a) unground seeds processed for 2 minutes (110 °C),

b) unground seeds processed for 6 minutes (130 °C).

2. Treatment with infrared light (IR) using GPL toplighting 1.2DR/WLBHO400V (Philips, Poland), used daily for growing plants in a greenhouse. Sample processing illustrated in Fig. 1. IR was carried out on lupine seeds of the varieties 'Derliat' and 'Rumba'. The seeds are spread on a glass tray 120mm away from the heat source in a thin, shallow layer to ensure even processing. The space above the lamp is enclosed with foil sheets to ensure a constant temperature, and the temperature is fixed during seed treatment. The seed treatment was carried out under two different time regimes: 10 minutes at 25 °C and 30 minutes at 35 °C, with the indicated temperatures reached at the end of the process (starting/room temperatures – 18 °C).

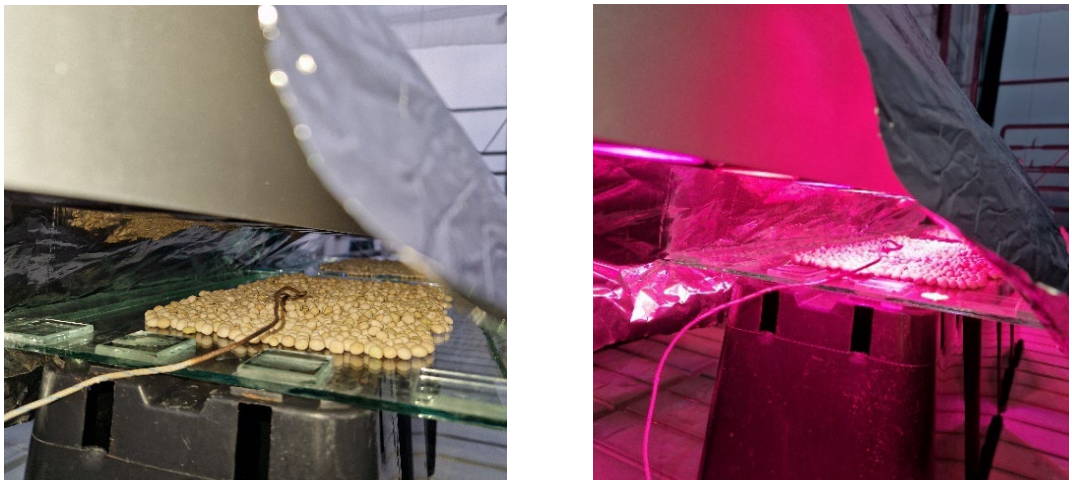


Figure 1. Experimental treatment of lupin seeds with IR light.

Chemical composition testing of lupine seeds was performed using standard methods: moisture content - LVS EN ISO 712-1:2024, protein content - Kjeldahl method, LVS EN ISO 5983-2:2009, fat content - ISO 11085:2015 using Soxhlet Extraction System. The determination of starch content and antinutrients was performed as described below.

Determination of the alkaloids content.

A sample weighting 1g (size 0.5 mm) is transferred to a flask. Then, 15 mL of a 2:1 petroleum and chloroform solution () and 1 mL of 15% sodium hydroxide solution are added. The solution is shaken for no less than 16 hours. Filter the sample through filter paper and add 10 mL of 0.1 N HCl to the filtrate. Shake the solution for 30–40 seconds. Fluid degradation occurs, resulting in a yellow and colorless solution containing alkaloids. Add 10 mL of 0.1 N HCl to the yellow solution, mix thoroughly-, and remove the colorless part. Repeat this process three times. From the alkaloid solution, take 10 mL and add 5 mL of a saturated $NaHCO_3$ solution. Leave it for 2–4 minutes, then add 5 mL of 0.01 N iodine solution and 0.5 g KJ. Close the flask and place it in a dark location for 15 min. Afterward, add 10 mL of 10% sulfuric acid and titrate with 0.01 N sodium thiosulphate, indicator starch solution. The alkaloids content is calculated using the formula:

$$A(\%) = \frac{(\mathrm{a} - \mathrm{b})\mathrm{xT}}{\mathrm{n}} x100, \quad (1)$$

where a – volume 0.01 N J_2, mL; b – volume 0.01 N $Na_2S_2O_3$, mL; T – the title 0.01 N J_2, 0.0004225; n – sample mass, g.

Determination of the urease activity

Inject the sample into the tube and add 10 mL of urea buffer solution. Seal the tube with a stopper, shake vigorously, and place it in a water bath at 30 ℃ ± 0.5 ℃ for 30 minutes. Immediately add 10 mL of hydrochloric acid, quickly cooling the solution to 20 ℃. Quantitatively transfer the content of the tube to a titration vessel by rinsing twice with 5 mL of destilled ater. Titrate the solution with 0.1 moL L^{-1} NaOH solution to a pH of 4.7. The urease activity is calculated relative to the empty sample as follows:

$$U = \frac{14\mathrm{xCx}(\mathrm{V0} - \mathrm{V1})}{30\mathrm{xm}}, \quad (2)$$

where V0 – volume of NaOH used to titrate the blank, mL; V1 – volume of NaOH used to titrate the test sample, mL; m – sample mass, g; C – NaOH concentration, mol L^{-1}.
The result is calculated the dry sample as follows:

$$U(dry) = U\mathrm{x}\frac{100}{100 - \mathrm{W}}, \quad (3)$$

W – moisture content of the sample, %.

Determination of starch content.

The sample is milled (Knifete 1095 Sample Mill Denmark) to a particle size of < 0.5 mm. To 2.5 g ± 0.05 g of the sample, add 25 mL of dilute hydrochloric acid (HCl = 0.309 mol L^{-1}), stir, and then add another 25 mL of the same hydrochloric acid. The sample is placed in a hot water bath at 100 ℃ with a shaker (GFL 1083, Germany) and shaken evenly. Immediately afterward, add 40 mL of cold water and cool the sample in a water bath at 20 ℃ ± 2 ℃. After cooling, add 5 mL of Solution I (prepared by

dissolving 10.6 g of potassium hexacyanoferrate (II) trihydrate in 100 mL of distilled water) and shake the sample for 1 minute. Then add 5 mL of Solution II (prepared by dissolving 21.9 g of zinc acetate dihydrate and 3 g of glacial acetic acid in distilled water, then refilling with distilled water to the 100 mL mark) and shake the sample again for 1 minute. Add distilled water to the mark, homogenize, and filter. If the filtrate is not completely clear, add an additional 5 mL of each solution. Measure the optical rotation of the sample (α = 1) using a 200 mm polarimeter tube in a Polarimeter ZUZI-430 (Beriain (Navarra), SPAIN). Calculation of the result in a dry sample

$$C(dry) = \frac{k(\mathrm{A} - \mathrm{A2})}{100 - \mathrm{W}}, \quad (4)$$

where A – reading from the machine; 2 – reading from the machine when ethanol is used; k – conversion coefficient from table – 10.87-other starches and mixtures; W – moisture content of the sample, %.

Statistical analyses

All analyses were performed in triplicate and the results were presented as mean with standard deviation. Data were processed with MS Excel 2016 software, *T-test* two-sample equal variance was used for the comparing of means, $p < 0.05$ is defined as a significant difference (Kristapsone, 2020).

RESULTS AND DISCUSSION

All varieties of lupin seeds were characterized by protein content, alcoloids and urease content with aim to find out varieties with higher suitability for fooder production and to identify varieties for wich antinutritive compounds exceed the amount allowed for consumption. The results assumed in Table 1.

Table 1. Comparison of protein and alcoloids content in lupin seeds.

Lupine varieties	Protein, %	Alkaloids, %	Urease activity, mg N g^{-1} at min 30 ℃
Haags Blau	31.56 ± 0.06	0.021 ± 0.362	0.38 ± 0.0476
Lejaskurzeme	41.99 ± 0.14	0.017 ± 0.362	0.36 ± 0.0356
Boruta	36.63 ± 0.19	0.019 ± 0.362	0.13 ± 0.0215
Probor	37.00 ± 0.20	0.029 ± 0.362	0.16 ± 0.0215
Derliat	33.37 ± 0.10	0.072 ± 0.362	0.17 ± 0.0215
Rumba	33.97 ± 0.12	0.042 ± 0.362	0.05 ± 0.0215
Sonet	31.89 ± 0.06	0.017 ± 0.362	0.24 ± 0.0215
Žulika	34.50 ± 0.12	0.025 ± 0.362	0.39 ± 0.0476

The results of the study show that the protein content of selected lupine varieties varied from 31.56% to 41.99%, which is similarly high compared to other studies, where it is about 35% of the dry weight (Duranty et al., 2008; Chukwuejim et al., 2023).

According to the standards accepted by several European countries and Australia, the alkaloid content in seeds intended for food or feed purposes (sweet varieties) must not exceed 0.02% of their dry weight (Frick et al., 2017). It was clarified, that lupin seeds of varieties ‘Lejaskurzeme’, ‘Sonet’, ‘Boruta’ contain a small amount of alkaloids and fall within this acceptable level. However, samples of varieties ‘Derliat’, ‘Rumba’,

'Žulika', 'Probor' exceed the allowed amount for animal feed, containing between 0.025% to 0.072% alkaloids. Two varieties with the highest alkaloid content - 'Derliat' and 'Rumba', were selected for processing. Urease activity in these samples was determined to be 0.17 and 0.05 mg N g^{-1} at min 30 ℃, respectively. Changes in chemical composition (in dry matter) before and after processing are reflected in Table 2.

Table 2. Chemical composition of lupin seeds before (control) and after treatment

Sample	Protein, %	Fat, %	Starch, %	Urease activity, mg N g^{-1} at min 30 ℃	Alkaloids, %
DERLIAT	33.37a	6.76a	6.32a	0.17a	0.072a
control	± 0.10	± 0.03	± 0.09	± 0.02	± 0.063
	after microwave treatment				
DERLIAT	33.41a	6.29b	5.82b	0.14a	0.068a
2 min MW	± 0.03	± 0.05	± 0.06	± 0.03	± 0.063
DERLIAT	33.64b	6.40b	6.18a	0.03b	0.056a
6 min MW	± 0.05	± 0.12	± 0.07	± 0.02	± 0.063
	after infrared treatment				
Derliat 2	33.61a	5.40b	5.21b	0.08b	0.03b
10 min	± 0.53	± 0.26	± 0.11	± 0.02	± 0.01
Derliat 1	34.11a	5.36b	5.04b	0.10b	0.03b
30 min	± 0.41	± 0.41	± 0.13	± 0.03	± 0.02
Rumba	34.81a	5.75a	4.46a	0.05a	0.042a
control	± 0.16	± 0.20	± 0.11	± 0.02	± 0.06
Rumba 2	34.65a	5.66a	5.21b	0.03a	0.01b
10 min	± 0.31	± 0.34	± 0.11	± 0.01	± 0.01
Rumba 1	35.21a	5.66a	4.74a	0.04a	0.01b
30 min	± 0.63	± 0.32	± 0.20	± 0.02	± 0.02

The application of microwave treatment, as can be concluded from the Table 2 insignificantly reduced the alkaloid content – a 5% decrease was observed after 2 minutes of treatment and a 22% after 6 minutes treatment. A significant decrease in urease activity was observed after 6 minutes of microwave treatment. The studies of Waseem et al. (2022) showed a 76% decrease of alkaloids in potato powder after 30 minutes treatment of microwaves at 55 °C. The results show that the protein content of the samples did not change significantly, but the fat content decreased ($p < 0.05$). These results allow us to conclude that high temperatures are not required for the reduction of alkaloids.

The results of the infrared treatment show that in the samples of the variety 'Derliat' the alkaloid content decreased from 0.07% to 0.03% after 10 min (a reduction of 57%) and remained at 0.03% even after 30 min of treatment. In the samples of the variety 'Rumba' the alkaloid content decreased from 0.04% to 0.01% (a reduction of 75%). The urease activity of the variety 'Derliat' samples decreased from 0.17 to 0.08 mg N min^{-1} at 30 °C already after 10min (a reduction of 53%) and did not change significantly after 30 min. Wiriyaumpaiwong et al. (2004) reported sufficient urease inactivation (< 0.3 pH difference) at infrared treatment with laboratory scale bulbs 650W, 160 °C for 10 min. Dondee et al. (2011) reported residual urease activities of soybeans as 70%, 52%, 39% with infrared treatments at 4 kW, 6 kW and 8 kW, respectively. It should be noted that

we obtained similar results within a lower power of infrared treatment - 0.4 kw, correspondingly also at a lower temperature. After treatment of lupins with infrared light, the content of protein, fat or starch did not change significantly. The results of Rehman & Shah (2025) studies of thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes show reductiction in the levels of antinutrients, along with an improvement in protein and starch digestibility. Maximum improvement was observed on cooking these food legumes at 121 °C for 10 minutes. Sesikashvili et.al. (2022) reported alkaloid content decrease by almost 60% under conditions of high-temperature (75–100 °C) micronization of legumes.

In this study was assessed changes in protein quality by comparing the amino acid content of the treated samples. A comparison of amino acid composition after different treatments is shown in the Table 3.

Table 3. Composition of amino acids before and after microwave (MW) and infrared (IR) treatments

Amino acids, g 100 g^{-1}	Derliat control	Derliat MW 6min	Derliat 1 IR 30 min	Derliat 2 IR 10 min	Rumba 1 IR 30 min	Rumba 2 IR 10 min
	average ± SD					
Aspartic acid	2.78 ± 0.44	3.03 ± 0.48	3.41 ± 0.02	3.46 ± 0.06	.40 ± 0.02	.47 ± 0.07
Glutamic acid	6.05 ± 0.97	6.62 ± 1.06	6.43 ± 0.09	6.63 ± 0.09	6.92 ± 0.04	6.74 ± 0.07
Serine	1.43 ± 0.23	1.53 ± 0.25	1.53 ± 0.07	1.55 ± 0.04	1.55 ± 0.08	1.53 ± 0.03
Glycine	1.21 ± 0.19	1.30 ± 0.21	1.09 ± 0.10	1.12 ± 0.05	1.07 ± 0.09	1.08 ± 0.04
Arginine	2.80 ± 0.45	2.96 ± 0.47	3.01 ± 0.09	2.93 ± 0.01	3.24 ± 0.07	3.18 ± 0.23
Threonine	0.90 ± 0.14	0.98 ± 0.16	1.01 ± 0.07	1.05 ± 0.06	1.05 ± 0.01	1.10 ± 0.05
Alanine	0.96 ± 0.15	1.05 ± 0.17	0.91 ± 0.05	0.96 ± 0.03	0.96 ± 0.02	0.87 ± 0.03
Proline	1.22 ± 0.20	1.31 ± 0.21	1.29 ± 0.09	1.27 ± 0.04	1.42 ± 0.01	1.32 ± 0.02
Histidine*	0.72 ± 0.11	0.77 ± 0.12	0.74 ± 0.04	0.71 ± 0.00	0.80 ± 0.03	0.74 ± 0.02
Tyrosine*	0.82 ± 0.13	0.86 ± 0.14	0.97 ± 0.06	1.01 ± 0.01	1.06 ± 0.01	1.02 ± 0.01
Valine*	0.94 ± 0.15	1.06 ± 0.17	1.22 ± 0.06	1.29 ± 0.02	1.38 ± 0.01	1.30 ± 0.01
Methionine*	0.19 ±0.03	0.20 ± 0.03	0.14 ± 0.01	0.16 ± 0.02	0.13 ± 0.03	0.12 ± 0.01
Cysteine*	0.35 ± 0.06	0.39 ± 0.06	0.25 ± 0.04	0.22 ± 0.00	0.20 ± 0.00	0.21 ± 0.02
Isoleucine*	1.01 ± 0.16	1.12 ± 0.18	1.36 ± 0.02	1.38 ± 0.03	1.47 ± 0.02	1.45 ± 0.03
Leucine*	1.87 ± 0.30	2.04 ± 0.33	2.61 ± 0.11	2.63 ± 0.13	2.69 ± 0.08	2.52 ± 0.12
Phenylalanine*	1.05 ± 0.17	1.17 ± 0.19	1.29 ± 0.02	1.34 ± 0.00	1.40 ± 0.02	1.35 ± 0.07
Lysine*	1.30 ± 0.21	1.39 ± 0.22	1.39 ± 0.07	1.39 ± 0.03	1.46 ± 0.00	1.50 ± 0.06
∑EAA**	8.25 ± 1.32	9.00 ± 1.44	9.97 ± 0.53	9.97 ± 0.24	10.59 ± 0.2	10.21 ± 0.2
∑EAA in total	32%	32.4%	34.8%	34.5%	35.4%	34.6%

*essential amino acid; **∑EAA – Sum of Essential amino acids.

Comparison of amino acids composition show that neither the individual amino acids nor the total amount of essential amino acids showed a statistically significant difference ($p > 0.05$). This could possibly be explained by the relatively short sample processing time at high (130 °C) temperature under microwaves and the influence of low temperature (25–35 °C) under infrared treatment. The proportion of essential amino acids in the total increases after processing, thus improving nutritional value of lupin seeds.

The quinolizidine alkaloids levels of bitter lupin seeds are traditionally reduced by soaking, cooking and washing lupin seeds for several days in large volumes of salted water. Optimum processing conditions comprised 18 h of soaking, 1 h cooking, 3 changes of water per day and 22 h of agitation/day This process removes between 88

and 97% of alkaloids (Carvajal-Larenas, 2013), but these are time and resource intensive treatments. The methods involving the presence of water such as soaking, boiling, autoclaving enhances reduction of antinutrients by leaching and hydrolysis (Suhag et.al., 2021) and can also reduce the water-soluble protein content.

As evidenced by the literature review (Dondee et al. 2011; Waseem et.al., 2022), infrared heating does not change the quality attributes of foods significantly, such as vitamins, protein, and antioxidant activities (Krishnamurthy et al., 2008, Tyagi et al., 2020). The results of the study show that both treatments have been sparing of amino acid changes, including sum of essential amino acids, but reduce as the alkoloid content of the samples same as the urease activity. However, processing methods need to be improved. The temperature in the microwave oven reached 130 °C within 6 minutes, and the sample in the center heated up faster, as evidenced by the change in its color to a darker shade. Therefore, the sample was processed unevenly, and it would be advisable to stir it during processing. Microwave processing power and duration has significant effect on the inactivation of antinutrients and needs to be critically evaluated for effective application on raw material. Infrared processing has more efficiently and at a more gentle temperature. Although both of these methods have advantages, compared to boiling, soaking, etc., more detailed studies are desirable to clarify the exposure conditions on different plant species and impact on other antinutrients such as tannins, trypsin inhibitor, phytic acid etc.

CONCLUSIONS

The results of the study show that treatment in a microwave oven for 6min and infrared radiation for 10 min reduces the content of alkaloids and urease activity in lupine seeds, without significantly changing either the protein content or the amino acid composition. Processing is simple, does not require a lot of resources such as water and time. They could be used for antinutrient reduction without loss of nutrients utilizing plant-based proteins more efficiently and reducing reliance on imported feed ingredients.

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